Two-hybrid and Related Systems

Secondary article

Stanley Fields, *University of Washington, Seattle, Washington, USA*

The two-hybrid system is a yeast-based genetic method to detect and analyse protein–protein interactions. Related systems have been developed to detect DNA–protein, RNA–protein and small molecule–protein interactions.

Article Contents

Introduction

Cellular processes such as replication, transcription, ribonucleic acid (RNA) processing, translation and secretion are mediated by complexes containing proteins or proteins and nucleic acids. The analysis of these complexes requires identification of the constituent components and the interactions among these components. The two-hybrid system (Fields and Song, 1989) is a genetic method that uses yeast to identify and characterize protein–protein interactions. Several features of this method have resulted in its widespread use in the last few years. First, the interaction of proteins from virtually any organism and from diverse cellular structures and pathways can be successfully detected. Second, the method results in the immediate availability of cloned genes for newly identified proteins. Third, the overall strategy that underlies this approach has proven to be generally applicable, allowing the development of related methods to analyse interactions of proteins with deoxyribonucleic acid (DNA), RNA or chemical ligands. Fourth, the site-specific transcriptional activators that are the basis of these approaches can be substituted by an increasing number of alternative proteins. These other proteins extend the methodology to the examination of interactions in cellular compartments other than the nucleus and the assay of interactions in a temporal fashion. Fifth, while the host for this system is usually the yeast *Saccharomyces cerevisiae*, any eukaryote may be feasible and analogous methods have been developed for use in prokaryotes.

Description of the Two-hybrid Assay

Nature of the hybrid proteins

The basis of the two-hybrid assay is the modular nature of transcriptional activators. Separate portions of these proteins are responsible for contacting DNA (the DNA-binding domain) and for contacting proteins of the basal transcription machinery (the transcription activation domain) (**Figure 1a**). By binding to *cis*-acting DNA elements, the DNA-binding domain targets an activator to the genes that it regulates. The activation domain is responsible for setting the level of expression of these regulated genes. Each of these two domains can carry out its activity independently of the other. In the two-hybrid system, a hybrid protein containing a DNA-binding domain fused to some protein 'X' will bind to a gene containing the cognate DNA element, but it will not effect transcription because it lacks an activation domain to recruit the transcriptional machinery (**Figure 1b**). A hybrid protein containing an activation domain fused to some protein 'Y' will fail to bind to a regulated gene because it lacks a DNA-binding domain, and it, too, will be inactive (**Figure 1c**). However, if both hybrid proteins are expressed in the same cell, and if the X and Y proteins noncovalently interact with each other, the activity of the transcription activator is reconstructed (**Figure 1d**), resulting in expression of a reporter gene containing sites for the DNA-binding domain.

Two DNA-binding domains are commonly used in this assay, one from the *S. cerevisiae* Gal4 protein and one from the *Escherichia coli* LexA protein. Transcriptional activation domains that are used include that from Gal4, the VP16 protein from herpes simplex virus, and the B42 activation domain from *E. coli*. The reporter gene whose expression is activated by the two-hybrid interaction is generally one whose expression is necessary for growth on appropriate plates, such as the yeast *HIS3* or *LEU2* gene required for growth in the absence of histidine or leucine, respectively. Additionally, the *E. coli lacZ* gene, encoding β-galactosidase, is also used as reporter, because this enzyme can be detected easily if its chromogenic substrate is present in the media.

Screening for proteins that interact

The principal use of the two-hybrid system is to screen for proteins that bind to some protein of interest, often called the 'bait' protein. In this application, a DNA-binding domain–X hybrid protein (often termed the 'bait') is coexpressed along with a library of all possible Y proteins fused to an activation domain. The library is generally

