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Minireview

Protein complementation assays: Approaches for the in vivo analysis of protein interactions

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ABSTRACT

The in vivo identification and characterization of protein–protein interactions (PPIs) are essential to understand cellular events in living organisms. In this review, we focus on protein complementation assays (PCAs) that have been developed to detect in vivo protein interactions as well as their modulation or spatial and temporal changes. The uses of PCAs are increasing, spanning different areas such as the study of biochemical networks, screening for protein inhibitors and determination of drug effects. Emphasis is given to approaches that rely on signals of spectroscopic nature (i.e. fluorescence or luminescence), the ones that are more directly related to bioimaging.

1. Introduction

The protein sequence universe is expanding at vertiginous speed as a result of decoding the genomes of many divergent organisms. One of the follow-ups of whole-genome sequencing projects consists in deciphering how this myriad of proteins interacts with each other. Proteins display high connectivity in the cell. In other words, proteins never act alone; on the contrary, they associate with other proteins to form stable or transient multiprotein complexes that execute a defined function. Importantly, aberrant protein interactions are related to many diseases and therefore, they have become important targets for the development of new chemical drugs. Overall, there is a requirement of techniques that allow studying protein binding as well as their specific inhibition in vivo because the cellular environment highly affects the establishment of these interactions.

Protein-fragment complementation assays (PCAs) might fulfil the above-mentioned needs because they are particularly well suited to detect protein interactions in the cell. They are based in the fusion of the hypothetical binding partners to two rationally designed fragments of a reporter protein [1]. The interaction between bait and prey proteins brings the split reporter fragments close enough to enable their non-covalent and specific reassembly followed by the recovery of its native structure and activity.

Different types of proteins have been used as protein reporters: dihydrofolate reductase (DHFR) [2], β-lactamase [3], TEV protease [4], green fluorescent protein (GFP) or its variants [5], luciferase [6], etc. Accordingly, the signal readout can be colour, cell survival or fluorescence, among others. PCAs were originally developed with DHFR, but the availability of fluorescent or luminescent proteins has significantly extended their applicability. DHFR enables the use of two different signals to report on protein binding: cell survival (using methotrexate, a DHFR inhibitor) or fluorescence (in the presence of a fluorescent substrate) [6]. On the other hand, if fluorescent [7] or luminescent proteins [8–10] are used, the signal is of spectroscopic nature. Particularly, when the reporter proteins are fluorescent proteins (FPs), we speak about bimolecular fluorescence complementation (BIFC).

Up to now, PCAs have been used in the study of in vivo protein–protein interactions (PPIs) at different levels: from the study of specific bindings in vivo to the screening of novel PPIs in different organisms, ranging from bacteria to animals or plants. Moreover, it has been demonstrated that BIFC is a very sensitive method that can be applied to measure spatial and temporal changes in protein complexes in response to drugs that activate or inhibit particular

Abbreviations: BIFC, bimolecular fluorescence complementation; PCA, protein-fragment complementation assay; GFP, green fluorescent protein; FP, fluorescent protein; DHFR, dihydrofolate reductase; PPI, protein–protein interaction; RET, resonance energy transfer; BIFC-RET, BIFC-based resonance energy transfer; FC, flow cytometry; AP/MS, affinity purification followed by mass spectrometry; Y2H, yeast two-hybrid

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cellular pathways as well as to identify compounds that interfere in protein–protein binding [11].

2. Fundamentals

The PCAs are based on the formation of a bimolecular complex when two non-active fragments of a reporter protein are brought together due to an interaction between bait and prey (where both are fused to the split domains of the reporter) (Fig. 1). The process starts with the interaction of the bait and prey proteins (complex 1). Importantly, this binding occurs in competition with alternative endogenous interaction partners present in the cell (complex 2). The interaction brings the two split fragments in proximity enabling their non-covalent reassembly, folding and recovery of protein reporter function.

The folding and activity of the protein reporter depends completely on the interaction between prey and bait. What's more, in the case of BIFC, the fluorescence signal qualitatively correlates with the interaction strength [12]. Thus, mutations that affect the binding interface cause a decrease in fluorescence emission. Therefore, BIFC can be used to map interaction surfaces in protein complexes.

A crucial requirement for the method is that the dissected fragments should not associate spontaneously in the absence of the binding proteins because this would render the method useless due to the presence of false positives. Studying the self-assembly of FPs, it has been concluded that, if they are expressed at high levels, in certain cases, the fragments can self-associate with each other regardless of the PPI [13]. This tendency depends on the fragments, the proteins fused to them and the cellular environment. Thus, it is really important to perform appropriate controls in PCAs to ensure the specificity of the detected signal. Also, to avoid self-association, it is advisable to express the protein fusions at low levels, close to those of the endogenous counterparts, if possible.

