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Review

Yeast-based assays for detecting protein-protein/drug interactions and their inhibitors

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ABSTRACT

Understanding cellular processes at molecular levels in health and disease requires the knowledge of protein–protein interactions (PPIs). In line with this, identification of PPIs at genome-wide scale is highly valuable to understand how different cellular pathways are interconnected, and it eventually facilitates designing effective drugs against certain PPIs. Furthermore, investigating PPIs at a small laboratory scale for deciphering certain biochemical pathways has been demanded for years. In this regard, yeast two hybrid system (Y2HS) has proven an extremely useful tool to discover novel PPIs, while Y2HS derivatives and novel yeast-based assays are contributing significantly to identification of protein-drug/inhibitor interaction at both large- and small-scale set-ups. These methods have been evolving over time to provide more accurate, reproducible and quantitative results. Here we briefly describe different yeast-based assays for identification of various protein-protein/drug/inhibitor interactions and their specific applications, advantages, shortcomings, and improvements. The broad range of yeast-based assays facilitates application of the most suitable method(s) for each specific need.

1. Introduction

Mounting evidence indicates that protein–protein interactions (PPIs) are key to understanding biological functions from the assembly of molecular machines and enzymatic complexes to the control of signal transduction pathways and cell–cell interactions (Vidal and Fields, 2014). Moreover deciphering PPI networks is critical for understanding pathological conditions and their molecular underpinnings (Kaltenbach et al., 2007; Lim et al., 2006; Silva et al., 2015; Suter et al., 2008). Thus identification of PPIs is of great importance to both academic community and pharmaceutical industry. However most biochemical methods such as affinity purification have proved too cumbersome to be used in high-throughput experiments. This increasingly called for a rapid, simple and reliable method by which PPIs could be identified in a research laboratory-scale as well as large-scale set-ups. Yeast two hybrid system (Y2HS) rightly met this challenge (Bartel et al., 1996; Formstecher et al., 2005; Ito et al., 2001; Rual et al., 2005; Stelzl et al., 2005; Uetz et al., 2000). The introduction of Y2HS (Fields and Song, 1989) as a genetic tool revolutionized what was then termed 'proteomics', so that this technique is currently the most commonly used method for investigating PPIs (Auerbach and Stagljar, 2005; Velasco-García and Vargas-Martínez, 2012). Accordingly at the time of publication of this article almost 1,310,000 research articles can be found in google scholar with the 'yeast two hybrid' appearing in their titles. Additionally Y2HS paved the way for designing strategies to discover inhibitors of the PPIs and protein-drug interactions; a prominent subject in drug discovery industry. Since the emergence of Y2HS, several modifications of the system provided solutions to the pitfalls of the early variant, including detection of a broader spectrum of PPIs, while others offered novel applications. Here we briefly discuss Y2HS and its modifications that conferred more sensitive and specific detection of various types of PPIs. Additionally other yeast based-assays which may be used as complement or alternative approaches to Y2HS variants will be introduced. We then describe yeast-based assays by which PPI inhibitors can be identified. Finally yeast-based approaches which identify protein-drug interaction will be outlined. Overall the aim of this article is presenting a broad repertoire of yeast-based assays as simple, inexpensive and reliable genetic tools for identification of PPIs, PPI inhibitors, and protein-drug interactions. Consequently exhaustive technical details have been avoided, although relevant sources were mentioned where necessary.

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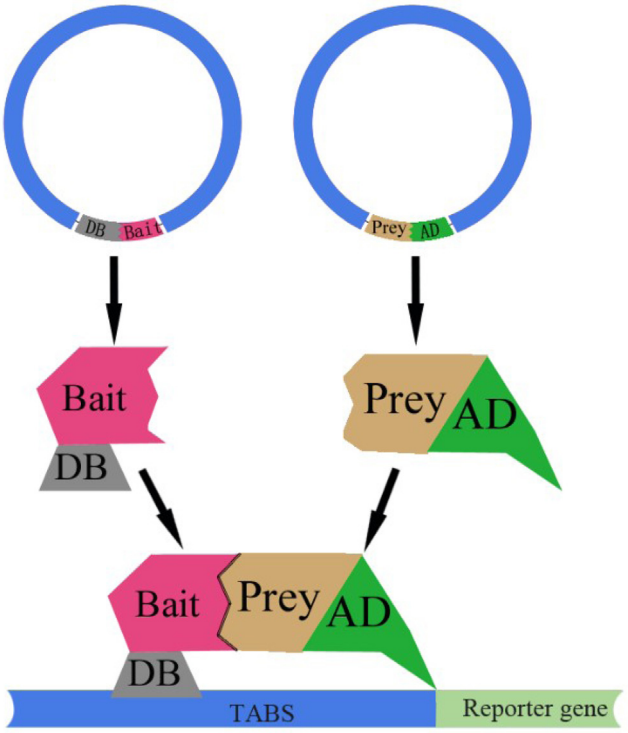

Fig. 1. The principle of 'yeast two hybrid system' (Y2HS). Transcription activators gene fragments; DNA binding domain (DB) and activating domain (AD) were independently fused to two potentially interacting protein genes (the bait and prey respectively) on two plasmids and the yeast strain transformed by the two plasmids. If the bait and prey interact, DB and AD are recruited in close proximity of each other, restoring transcription factor activity and thereby inducing the expression of the reporter gene. This usually demonstrates a particular phenotype such as growth on certain media. TABS stands for transactivating binding sequence.

