Encyclopedic Reference of Genomics and Proteomics in Molecular Medicine

The Two-Hybrid System

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Synonyms: Interaction trap

Definition

Experimental procedure to detect protein-protein interactions that uses two fusion proteins ("hybrids") which reconstitute an active transcription factor (or some other active protein) when the two fusion proteins interact (see Fig. 1).

It has been estimated that more than 50% of all protein interactions described in the literature have been detected using the yeast-two hybrid system

Characteristics

The **yeast two-hybrid system** was first described 1989 by S. Fields and O-K. Song (1). The basic concept emerged form previous experiments on transcription factors which usually contain separable DNA-binding domains (DBD) and transcriptional activation domains (AD) (Fig.1). This property can be exploited to detect protein-protein interactions.

Basic Principle of the classical Yeast-Two Hybrid System (Fig. 1)

In order to test if two proteins X and Y interact, they are expressed as fusion proteins with a transcriptional activating domain (AD, prey), and a DNA-binding domain (DBD, bait), respectively. The term "two-hybrid" derives from these two chimeric proteins.

The bait and prey fusions are co-expressed in yeast where a physical interaction between X and Y leads to the reconstitution of a functional transcription factor that binds to the promoter of a reporter gene (Fig.1). Consequently, transcription of the reporter gene is switched on. A protein-protein interaction is therefore translated into reporter gene activity which can be detected or measured. The interaction must take place in the nucleus for the subsequent activation of the reporter gene. For this purpose non-nuclear proteins are targeted to the nucleus.

The two-hybrid system can be used for the detection of essentially any protein-protein interaction, independent of the function of the corresponding proteins (but see limitations below).

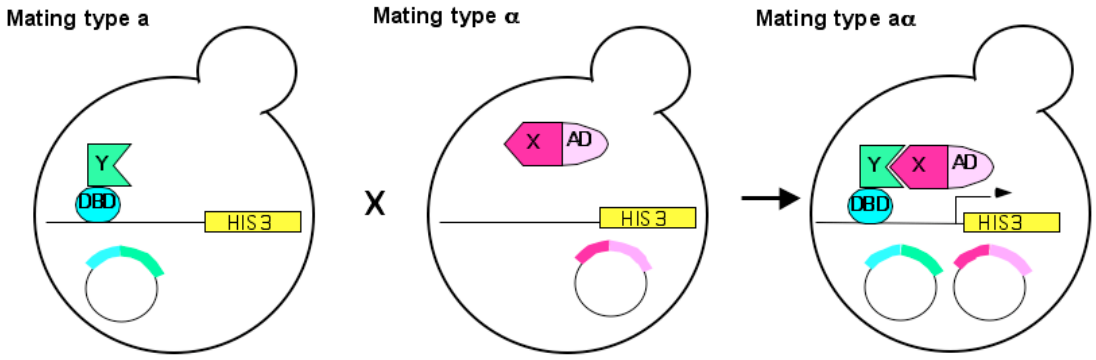

Figure 1: Classical Yeast Two-Hybrid System. A protein of interest Y is expressed in yeast as a fusion to a DNA-binding domain (DBD, "bait"; circles denote expression plasmids). Another protein of interest X is fused to a transcriptional activation domain (AD, "prey"). The two yeast strains are mated to combine the two fusion proteins in one cell. If proteins Y and X interact in the resulting diploids cells, they reconstitute a transcription factor which activates a reporter gene (here: HIS3) and therefore allows the cell to growth on selective media (here: media lacking histidine).

Variations of the two-hybrid system

Based on the two-hybrid paradigm numerous variations have been developed to overcome the limitations of the classical yeast two-hybrid system. An more comprehensive overview of variations is shown in Table 1. Only some important variations are described as follows:

Reverse two-hybrid and split hybrid system: The "reverse" two-hybrid system has been invented to select for disrupted two-hybrid interactions e.g. by mutations, drugs or competing proteins (Fig.2). In this system the interaction of X and Y proteins induces the transcription of a reporter gene that confers toxicity to the yeast.