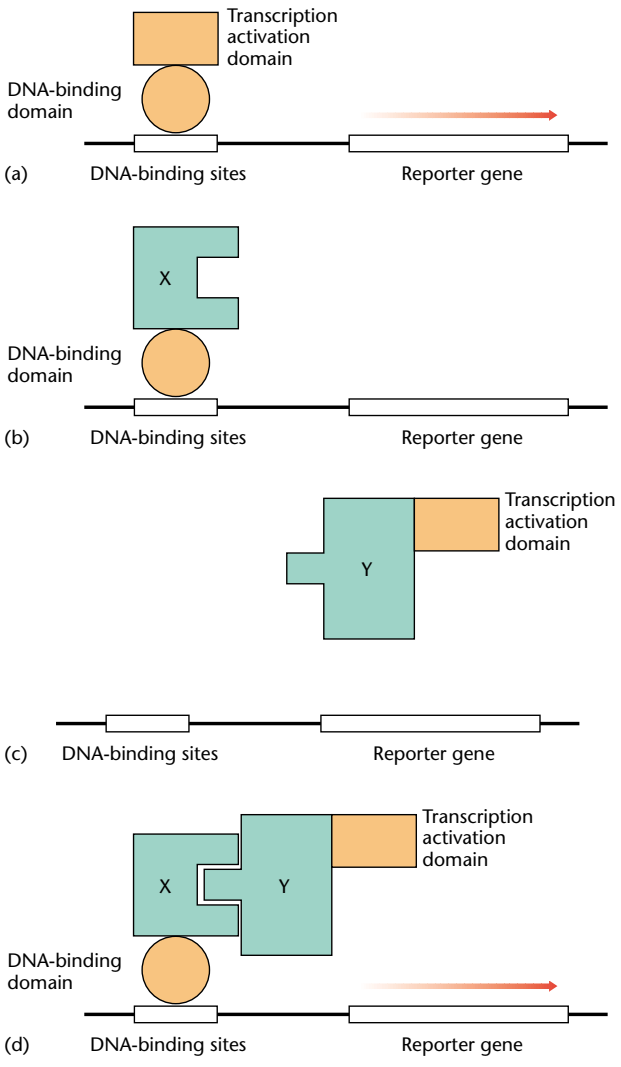

Figure 1 The two-hybrid system. (a) A transcriptional activator may contain a DNA-binding domain (filled circle) and a transcription activation domain (filled rectangle). (b) A hybrid protein composed of a DNA-binding domain and a protein 'X' does not activate transcription if X does not have an activation domain. (c) A hybrid protein composed of an activation domain and a protein 'Y' does not activate transcription because it does not bind to the DNA-binding sites. (d) Interaction between X and Y reconstitutes the function of the activator and leads to expression of the reporter gene.

constructed from complementary DNA (cDNA) corresponding to the messenger RNA (mRNA) population of cells likely to express proteins interacting with the bait. Yeast transformants are plated under conditions that allow growth only when there is expression of the reporter gene coding for an essential metabolic enzyme. Positive colonies from this selection are secondarily tested for β-galactosidase activity. Typically, over a million transformants are screened to detect the few colonies that are positive for reporter gene expression. The commercial availability of libraries derived from many different organisms and tissue sources has made this application particularly accessible to the biological community.

When interactions are detected, it is convenient to use the two-hybrid assay to delineate the domains of the X and Y protein that interact, by constructing truncated versions of these proteins and testing them for reporter gene activity. Often, this type of information can be obtained directly from the initial search results if multiple clones are identified encoding the same protein; the domain that is included in all of the clones defines a minimal interacting region. In addition, it is possible to use the two-hybrid approach to identify specific amino acid residues necessary for an interaction by introducing specific mutations into the gene encoding one of the interacting partners and screening for those that eliminate reporter gene activity.

Advantageous features of the assay

The following features have led to the widespread popularity of the two-hybrid assay.

1. The X and Y proteins can be derived from any organism, including those not typically amenable to genetic approaches. Indeed, interesting interactions have even been found in some cases when X and Y are from different organisms.
2. The method can be used with X and Y proteins that normally localize to any cellular compartment; successful two-hybrid signals have been detected with nuclear, cytoplasmic, mitochondrial, membrane-associated and extracellular protein pairs.
3. No inherent size limitations exist, and no precise spatial configuration of the X and Y proteins is required. Thus, a protein can be reduced to successively smaller domains that retain the capacity to interact with its partner.
4. The assay is highly sensitive, detecting interactions with apparent affinities of up to $\sim 10\,\mu mol\,L^{-1}$. This sensitivity is likely a consequence of multiple factors: the overproduction of the hybrid proteins; the use of reporter genes whose expression is driven by multiple high-affinity binding sites; the stabilization of interactions by other components of the transcription machinery; and the use of stable reporter proteins for which a low abundance is sufficient for phenotypic selection. Thus, transient protein–protein interactions can be converted via a transcriptional assay to a simple growth selection.

Limitations

Several features of the two-hybrid approach impose limitations on the nature of the interactions that can be analysed.

