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Uncovering the In Vivo Proxisome Using Proximity-Tagging Methods

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The development of new approaches is critical to gain further insights into biological processes that cannot be obtained by existing methods or technologies. The detection of protein–protein interaction is often challenging, especially for weak and transient interactions or for membrane proteins. Over the last decade, several proximity-tagging methodologies have been developed to explore protein interactions in living cells. Among those, the most efficient are based on protein partner modification, such as biotinylation or pupylation. Such technologies are based on engineered variants of enzymes like peroxidases or ligases that release reactive molecules, in the presence of specific substrates, that bind surrounding proteins. Fusing a protein of interest (POI) to these enzymes allows the definition of an unbiased "proxisome," that is, all of the proteins in interaction or in close vicinity of the POI. Here, the different proximity-labeling tools available are described and comprehensive comparison to discuss advantages and limitations is provided.

1. Introduction

By analogy with a theater play, biological processes require different actors assigned to perform specific tasks in space and time. In living cells, such actors are mostly proteins that "communicate" between each other. Communication is achieved through different types of interactions such as weak, transient, stable, or long interactions, resulting in specific biological effects. Protein–protein interaction (PPI) can lead to conformational effects which enable the formation of a structural complex, activate or inactivate a protein, create a new biding site for the interaction with other binding partners or a substrate, serve as regulatory pathway, or allow subcellular relocalization.

Detection of PPIs, in space and time, is therefore critical for deciphering each step of a biological process. A number of methods for assaying PPIs in vivo have been developed and are routinely used in laboratories. Co-immunoprecipitation (Co-IP) represents one of the most standard methods of identifying interacting partners in vivo.[1,2] Briefly, a protein of interest (POI) is immunoprecipitated from a cellular protein extract by using specific antibodies immobilized on beads (**Figure 1**a). While unbound proteins are washed out, binding partners are co-precipitated and can be visualized and identified by Western Blot or mass spectrometry analysis. It is worthy to note that Co-IP is a variant of the pull-down assay, which used a tagged bait protein to capture protein complexes instead of antibodies. Tandem affinity purification (TAP) is also a systematic approach to detect PPIs at near proteome scale under in vivo conditions. TAP consists in two consecutive purifications by using two different tags fused to a bait protein, then considerably reducing the amount of nonspecific contaminants.[3,4] Additional methods such as two-hybrid systems, including bacterial (bacterial adenylate cyclase-based two-hybrid [BACTH]) and yeast (yeast two-hybrid assay [Y2H]) two-hybrid assays (see ref. [5] for a comparative review), are powerful genetic approaches to characterize PPIs in native or near-native context.[6–13] POIs are fused to the two isolated fragments from the *Bordetella pertussis* adenylate cyclase[6,8–10,13] or from the Gal4 transcription factor[7,11,12] for BACTH and Y2H, respectively (Figure 1b). Physical association between tested protein pairs restores the activity of the adenylate cyclase or Gal4 that can be visualized by a transcriptional-activated reporter. Other interaction-mediated reconstitution-based methods exist, such as the TOXCAT or GALLEX systems for studying transmembrane helix–helix oligomerization in a natural membrane environment.[14–16] With a similar conceptual approach, Förster resonance energy transfer (FRET) is a well-suited biophysical method for the investigation of PPIs that occur between two proteins positioned within 10 nm of each other, allowing study of molecular interactions in real time.[17,18] In brief, FRET relies on the energy transfer from a donor fluorescent molecule (i.e., diminution of fluorescence intensity) to an acceptor when these two probes are separated by 10 nm or less[19–22] (Figure 1c). Then, PPIs or conformational changes within a protein (if the two probes are fused to the same protein) can be visualized in real time, by using a fluorometer or a fluorescence microscope. Furthermore, this method can be extend to several practical applications in biology such as the detection of protein cleavage, changes in micro-environment, or quantitative analysis of protein interactions (see refs. [23,24] for comprehensive reviews).

Although these methods have improved our knowledge about biological processes by the characterization of number of PPIs, some important limitations are still present. Due to the nature of stringent purifications, co-precipitation-based methods

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