Split Beta-Lactamase Complementation Assay

A Search for the Molecular Better Half!

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Mapping protein–protein interactions occurring in a living cell can provide answers to a plethora of questions in biological research. Split beta-lactamase based protein fragment complementation assay provides an opportunity to analyze such interactions in both *in vitro* and *in vivo* environments. This flexible and robust assay holds potential to create a difference in our understanding of cellular processes.

Keywords
Protein fragment complementation, beta-lactamase, protein-protein interaction.

Introduction

Protein–protein interactions form the basis of cellular processes that occur inside a living cell. Recent advances in biological research have strongly instigated the need to map biochemical networks for improved understanding of living cells. Apart from prediction and determination of protein structures to ascribe them potential functions, characterization of proteins also needs to address how and when the proteins interact with other counterpart proteins to bring about the much-desired orchestration in the cellular physiology. Simultaneously, the lack of knowledge of a large number of novel genes discovered after the sequencing of numerous genomes has created a vacuum due to lack of information pertaining to biological relevance of their encoded proteins. Consequently, an important goal of modern biology now is to assign relevance to the genotypic information by linking it to the phenotypic information. This poses a pressing demand for simple yet high-throughput functional assays that can rapidly assign the functions to the proteins involved in different cellular processes by studying their interaction patterns with other proteins *in vivo*.

Protein Fragment Complementation: What's in a Name?

Protein fragment complementation assays (PCAs) have emerged as an exciting option for such applications and tend to offer a

complete solution to study protein–protein interactions in a high-throughput format. These assays comprise of a protein molecule, which is rationally split into two fragments that do not interact with each other independently but can refold and reconstitute the activity of the native protein when brought together by interacting partners fused to them covalently. The reconstituted activity thus obtained can be used to select such interacting pairs (*Figure* 1). To name a few, PCAs employing proteins like murine dihydrofolate reductase (mDHFR), TEM-1 beta-lactamase (TEM-1 Bla), luciferase, beta-galactosidase, GFP, g3p of M13 filamentous phage, etc. have been used successfully for studying protein–protein interactions in bacterial hosts.

TEM-1 Beta-Lactamase: The Molecule of Choice

The focus of this article is the use of TEM-1 neta-lactamase (TEM-1 Bla; EC: 3.5.2.6) in PCAs. TEM-1 Bla is an *E. coli*-

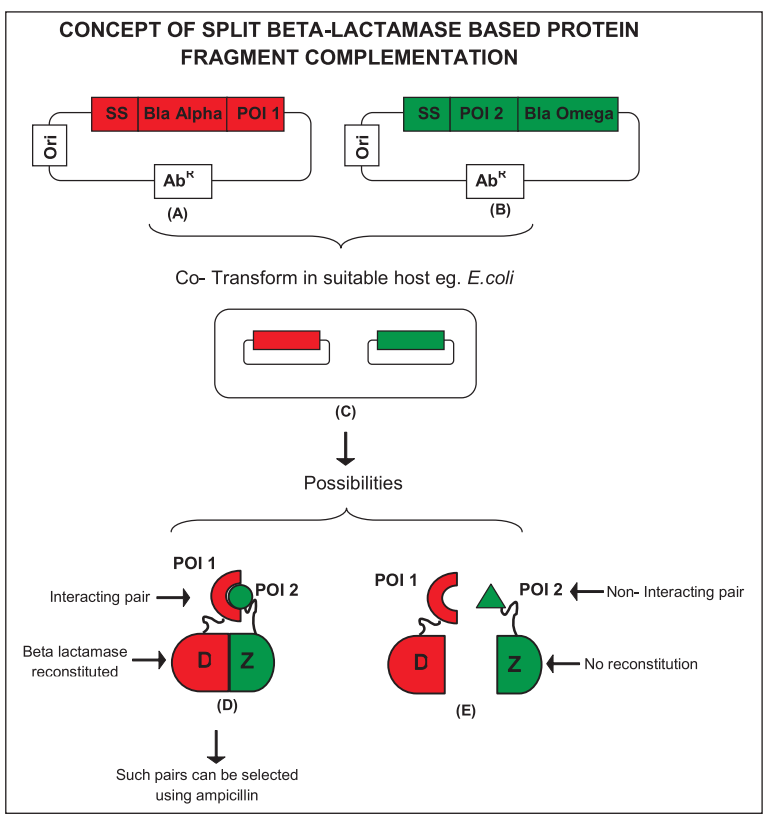

Figure 1. Concept of split beta -lactamase protein fragment complementation assay. (A) and (B) are vector systems involved in the assay. As an example, a vector system for bacterial host is described here. (C) Co-transformation of complementation vectors in appropriate bacterial host. (D) and (E) are types of interactions expected in the system. Pairs could be interacting thereby leading to reconstitution of beta-lactamase or *vice versa*.

In 1940, Abraham and Chain first described the presence of a substance in bacterial extracts that could inactivate the drug Penicillin – this was called Penicillinase (now referred as Beta-lactamase).

derived plasmid-borne resistance determinant to beta-lactam-based drugs which was discovered in 1965. The protein is named after the patient Temorina who suffered from urinary tract infection untreatable with ampicillin. Although clinically it has remained a barrier in the treatment against a variety of infectious agents by making them resistant to drugs, in biological research it has been exploited very well for use as a selection marker in DNA-cloning experiments. Apart from its use as a direct selection marker, TEM-1 Bla has been modified and used successfully in different formats for studying protein–protein interactions. TEM-1 Bla is a small (286 amino acids, ~29 KDa), monomeric and well-expressing molecule in both bacterial and mammalian cells for which a variety of sensitive substrates are available and these properties make it well suited for PCAs in both prokaryotes and eukaryotes. In addition, a variety of colorimetric and fluorogenic TEM-1 Bla substrates, compatible with prokaryotic and eukaryotic systems, are available for achieving high sensitivities while assaying the activity of this enzyme.