1. Membrane or secreted proteins that are incapable of folding intracellularly or entering into the nucleus are not amenable to a transcription-based assay.
2. Interactions dependent on a posttranslational event that does not occur in yeast, such as a specific phosphorylation, will not be detected (but see modifying and bridging proteins, below).
3. Protein fusions that are either toxic to or unstable in yeast cells are unsuitable.
4. Almost all two-hybrid systems have the transcription factor domain fused to the N-terminus of the hybrid, which may occlude an interaction dependent on a free N-terminal domain of the X or Y protein.
5. Other yeast proteins may bind to the X or Y protein and effectively titrate it, making it unavailable in the assay.
6. It is commonly observed that small domains sufficient for interaction yield significantly greater transcription signals than the larger intact proteins containing these domains. While in certain cases this difference may reflect enhanced accessibility of the interacting domain, as may occur normally upon a conformational change, in many cases it may simply be due to more efficient translation, folding, nuclear import, or activation domain accessibility with these isolated domains.

Permutations of the Original Assay

The two-hybrid assay has been frequently modified in order to expand its potential uses in the detection of protein–protein interactions.

Other than yeast-based systems

While originally developed for use in yeast, the two-hybrid assay should function in any eukaryotic organism, as the principles of transcriptional activation appear to be widely conserved. In particular, mammalian cells have been used to delineate the domains of two proteins necessary for an interaction and for confirming interactions detected initially in a yeast screen. However, due to difficulties inherent in selection methods when applied to mammalian cells, these approaches have not typically been used in primary screens to identify partner proteins. Conversely, it should be noted that interactions among numerous mammalian proteins have been successfully detected in yeast-based versions of the assay.

Bacterial versions of the assay have been developed that exploit the requirement for dimerization or cooperative binding of prokaryotic transcriptional regulators, but these systems have also not yet been widely used.

Reverse assays

In reverse two-hybrid approaches, the reporter gene encodes a protein toxic to yeast under some defined condition, preventing growth of yeast cells expressing a pair of interacting proteins. Consequently, treatments that disrupt the two-hybrid interaction can be directly selected. For example, expression of the *URA3* gene is toxic to yeast in the presence of the compound 5-fluoroorotic acid (5-FOA). With *URA3* as the reporter gene, the reverse two-hybrid system can be used to identify mutations in either the X or Y protein that abolish or severely diminish the interaction (Vidal *et al*., 1996). Similarly, the expression of proteins, peptides or drugs that disrupt the interaction can be selected for by growth of the yeast on 5-FOA plates. Another reverse assay, termed the split-hybrid method, uses the *E. coli* TetR repressor as the reporter protein expressed due to the two-hybrid interaction (Shih *et al*., 1996). This repressor in turn binds to and inhibits the expression of a *HIS3* gene that contains the TetR DNA-binding sites. Mutations that disrupt the interaction decrease the level of *TetR* repressor expression and consequently lead to increased transcription of *HIS3*, which can be selected on plates lacking histidine.

Differential screening

A protein often binds to multiple other proteins, and these interactions may be mediated by separate domains of the polypeptide. Thus, it should be possible to identify mutations that affect binding to one partner protein but not to another, such as those in a signalling protein like Ras that affect its interaction with only one of multiple effector proteins.

An elegant method of identifying mutations that act differentially makes use of two different DNA-binding domain hybrids, one based on Gal4 and one on *LexA*, that bind to two different reporter genes (*URA3* and *lacZ*, respectively) in the same strain (Inouye *et al*., 1997). In the presence of an activation domain hybrid with a protein Y that binds to both X_1 and X_2, the transformants are Ura^+ and $LacZ^+$. However, mutants of the protein Y unable to bind to protein X_1 but still capable of binding to protein X_2 are scored as Ura^- and $LacZ^+$.

Modifying and bridging proteins

Interactions dependent upon either a posttranslational modification that does not occur in yeast or the presence of another nonyeast protein cannot be analysed with the standard two-hybrid assay. To study interactions that require tyrosine phosphorylation, Osborne *et al.* (1995) coexpressed a protein tyrosine kinase in yeast. This enzyme phosphorylated the γ subunit of the immunoglobulin E (IgE) receptor, which was fused to a DNA-binding domain, leading to the identification of an interacting

protein containing an SH2 (Src homology 2) domain. It should be noted, however, that in cases where a protein possessing intrinsic protein tyrosine kinase activity is fused to the DNA-binding domain, phosphorylation-dependent interactions may occur in yeast. For example, certain combinations of mammalian cell surface receptors and adaptor proteins have yielded a two-hybrid signal which was dependent on a kinase active receptor.

