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I-DIRT: A Novel Method to Distinguish Specific Protein Interactions from Contaminants in Biological Complexes

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Technology Summary

There have been significant advances in protein research, specifically in the increased ease in isolating protein complexes from biological systems of interest using an affinity-tagged member, and then identifying the constituents of those same complexes using mass spectrometry. The main problem is the co-enrichment of proteins that associate non-specifically with the affinity-tagged complex of interest.

Our scientists have developed a method to overcome this challenge using metabolic labeling, termed Isotopic Differentiation of Interactions as Random or Targeted or I-DIRT for short. First, cells that contain the affinity-tagged protein are grown in regular media, while cells that do not contain the tagged protein are grown in a heavy isotopic medium so that the resulting proteins are labeled at a specific amino acid with a heavier isotope that can be distinguished easily as a visible mass shift during mass spectrometry. The two cell cultures are combined in equivalent amounts, lysed, and a standard immunoisolation procedure is used to obtain the affinity-tagged protein of interest along with its partners. The resulting complex is then digested and analyzed by mass spectrometry to identify those proteins that have a large ratio (60% or more) of light isotopes, which are the proteins that specifically interact with the tagged member.

Advantage

- This method enables researchers to isolate protein complexes under non-stringent conditions, thus preserving the integrity of the complex, while allowing for the discrimination between specific and non-specific interactors within the complex. This method is straightforward, efficient, and adaptable to most biological systems that commonly use immunoisolation methods.

Area of Application

- Proteomic research, specifically the analysis of isolated protein complexes from biological systems utilizing mass spectrometry for individual protein identification.

Stage of Development

- Proof of concept – the method has been used to successfully identify specific interactions within a yeast DNA polymerase complex and a bacterial RNA polymerase complex.

Lead Inventors

- Dr. Brian Chait & Dr. Michael Rout

Patent Information

- U.S. Patents 7,968,299 (issued June 28, 2011) and 8,227,198 (issued July 24, 2012)

References

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