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Review

Identifying components of protein complexes in *C. elegans* using co-immunoprecipitation and mass spectrometry

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ABSTRACT

Mass spectrometry-based proteomics is rapidly becoming an essential tool for biologists. One of the most common applications is identifying the components of protein complexes isolated by co-immunoprecipitation. In this review, we discuss the co-immunoprecipitation, mass spectrometry and data analysis techniques that have been used successfully to define protein complexes in *C. elegans* research. In this discussion, two strategies emerged. One approach is to use stringent biochemical purification methods and attempt to identify a small number of complex components with a high degree of certainty based on MS data. A second approach is to use less stringent purification and identification parameters, and ultimately test a longer list of potential binding partners in biological validation assays. This should provide a useful guide for biologists planning proteomic experiments.

Contents

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1. Introduction

The power of proteomics is revolutionizing biological research. Many *C. elegans* scientists are wondering how to harness this emerging technology for their own studies. One of the most common uses of mass spectrometry-based proteomics is the identification of individual proteins from samples containing many proteins. This is especially useful for identifying members of purified protein complexes. Traditionally, a worm geneticist could identify genes acting in the same pathway by mutagenic or RNAi-based screens for animals with a mutant phenotype. However, such genetic screens are limited by an inability to determine if the gene products are interacting physically. Generally, a biochemical purification procedure that isolates a small selection of proteins is required to identify physically interacting components of a complex, although in practice the final sample often contains non-specific 'background' proteins that are not members of the complex. With the development of efficient protein separation technologies, the extreme sensitivity of the modern mass spectrometer can aid in the identification of true complex members. Understanding the relationship between methods used for complex purification, protein identification and complex determination is of key importance in designing a successful experiment.

There are three critical decisions to be made when designing an experiment. 1) How will the protein complex be isolated? 2) How will proteins be identified? 3) How will members of the complex be distinguished from non-specific background proteins? After a discussion of these questions, we will examine published work to see how different approaches affect results.

2. Isolation of the protein complex by co-immunoprecipitation (co-IP)

This question focuses on the biochemical purification steps used to isolate the protein complex. The worm lysate contains thousands of proteins. The goal of purification is to generate a sample containing only the complex of interest. In this regard, the worm poses a challenge. Although the genome and proteome of *C. elegans* is smaller than those of a mammal, proteomic analysis may not be any easier. With larger animals you can reduce the variety of proteins in a sample by surgically removing a tissue of interest. However, when we lyse an adult worm all 959 somatic cells and the germ cells are present. (Imagine your response to a colleague grinding up an entire adult mouse to study kidney proteins.) This reduces the relative amount of desired complex and increases the number of background proteins that need to be removed.

Harvesting synchronized worm cultures can help reduce complexity. The groups studying the kinetochore during early development enriched for embryos by dissolving synchronized adults in bleach and collecting the bleach-resistant embryos [1,2]. Techniques such as gel filtration and differential centrifugation may be used to enrich for organelles [3]. Membrane-associated or DNA binding complexes present specific challenges to complex purification [4,5].

A popular way to isolate a protein complex from worm lysate is by co-immunoprecipitation. The co-IP can be performed in a single-step or as part of a tandem purification. An antibody targeting a known member of the complex is bound to a bead and incubated with lysate in order to extract the target from the solution along with the other members of the complex. The antibody used for the co-IP can recognize the protein of interest or a protein tag that has been genetically fused to the protein of interest. The advantage of using an antibody against the protein of interest is that the protein expression is not altered; the disadvantages are mostly practical, e.g. more time required to synthesize and/or purify the peptide or protein antigen and then to immunize animals and harvest the antibodies. These polyclonal antibodies are limited in supply and unique to each immunized animal. Also, one cannot be certain that the antibodies will be specific enough for useful separation. Alternatively, a molecular "tag" can be added to a protein of interest expressed from a transgene and purified using an antibody against the tag. The tag can be used for a single-step purification or a multi-step or tandem purification. Many different tags have been used in *C. elegans* [6–9]. As the functional impact of adding a tag usually is not known, different tags attached at either the N or C terminus can be constructed. The ability of a tagged protein to rescue the phenotype of a null mutant is a sign that the tagged protein is functional. Commonly, green fluorescent protein (GFP)-tagged proteins are generated to determine protein localization in *C. elegans*. GFP can also be used for single-step purification [10]. This is an exciting option as many strains generated solely to obtain localization data can now be used to identify binding partners. Combining a localization tag with a purification tag, as done with the multipurpose Localization and Affinity Purification (LAP) tag [7], provides an improved purification ability without sacrificing the localization information *C. elegans* biologists have come to expect (see Section 5.1, Identifying a kinetochore regulatory complex).

There are several caveats to tagging proteins. Gene expression may not be regulated under the same promoter and the copy number of the gene could be much higher so the protein may be over-expressed, increasing non-specific interactions. Additionally, the tag itself may interfere with the protein's normal function, translation and turnover rates or prevent the formation of protein complexes. A major benefit to using tags is the commercial availability of well-characterized antibodies which should help to reduce the effort required to optimize the purification.

Isolation of protein complexes often fails due to inadequate sample preparation. Possible explanations for failure include acquiring undetectable amounts of the complex or destruction of the complex during purification. Although the expression level of the target protein and the characteristics of the antibody have a major influence, using a higher amount of worm lysate should increase the likelihood of success. For a co-IP of abundant proteins, 10–20 mg of lysate was adequate [11,12], while 40 g was used for a less abundant membrane protein [4]. Liquid culture of worms can be carried out to generate large quantities of worms [13]. However, as behavior and physiology may be altered by liquid culturing, it is possible that some complexes will be different compared to complexes