In several cases, coexpression of a third protein that either mediates an interaction or induces a conformational change in one of the proteins allows an interaction to be identified. For example, the binding of the cytoplasmic domain of the epidermal growth factor receptor to the Sos protein could be observed when the Grb2 protein was coexpressed. Coexpression in yeast of either growth hormone or vascular endothelial growth factor, which are secreted in their normal mammalian cell environment, led to a two-hybrid signal when the extracellular domain of the cognate receptor was present as both a DNA-binding domain and an activation hybrid.

Finally, interactions mediated by small ligands are amenable to two-hybrid analysis. Chiu *et al*. (1994) demonstrated that proteins could be identified that interact with the complex of the protein FKBP12 and the immunosuppressant rapamycin.

Peptides

Although two-hybrid searches typically involve libraries encoding proteins, Yang *et al*. (1995) made use of an activation domain fusion library that expressed random 16-residue long peptides. Screening this library for peptides able to bind to the retinoblastoma protein (Rb) identified seven interacting peptides, all of which contain the Leu-X-Cys-X-Glu motif found in Rb-binding proteins. Furthermore, mutagenesis of two of these peptides led to changes in transcriptional activity in the two-hybrid assay, allowing the delineation of residues particularly important for binding to Rb.

In a related approach, Colas *et al*. (1996) used a library of conformationally constrained 20-residue peptides displayed in the active site loop of *E. coli* thioredoxin, and screened for those that bound to human cyclin-dependent kinase 2 (Cdk2). Importantly, peptides so identified recognized different epitopes on the Cdk2 surface and were able to inhibit Cdk2 kinase activity, suggesting possible applications for drug discovery.

One-hybrid Assay

The one-hybrid assay is a method to clone genes encoding proteins that bind to a specific DNA element (Wang and Reed, 1993). Such elements may be sequences that regulate transcriptional activation or repression, DNA replication, localization of chromosomes to sites in the nucleus, or other DNA functions. Conceptually, the one-hybrid approach is similar to that of two-hybrid experiments, and it takes advantage of many of the same procedures and reagents.

In a one-hybrid experiment, the DNA element whose binding proteins are sought controls the transcription of one or more genes whose expression can be selected. The element is generally narrowed to its minimal functional unit by biological assays in the relevant organism, and then multimerized copies of it are inserted in the 5′-upstream region of reporter genes such as *HIS3* or *lacZ* that lack their own activator binding sites. These reporter genes may be either integrated into yeast chromosomes or maintained on plasmids. The resulting yeast strain is transformed with a library of plasmids encoding proteins fused to an activation domain. Expression of a DNA-binding domain that interacts with the multimerized DNA element gives rise to transformants that are histidine prototrophs or positive for β-galactosidase activity by virtue of the fusion of this domain to the activation domain.

This approach requires that yeast does not possess a transcription factor that recognizes the DNA element to activate transcription, and thus reporter strains must be tested prior to introduction of the library. Furthermore, false positives can arise due to interactions with nonspecific DNA-binding proteins. These can be eliminated by constructing a second reporter strain that is identical to the first, except for a small mutation in the DNA element that abolishes its interaction with its cognate binding factor, as assessed in a biological assay. Only activation domain hybrids that yield reporter gene activity in the first strain but not the second are retained for further study.

The one-hybrid assay, like its predecessor, is sensitive, rapid and results in the immediate availability of the cloned gene for, in this case, the DNA-binding protein. It can be used with any of the wide range of activation domain libraries currently available. Sequences of newly identified DNA-binding domains can be compared to other such domains that have been previously characterized. Full-length cDNAs for these proteins can be obtained and used for transfection into cells with reporter plasmids to assay for effects of transcriptional activity, or translated into protein for use in gel mobility shift assays and other *in vitro* binding assays.

RNA Three-hybrid Assay

The RNA three-hybrid system is a method of detecting and analysing RNA–protein interactions, such as those important in RNA splicing, translation, early development and the life cycle of RNA viruses. In this assay, a hybrid RNA molecule functions as a bridge between two hybrid proteins. One hybrid protein contains an RNA-binding

domain fused to a DNA-binding domain, while the other hybrid protein contains a second RNA-binding domain fused to a transcription activation domain. The hybrid RNA carries the cognate recognition sites for the two RNA-binding domains, and thus links the three components together to stimulate transcription of the reporter gene.