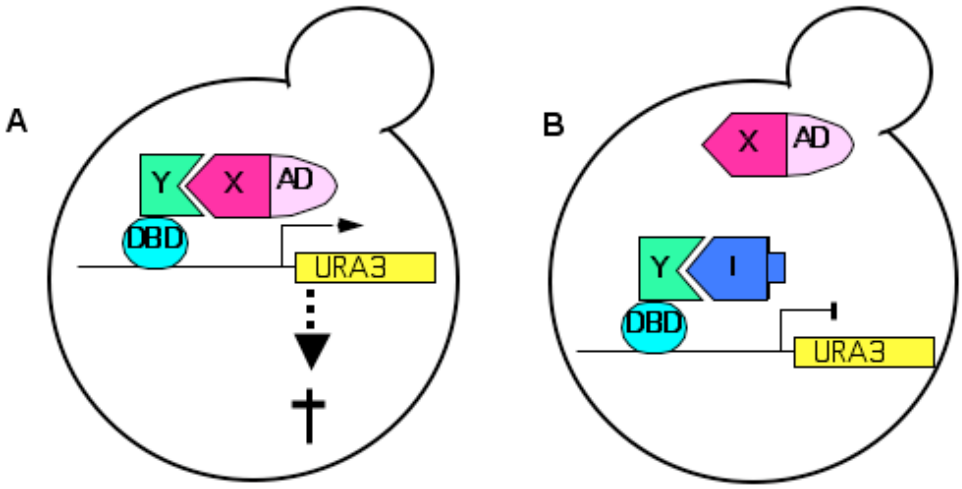

Figure 2. Reverse Two-Hybrid System. The interaction of bait and prey in the yeast cell is lethal (A), selecting for yeasts were the interaction is disrupted (B). Selection can be induced by the addition of FOA (5-fluoro-orotic acid) which is converted to the toxic compound 5-fluorouracil by the URA3 gene product.

***Three-hybrid System**:* In this yeast two-hybrid variation a third protein (Z) is expressed along with the DBD and AD fusions (Fig.3). Expression of the reporter gene is used to select for interactions that occur only in the presence of this protein.
SenGupta *et. al.* (1996) developed a three-hybrid system to detect and analyze RNA-Protein interactions in which the binding of a bifunctional RNA molecule links the DBD and AD hybrid-proteins and activates transcription of the reporter gene. This system is known as ***RNA three-hybrid system*.**

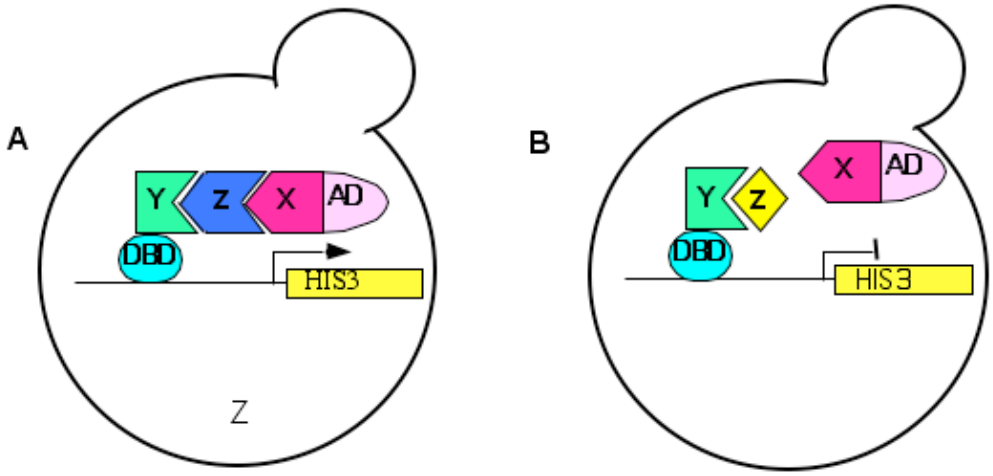

Figure 3. Three-Hybrid System. A third protein (Z) is expressed along with the DBD and AD fusions. Expression of the reporter gene is used to select for interactions that occur only in the presence of this protein (A). This third protein alternatively can prevent the formation of a two-hybrid complex (B). Alternatively, Z may be a hybrid RNA molecule with part of the sequence binding to Y and the other to X ("RNA three-hybrid").

***SOS Recruitment system (SRS, Fig. 4)**:* This membrane-associated two-hybrid system makes use of the Ras pathway in yeast (Aronheim *et. al.* 1997). When localized at the plasma membrane, the yeast Ras guanyl nucleotide exchange factor (RGEF) cdc25 stimulates GDP/GTP exchange on Ras and promotes downstream signalling events that ultimately lead to the cell growth. A mutant yeast strain harbouring the temperature sensitive cdc25-2 allele is still able to grow at 25°C but fails to grow at 36°C. However, the human RGEF (hSOS) when targeted to the plasma membrane efficiently complements the mutation, leading to cell growth at 36°C. In the SRS the translocation of hSOS is dependent on a protein-protein interaction: the bait X is fused to C-terminally truncated hSOS, which is active but unable to target to the plasma membrane. The bait is co-expressed with a prey Y, which can either be an integral membrane protein or a soluble protein that is anchored to the membrane by means of a myristoylation signal.

