Yeast Two-Hybrid System:

When two proteins interact with each other, their corresponding genes are known as interacting genes. The yeast two-hybrid system uses a reporter gene to detect the physical interaction of a pair of proteins inside a yeast nucleus.

The two-hybrid method is based on the observation that most of the transcriptional proteins (i.e. the proteins involved in promoting transcription of a gene) contain two distinct domains—DNA binding domain and transcriptional activation domain. When these two domains are physically separated, the protein loses its activity. However, the same protein can be reactivated when the domains are brought together. These proteins can bind to DNA and activate transcription.

The target protein is fused to a DNA-binding domain to form a bait. When this target protein binds to another specifically designed protein namely the prey in the nucleus, they interact, which in turn switches on the expression of the reporter gene (Fig. 5.15). The reporter genes can be detected by growing the yeast on a selective medium.

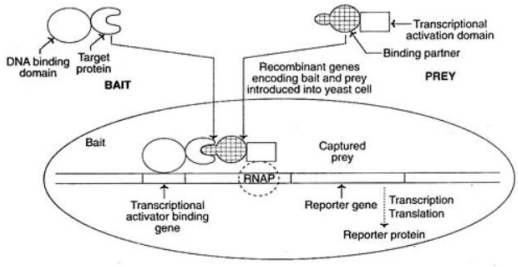

Fig. 5.15 : Elucidation of protein-protein interaction by yeast two-hybrid system (RNAP-RNA polymerase)

It is possible to generate the bait and prey fusion proteins by standard recombinant DNA techniques. A single baid protein is frequently used to fish out interacting partners among the collection of prey proteins. A large number of prey proteins can be produced by ligating DNA encoding the activation domain of a transcriptional activator to a misture of DNA-fragments from a cDNA library.

The two-hybrid system is an artificially constructed genetic system intended to facilitate the detection and assessment of protein–protein interactions. In the two-hybrid system a host organism, typically yeast or bacteria, is engineered so as to contain three components. These are a first protein fused to a DNA-binding domain of known specificity (hybrid 1); a second protein fused to a transcriptional–activation domain (hybrid 2), that can interact with the first protein, constituting a functional, albeit composite, transcription factor; and one or more reporter genes transcribed based on the binding of the

composite transcription factor. Many permutations of the two-hybrid paradigm have been developed, and two-hybrid systems have become a mainstay of proteomic investigations.

Pioneered by Stanley Fields and Ok-Kyu Song in 1989, the technique was originally designed to detect protein–protein interactions using the Gal4 transcriptional activator of the yeast *Saccharomyces cerevisiae*. The Gal4 protein activated transcription of a gene involved in galactose utilization, which formed the basis of selection. Plasmids are engineered to produce a protein product in which the DNA-binding domain (BD) fragment is fused onto a protein while another plasmid is engineered to produce a protein product in which the activation domain (AD) fragment is fused onto another protein. The protein fused to the BD may be referred to as the bait protein, and is typically a known protein the investigator is using to identify new binding partners. The protein fused to the AD may be referred to as the prey protein and can be either a single known protein or a library of known or unknown proteins. In this context, a library may consist of a collection of protein-encoding sequences that represent all the proteins expressed in a particular organism or tissue, or may be generated by synthesising random DNA sequences. Regardless of the source, they are subsequently incorporated into the protein-encoding sequence of a plasmid, which is then transfected into the cells chosen for the screening method. This technique, when using a library, assumes that each cell is transfected with no more than a single plasmid and that, therefore, each cell ultimately expresses no more than a single member from the protein library. If the bait and prey proteins interact (i.e., bind), then the AD and BD of the transcription factor are indirectly connected, bringing the AD in proximity to the transcription start site and transcription of reporter gene(s) can occur. If the two proteins do not interact, there is no transcription of the reporter gene. In this way, a successful interaction between the fused protein is linked to a change in the cell phenotype. There are a number of domains from which to choose the BD, bait and prey and AD, if these are to remain constant. In protein–protein interaction investigations, the BD may be chosen from any of many strong DNA-binding domains such as Zif268. A frequent choice of bait and prey domains are residues 263–352 of yeast Gal11P with a N342V mutation and residues 58–97 of yeast Gal4, respectively. These domains can be used in both yeast- and bacterial-based selection techniques and are known to bind together strongly.