In the most widely used version of this assay (SenGupta *et al*., 1996) (**Figure 2**), the first hybrid protein consists of the LexA protein fused to the coat protein of the *E. coli* bacteriophage MS2, a protein that binds to a stem–loop structure in the phage RNA. The second hybrid protein consists of the Gal4 activation domain linked to an RNA-binding protein, 'Y'. The hybrid RNA is expressed under the control of a yeast polymerase III promoter and consists of two sites for binding of the MS2 coat protein linked to an RNA sequence 'X'. The RNA X and RNA-binding domain Y vary in different experiments. The yeast *HIS3* gene and the *E. coli lacZ* gene, under the regulation of multiple LexA-binding sites, serve as reporters.

The primary applications of this assay include the isolation of proteins that bind to a specific RNA element, and the isolation of RNA ligands that interact with a specific RNA-binding protein. For the first application, the RNA X element must be first identified by experiments in the relevant organism, and then DNA encoding this sequence is cloned into the RNA expression vector adjacent to the MS2 coat protein binding sites. An activation domain library is transformed into yeast containing the plasmid encoding the RNA hybrid, and selection is carried out for histidine prototrophy. Libraries suitable for this purpose are identical to those for two-hybrid approaches. Proteins identified in a three-hybrid selection are characterized to determine if their binding is specific to the RNA X element by assaying for three-hybrid reporter gene activity when they are paired with hybrid RNAs containing small mutations in X.

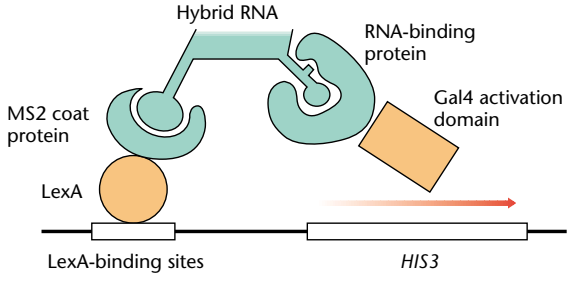

Figure 2 The three-hybrid system to detect RNA–protein interactions. A hybrid protein of the LexA protein fused to the bacteriophage MS2 coat protein localizes to the regulatory region of the reporter gene by binding to the LexA-binding sites. A hybrid protein of the Gal4 transcription activation domain fused to a second RNA-binding protein will activate transcription of the reporter gene when in close proximity to the gene's regulatory region. A hybrid RNA containing cognate binding sites for the coat protein and the other RNA-binding protein bridges the two hybrid proteins and results in expression of the reporter gene.

In a second application of this method, a library of hybrid RNAs is generated in which total cDNA, genomic DNA or synthetic oligonucleotides are cloned into the RNA vector adjacent to the sequence encoding the MS2 coat protein binding sites. This library is introduced into a three-hybrid reporter strain expressing an activation domain hybrid with an RNA-binding domain of interest, and selection and screening carried out as described above.

This three-hybrid approach may be useful for dissecting known RNA–protein interactions, defining minimal domains and pinpointing amino acid residues or nucleotides necessary for binding. It may also permit the rapid screening of inhibitors for an RNA–protein interaction, such as are implicated in viral replication or assembly.

Small Molecule Three-hybrid Assay

Molecules other than RNA can be used to bridge two hybrid proteins. Licitra and Liu (1996) demonstrated that a dimer of organic ligands can also be used for this purpose. In their three-hybrid system, the protein fused to the DNA-binding domain is the protein domain that binds to ligand A, and the protein fused to the transcription activation domain is the protein domain that binds to ligand B (**Figure 3**). The hybrid ligand A–B is a chemically synthesized covalently linked heterodimer of the ligands A and B.

