Simposio Internacional: Biointeractómica

***International Symposium**: Biointeractomics*

Sevilla, 30 y 31 de octubre de 2012
Sevilla, October 30-31, 2012

Quantitative Proteomics: A Strategic Ally to Map Centrosomal Protein Interaction Networks

Juan P. Albar

Proteins involved in highly regulated processes, use to work integrated in networks. These networks are formed by multi-domain proteins showing a number of “modules” that are used to accomplish a varied set of interactions. The centrosome is, indeed, a paradigm of a cellular “hub” coordinating many of these protein networks. The centrosome organizes the cytoskeleton of microtubules, and it is also responsible for cell polarity and the formation of the mitotic spindle during cellular division. The characterization of interaction mechanisms and regulation of different protein families, such as the Polo-like (PLK2, 3 and 4) and Aurora kinases, the tubulin binding or microtubule related proteins XMAP, TACC3, NA-14 and DLC8 among others, has been focused by the CAM Centrosome Consortium.

Affinity Purification combined with Mass Spectrometry is arguably the most widely employed technique to characterize protein-protein interactions. Among other advantages, it offers high throughput and sensitivity and allows the characterization of protein interaction networks under physiological conditions. The main challenge of this approach is to distinguish *bona fide* binders from background contaminants that unavoidably result from any purification procedure. In this regard, the combination of quantitative proteomics and affinity purification emerges as one of the most powerful, yet relatively simple, strategies to characterize protein-protein interactions.

The protein NA14 is a key adaptor protein mediating the intermolecular interactions of microtubules and Spastin. To gain insight into its structure and function, we have expressed, purified and characterized human NA14 and some variants. In addition, to characterize interaction partners of the centrosomal protein NA14, we have used Stable Isotope Labeling by Amino Acids in Cell Culture (SILAC) in combination with tag-based affinity purification of the target protein. Quantitative information derived from the SILAC labeling was shown to be effective at filtering out false interaction partners in this experimental setup.