3. Strategy design

The particular design of the PCA experiment might strongly determine its results. Different aspects have to be taken into account:

3.1. Protein reporter

The selection of the protein reporter will depend on the goal of the study and the bait protein. The Table 1 summarizes the different enzymes that have been used. Particularly, DHFR has been widely used in experiments involving library selections, whereas FPs or luminescent proteins are more suitable for studying a specific protein interaction: location, dynamics, inhibition or surface mapping. The simplest reporters are FPs because the readout is directly provided by the protein fluorophore and there is no need of any substrate (as in the case of luminescent proteins or DHFR). One important feature to consider when using FPs as reporters is that their reassembly is usually irreversible [12,14,15]. However, several studies suggest that the bimolecular complex can be partially reversible [16–18]. Nevertheless, in those cases, it has not been demonstrated convincingly that the signal fading is linked to a dissociation of the fusion proteins. Besides, during in vivo experiments, other possibilities such as proteosomal degradation cannot be excluded [19]. On the other hand, one cannot disregard that BIFC provide a stable fluorescence signal that is especially useful when working with weak or transient PPIs.

3.2. Protein reporter fragments

The fragmentation pattern for the chosen protein reporter is usually well defined [5]. Each protein requires specific breaking points that allow the non-covalent protein reconstitution while minimizing the spontaneous folding.

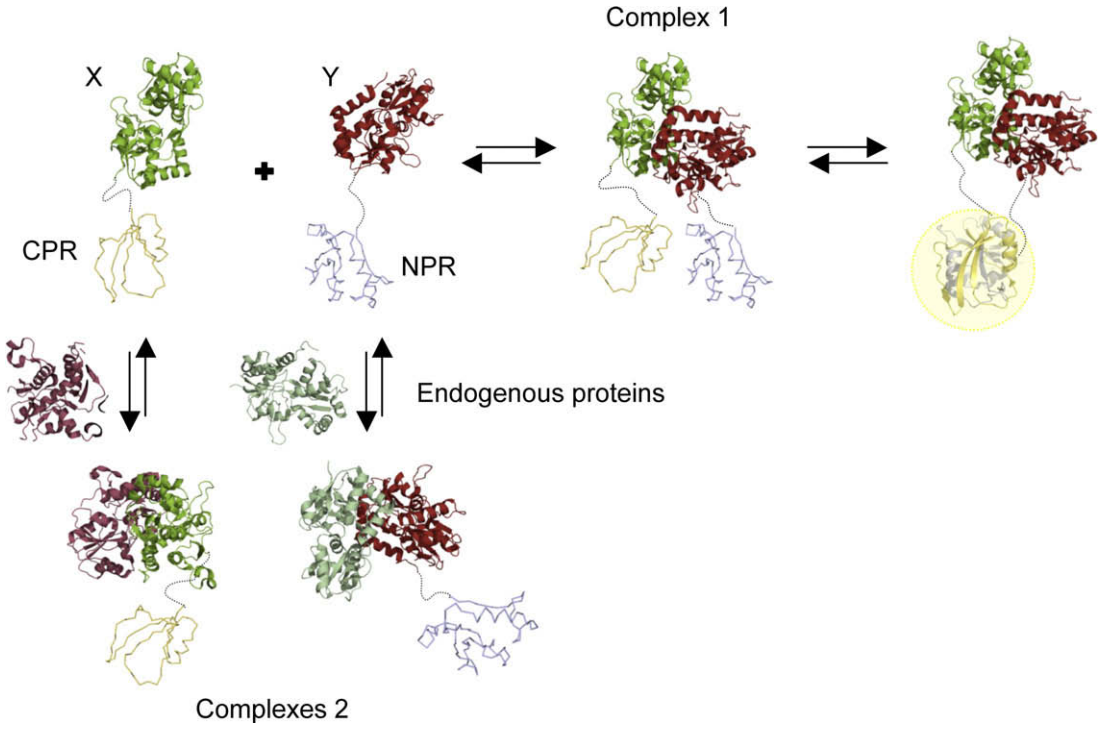

Fig. 1. Pathway for protein complementation assays (PCAs) complex formation. In PCAs each interaction partner (bait, X and prey, Y) is fused to a fragment of the reporter protein (N-terminal fragment, NRP and C-terminal fragment, CPR) and both fusions are co-expressed in the cell. The binding between X and Y (complex 1) brings the two split domains of the reporter together enabling its reassembly and recovery of its activity. It has to be taken into account that this process occurs in competition with the alternative endogenous interaction partners present in the cell (complex 2).