Split-ubiquitin System: Johnsson and Varshavsky (1998) have developed a cytoplasmic two-hybrid assay based on ubiquitin. Ubiquitin is a small protein of 76 amino acids which acts as a "tag" for protein degradation. Proteins fused to ubiquitin are rapidly cleaved in vivo by ubiquitin-specific proteases (UBPs).
If the carboxy terminal of ubiquitin (Cub) is fused to a reporter protein and co-expressed with the amino-terminal fragment (Nub), the two halves will reconstitute the native ubiquitin, resulting in the cleavage of the reporter protein. For its adaptation to detect protein-protein

interactions a mutant Nub, unable to interact with Cub on its own, was fused to one protein and a Cub-reporter hybrid was fused to its prospective interaction partner (Fig.5). Interaction between the two proteins allows ubiquitin to be reconstituted, leading to cleavage and release of the reporter gene.

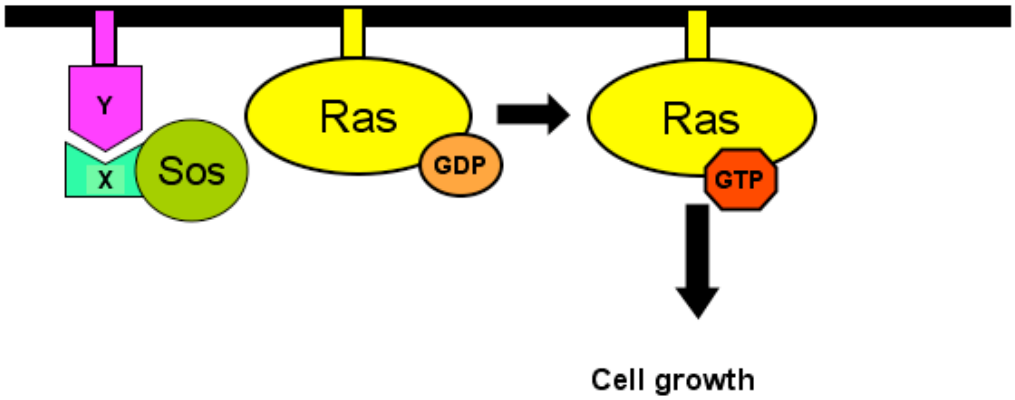

Figure 4. Sos Recruitment System. Protein X is fused to a human Ras guanyl exchange factor, Sos. Putative interaction membrane protein Y (or localized to the membrane by a myristoylation tag) is co-expressed. Interaction between X and Y recruits Sos to the membrane, where it stimulates guanyl nucleotide exchange on Ras. GTP-bound Ras stimulates cell growth.

As the formation of split-ubiquitin and subsequent cleavage by UBPs do not depend on any special localization of the proteins this system is suitable to the investigation of membrane proteins. Up to date two membrane based split-ubiquitin systems have been described: ***Ura3 based split-ubiquitin system*** and the ***transactivator based split-ubiquitin system***.
A further advantage of this system is that the signal for an interaction can be changed by changing the nature of the reporter protein (eg. transcription factor or enzyme activated by ubiquitin cleavage). Furthermore, the small size of the ubiquitin fragments in the hybrid proteins may also be advantageous because it minimizes the possibility of steric hindrance.
Up to date it is the most widely used of the alternative yeast-based two-hybrid systems.