2. Yeast two hybrid system (Y2HS)

The principle of Y2HS is based on the fact that two domains of transcription activators (TA); DNA binding domain (DBD) and transcription activation domain (TAD) do not need to be physically connected in order to activate transcription. If the two domains locate in close proximity, they can still function as TA. Fusion of two potentially interacting proteins to each of the TA domains therefore facilitates the proximity of TA domains required for the functionality of TA and thereby initiates transcription of a reporter gene such as *LacZ* as read-out. This was first shown for the transcription activator Gal4p. The protein which is fused to DNA-binding domain was named the "bait" and the protein which was fused to the activating domain was called the "prey" (Chien et al., 1991; Fields and Song, 1989) (Fig. 1). A yeast strain lacking the transcription activator Gal4p and its negative regulator Gal80p will be co-transformed by two plasmids each of which expresses either the bait fusion or the prey fusion. An alternative method for co-transformation of two plasmids in Y2HS is mating two strains each harboring each of the plasmids involved, which can then mate and grow in selective media for both plasmids. The advantages include the need for only single transformation, direct selection for interaction, and picking up fewer false positive colonies as a result of using two reporter genes (Bendixen et al., 1994). Large scale Y2HS can be implemented in two formats; library and matrix approaches. Each scheme has its own advantages and disadvantages but they usually complement each other (details in (Auerbach and Stagljar, 2005)). Many variants which can influence Y2HS results are as follows: (i) multiple reporter genes, (ii) episomal bait and prey plasmids with different copy numbers, (iii) various bait and prey promoters, (iv) different bait and prey vectors, (v) alteration of the exact composition of the medium, (vi) the identities of the DNA-binding domain, the activation domain, and the reporter genes, (vi) the effect of steric hindrance, (vii) carrying out Y2HS in the physiological condition versus stress conditions, (viii) genes which may compete with bait or prey for binding or somewhat affect the detection (reviewed in (Stynen et al., 2012)).

Y2HS has not only been used for global PPI identification (though with some limitations; see Y2HS limitations) but also specifically applied to determine PPIs involved in certain pathological conditions such as neurodegenerative diseases and ataxias (Kaltenbach et al., 2007; Lim et al., 2006; Suter et al., 2008). Additionally Y2HS was successfully employed to map the pathogen-host interactions for a number of life-threatening pathogens e.g. *Campylobacter jejuni*, human cytomegalovirus, acquired immunodeficiency virus, *Bacillus anthracis*, *Francisella tularensis* and *Yersinia pestis* (Silva et al., 2015). Furthermore Y2HS has been instrumental in understanding molecular pathways and functions in yeast. Deciphering proteins involved in mitotic spindle formation and its relation with other cellular pathways exemplifies this application (Suter et al., 2008; Wong et al., 2007). Y2HS is usually considered to be complementary to other affinity purification methods such as tandem affinity purification (Brückner et al., 2009; Stynen et al., 2012).

Since its emergence, minor modifications were introduced which are summarized in Table 1, other variants with major improvements have been discussed in Section 2.2.

2.1. Y2HS limitations and approaches for overcoming them

Although highly efficient as a convenient method for PPIs detection, Y2HS suffers from a number of drawbacks; a summary of these and solutions for them is presented in Table 2 (Bartel et al., 1993; Brückner et al., 2009; Fukada et al., 2005; Gietz, 2006; Koegl and Uetz, 2007; Petschnigg et al., 2011; Serebriiskii and Golemis, 2001; Stagljar and Fields, 2002; Stynen et al., 2012; Vidalain et al., 2004).

2.2. Different read-outs report PPIs

Initially transcription activation (yeast growth and colony color in certain media) were read-outs which reported the occurrence of a PPI, later other read-outs were introduced such as fluorescence or luminescence. In the following sections different modifications of Y2HS are classified according to the read-out system which were used.

2.2.1. Y2HS modifications with transcription activation as the read-out

As mentioned earlier a limitation of the conventional Y2HS is that transactivator proteins cannot be used as a bait. To overcome this problem a variety of methods have been suggested such as: "repressed transactivator system" (RTA). This method however could not detect general transcriptional factors (GTFs) which are known target of VP16, a transcriptional activator *in vitro* (Hirst et al., 2001). An alternative method for RTA is a method which makes use of the RNA polymerase III (RNA Pol III). In the traditional Y2HS, the RNA polymerase II (RNA pol II) is recruited to promote transcription. Some proteins are able to auto-activate RNA pol II regardless of any interactions, it is therefore possible to use Y2HS which are developed based on the RNA pol III transcription system instead (Marsolier et al., 1997; Petrascheck et al., 2001; Sieber et al., 2004; Stynen et al., 2012). A further alternative strategy for identification of proteins interacting with transactivator bait proteins is yeast one hybrid system (Y1HS); a method initially derived from Y2HS to identify proteins interacting with a particular DNA sequence (reviewed in (Liao and Fang, 2000; Reece-Hoyes and Walhout, 2012)). This method was then slightly modified to accom-