Other genetic systems like the yeast two-hybrid system rely on reconstitution of protein upon interaction followed by its transport in the nucleus to initiate the transcription of the reporter gene. As a result, such assays are unable to provide information about kinetics of the interactions and tend to miss out the transient interactions between proteins. TEM-1 Bla-based PCA can capture both transient and long-lived interactions due to its ability to generate signal immediately upon specific interaction. Simultaneously, such an assay also allows for enzymatic amplification of the signal thereby providing more accurate readouts during the study. Thus, this system holds potential to monitor a diverse range of biological interactions.

Experiment Design: The Hunt Begins

Given all the favorable attributes of TEM-1 Bla, the molecule has been closely studied and rationally split into two fragments namely Bla-alpha (1-197 residues) and Bla-omega (198–286 residues) [1, 2]. This splitting is governed by the underlying

principle that the fragments generated should not fold spontaneously without being brought close together by interactions between the proteins to which they are fused. Additionally, the splitting should not destroy the catalytic center of the enzyme making it permanently inactive. The suggested breakpoint after residue 197 falls in the exposed loop from Thr195 to Ala202 between two alpha helices and the fragments thus generated have been found to complement for activity only when brought closer by specific interactors attached to them covalently. Also, the breakpoint happens to be far from the catalytic site residue Ser70, thereby preserving its integrity. Other breakpoints have also been suggested but Glu197/Leu198 has been used widely.

The concept of reassembly of protein fragments to yield functionally active molecules was noticed over more than 60 years ago with ribonuclease (1958) and Beta galactosidase (1967).

It has been observed that the fragments generated by splitting the protein have less specific activity upon complementation as compared to the native wild-type enzyme. In the case of TEM-1 Bla, this has been addressed by incorporating additional three amino acid residues Asn-Gly-Arg after Glu197 in the Bla-alpha fragment. These residues have been found to improve signal-to-noise ratio and enhance the specific activity of the enzyme molecule obtained after complementation. To provide additional stability to the molecule, another mutation Met182Thr has been described [1, 2].

For studying protein–protein interactions in bacterial host, the two fragments, viz., Bla-alpha and Bla-omega are cloned in two separate plasmids under appropriate signal sequences for transport into the periplasm (*Figure* 1). When a pair of interacting protein partners is cloned after C-terminal of Bla-alpha and N-terminal of Bla-omega, and co-transformed in appropriate host cell, the protein products thus produced interact specifically in the bacterial periplasm and reconstitute enzyme activity to confer resistance to beta-lactam-based drugs like ampicillin. Consequently, such interacting pairs can be selected with ease. However, if the fused proteins do not interact, no resistance would be conferred and such co-transformants would be readily eliminated from screen (*Figure* 1). Therefore, split beta-lactamase-based PCA can be used for mapping biochemical networks in the

An experiment is a question which science poses to Nature, and a measurement is the recording of Nature's answer.

– *Max Planck*

cellular systems by rapidly screening protein of interest (fused to one Bla fragment) against a library of DNA molecules (fused to another Bla fragment) encoding other cellular proteins.

A Word of Caution: Controls are Important!

Although PCAs like split TEM-1 beta-lactamase can be very informative about the protein–protein interaction patterns, but these could be equally erroneous if performed without proper controls. To ensure that the experimental biases due to non-specific interactions are minimal, a set of controls must be performed [3]. These controls should include (a) negative and positive interacting pairs; the assay should be first validated using a set of already characterized interacting and non-interacting pairs to obtain positive and negative readouts respectively, (b) fragment swapping; the observed complementation should occur irrespective of the Bla fragment to which the test proteins are fused, and (c) competition – the PCA signal should be diminished upon over-expression of one or the other test proteins due to competition.

Success Stories

The applicability of split beta-lactamase complementation has been extensively validated for studying protein–protein interactions in mammalian cells [1, 2]. The system has also been successfully used to screen antibody libraries against the protein of interest to select for specific antibodies [4]. Alternatively, it has been utilized for mapping epitopes of one antibody by screening it against the target antigen fragment library [4]. Essentially, this assay has been shown to provide highly specific readouts while studying protein–protein interactions.

Future Prospects: The Road Ahead

PCAs like split beta-lactamase could be very promising in mapping a plethora of biochemical interactions occurring in the cellular environment. Owing to the high-throughput nature of these assays, they can prove to be rapid and easy alternatives for

studying functions of a large number of novel genes discovered after sequencing of large genomes. Such assays can find use in screening cDNA libraries against specified targets. Additionally, split beta-lactamase complementation can be exploited for use as protein biosensors for developing point-of-care diagnostics based on homogeneous assays utilizing specific antigen (present in patient sample) and antibody (fused to Bla fragments) interactions [5]. Furthermore, use of such an assay is being explored for studying complex libraries-against-libraries to allow simultaneous screening of the entire genomes in one go. Such PCAs are a very flexible and robust technology and it is envisaged that they will find many more applications in modern biology in the near future.

Suggested Reading

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