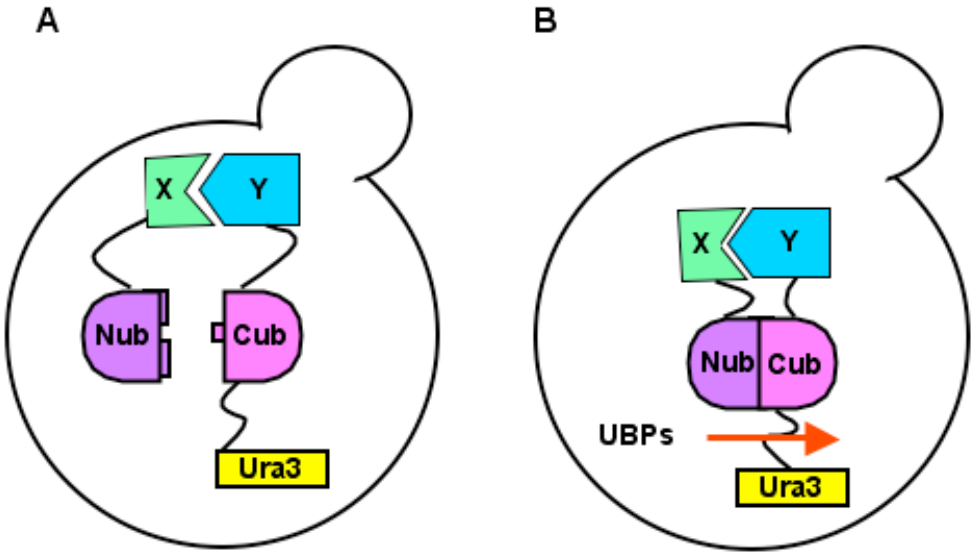

Figure 5. Spit Ubiquitin System. Proteins fused to ubiquitin are rapidly cleaved *in vivo* by ubiquitin specific proteases (UBPs). A mutant Nub is fused to protein X and the Cub-reporter gene hybrid is fused to protein Y (A). Interaction between both proteins reconstitutes ubiquitin and leads to cleavage and release of the reporter gene (URA3) (B).

Advantages and limitations of the yeast two-hybrid system

The yeast two-hybrid system became one of the most popular technologies for the detection of protein-protein interactions because it is fairly simple, rapid and inexpensive (avoids the costly protein purification and antibody development needed in the traditional biochemical methods). No previous knowledge about the interacting proteins is necessary for a screen to be performed. Finally, the system can be scaled up to high-throughput usage.

Limitations. Some classes of proteins are not suitable to analysis by the yeast two-hybrid system. For example, transcriptional activators may activate transcription without any interaction. Another class of troublesome proteins are those containing hydrophobic transmembrane domains which may prevent the proteins from reaching the nucleus. To overcome this limitation one of the alternative membrane-associated two-hybrid systems may be used.
Other proteins may require modification by cytoplasmic or membrane associated enzymes in order to interact with binding partners. Alternative methods could also help in this case.

False positives and false negatives

The two-hybrid system has a tendency to produce false positives, that is reporter gene activity where no protein-protein interaction is involved. Frequently, such false positives are caused by bait proteins that act as transcriptional activators. Other false positives may be caused by proteins that lead to non-specific interactions for largely unknown reasons. Some bait or prey proteins may affect general colony viability and hence allow a cell to grow under selective conditions and activate reporter gene activation. Mutations or other random events of unknown nature may be invoked as potential explanations as well. Overall, extremely few cases of false positives can be explained mechanistically.

A number of procedures have been developed in order to identify or avoid false positives altogether, including the utilization of multiple reporters, independent methods of specificity testing or simply repeating assays to make sure a result is reproducible.

False negatives involves physiological protein-protein interactions that are not detected by two-hybrid assays. They may arise by sterical hindrance of the two fusion proteins so that transcriptional activation is prevented. Other explanations include instability of proteins or failure of nuclear localization.

Comparison to other methods

	Advantages	**Disadvantages**
Two-Hybrid	simple and inexpensive Coverage of low abundant proteins	significant risk of false positives
Mass Spectrometry	Identification of protein complexes	Expensive and time consuming purification required
In vitro binding	defined conditions	potentially non-physiological conditions
Protein Chips	defined conditions Potentially highly parallel	requires purification of proteins

Clinical relevance and applications

Two-hybrid systems are useful for the investigation of protein-protein interactions, protein-nucleic acid and protein-small molecule interactions. However, the technology is mainly used in basic research in order to understand the function of proteins. Many protein-protein interactions can cause disease when they are disrupted (e.g. hemoglobin is a tetramer that binds oxygen only normally when bound to other hemoglobin subunits). Protein interactions are now being identified by biotech and pharma companies as potential drug targets, especially in combination with screens for small-molecule ligands that can disrupt or modulate these protein interactions.

References

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Online resources

http://www.fccc.edu/research/labs/golemis/interaction-trapinwork.html
http://depts.washington.edu/sfields/projects/YPLM/
http://www.two-hybrid.org