In one application of this assay (Licitra and Liu, 1996), the heterodimeric ligand was composed of the steroid hormone agonist dexamethasone linked to the immunosuppressant FK506. One hybrid protein consisted of LexA fused to the hormone-binding domain of the glucocorticoid receptor (which binds dexamethasone); the other hybrid protein consisted of the transcriptional activator B42 fused to FKBP12 (which binds FK506). The method was also shown to be capable of identifying a cDNA clone for FKBP12 in a screen for FK506-binding proteins carried out in the presence of the hybrid ligand. This assay is dependent on the permeability of yeast cells to the ligand,

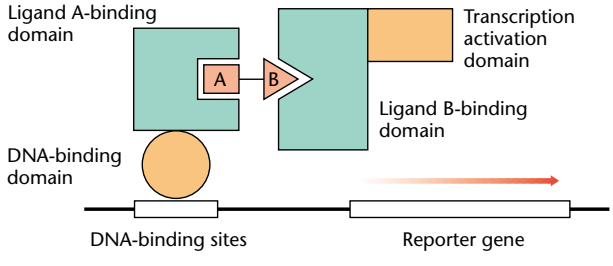

Figure 3 The three-hybrid system to detect the interaction of a protein with a small molecule. A hybrid protein of a DNA-binding domain with a protein domain that binds to ligand A, and a hybrid protein of an activation domain with a protein domain that binds to ligand B, are bridged by a heterodimer of A linked to B.

and has limitations for membrane-associated proteins similar to the two-hybrid system. With the use of synthetic combinatorial libraries of small organic molecules covalently linked to a known ligand, the assay has the potential to identify new compounds that may have therapeutic uses.

The chemical three-hybrid assay is a specific version of the more general use of heterodimeric ligands to bring together two hybrid proteins containing ligand-binding domains. In its application to transcription, a dimer of the immunophilins FK506 and cyclosporin A was shown to bridge a Gal4 DNA-binding domain–FKBP12 hybrid and a VP16 activation domain–cyclophilin hybrid, resulting in activation of a reporter gene (Belshaw *et al*., 1996). Similar chemical inducers of dimerization were also capable of activating signalling pathways by aggregating intracellular domains of receptors; of recruiting a protein such as the Fas receptor to the plasma membrane where it becomes active; and of inducibly targeting a protein to the nucleus (Belshaw *et al*., 1996).

Alternative Protein Interaction Systems

The two-hybrid assay requires that both hybrid proteins must be capable of entering the nucleus as correctly folded and modified proteins, and that the DNA-binding domain hybrid does not itself activate transcription. To overcome these limitations, several investigators have developed alternative assays that exploit the logic of the two-hybrid system to reconstitute the function of a protein other than a transcriptional activator. The ubiquitin-based split-protein sensor system (Johnsson and Varshavsky, 1994) uses two inactive fragments of ubiquitin, which is normally attached to proteins as part of a degradation pathway. When these fragments are fused to a pair of interacting proteins, ubiquitin activity is restored, leading to proteolytic cleavage of a reporter protein.

In the Sos recruitment system, localization of the human Sos protein to the yeast plasma membrane through a protein–protein interaction provides the assay (Aronheim *et al*., 1997). Sos is a mammalian guanyl nucleotide exchange factor that can complement a temperature-sensitive version of the yeast Cdc25 protein, provided that Sos is present at the yeast membrane. This membrane targeting occurs via the interaction of a protein 'X', fused to a cytoplasmic Sos protein, with a protein 'Y', fused to a myristoylation signal. Successful use of this system in a search has been demonstrated, with the bait protein c-Jun fused to Sos, and a library of human proteins fused to the membrane targeting signal.

A mammalian cell-based assay takes advantage of intracistronic complementation of the *E. coli* β-galactosidase protein (Rossi *et al*., 1997). Nonfunctional weakly complementing β-galactosidase peptides can assemble to form an active enzyme by virtue of the interaction of two proteins fused to these peptides. Finally, fluorescence resonance energy transfer (FRET) approaches using hybrid proteins may provide highly sensitive assay systems. Miyawaki *et al*. (1997) used two green fluorescent proteins (GFP) of differing emission wavelengths. These GFPs were fused to a calcium sensor composed of calmodulin and calmodulin-binding peptide, and changes in calcium concentration could be detected by changes in FRET mediated by alterations in the conformation of the calcium-binding domains. These two approaches have the advantages that different cellular compartments can serve as the site of the interaction and that real-time analysis of interactions may be feasible.

Conclusions

Because of the ease with which the two-hybrid system can be used to detect new interactions among proteins, efforts are beginning for scaling up this technology to analyse ever larger sets of proteins. Current or planned projects focus on detecting interactions of the proteins encoded by the bacterium *E. coli*, the yeast *S. cerevisiae*, the nematode *Caenorhabditis elegans* and the fruitfly *Drosophila melanogaster*. Additional projects are underway in biotechnology companies to apply large scale two-hybrid methodology to human proteins.

Finally, other uses of hybrid proteins in understanding macromolecular interactions are likely to be developed. These new approaches should continue to extend the types of interactions that can be analysed, the organisms that can conveniently be used as hosts, and the compartments within the cell where interactions can be detected.

References

Aronheim A, Zandi E, Hennemann H, Elledge SJ and Karin M (1997) Isolation of an AP-1 repressor by a novel method for detecting protein–protein interactions. *Molecular and Cellular Biology* **17**: 3094–3102.

Belshaw PJ, Ho SN, Crabtree GR and Schreiber SL (1996) Controlling protein association and subcellular localization with a synthetic ligand that induces heterodimerization of proteins. *Proceedings of the National Academy of Sciences of the USA* **93**: 4604–4607.

Chiu MI, Katz H and Berlin V (1994) RAPT1, a mammalian homolog of yeast TOR, interacts with the FKBP12/rapamycin complex. *Proceedings of the National Academy of Sciences of the USA* **91**: 12574–12578.

Colas P, Cohen B, Jessen T *et al*.(1996) Genetic selection of peptide aptamers that recognize and inhibit cyclin-dependent kinase 2. *Nature* **380**: 548–550.

Fields S and Song O (1989) A novel genetic system to detect protein–protein interactions. *Nature* **340**: 245–246.

Inouye C, Dhillon N, Durfee T, Zambryski PC and Thorner J (1997) Mutational analysis of *STE5* in the yeast *Saccharomyces cerevisiae*: application of a differential interaction trap assay for examining protein–protein interactions. *Genetics* **147**: 479–492.

Johnsson N and Varshavsky A (1994) A split ubiquitin as a sensor of protein interactions *in vivo*. *Proceedings of the National Academy of Sciences of the USA* **94**: 10340–10344.

Licitra EJ and Liu JQ (1996) A three-hybrid system for detecting small ligand–protein receptor interactions. *Proceedings of the National Academy of Sciences of the USA* **93**: 12817–12821.

Miyawaki A, Llopis J, Heim R *et al*. (1997) Fluorescent indicators for Ca^{2+} based on green fluorescent proteins and calmodulin. *Nature* **388**: 882–887.

Osborne MA, Dalton S and Kochan JP (1995) The yeast tribrid system – genetic detection of *trans*-phosphorylated ITAM-SH2-interactions. *Bio/Technology* **13**: 1474–1478.

Rossi F, Charlton CA and Blau HM (1997) Monitoring protein–protein interactions in intact eukaryotic cells by β-galactosidase complementation. *Proceedings of the National Academy of Sciences of the USA* **94**: 8405–8410.

SenGupta DJ, Zhang B, Kraemer B *et al*. (1996) A three-hybrid system to detect RNA–protein interactions *in vivo*. *Proceedings of the National Academy of Sciences of the USA* **93**: 8496–8501.

Shih H, Goldman PS, DeMaggio AJ *et al*. (1996) A positive genetic selection for disrupting protein–protein interactions: identification of CREB mutations that prevent association with the coactivator CBP. *Proceedings of the National Academy of Sciences of the USA* **93**: 13896–13901.

Vidal M, Brachmann RK, Fattaey A, Harlow E and Boeke JD (1996) Reverse two-hybrid and one-hybrid systems to detect dissociation of protein–protein and protein–DNA interactions. *Proceedings of the National Academy of Sciences of the USA* **93**: 10315–10326.

Wang MM and Reed RR (1993) Molecular cloning of the olfactory neuronal transcription factor Olf-1 by genetic selection in yeast. *Nature* **364**: 121–126.

Yang M, Wu Z and Fields S (1995) Protein–peptide interactions analysed with the yeast two-hybrid system. *Nucleic Acids Research* **23**: 1152–1156.

Further Reading

Bartel PL and Fields S (eds) (1997) *The Yeast Two-Hybrid System*. Oxford: Oxford University Press.

Brachman RK and Boeke JD (1997) Tag games in yeast: the two-hybrid system and beyond. *Current Opinion in Biotechnology* **8**: 561–568.

Brent R and Finley RL Jr (1997) Understanding gene and allele function with two-hybrid methods. *Annual Review of Genetics* **31**: 663–704.

Frederickson RM (1998) Macromolecular matchmaking: advances in two-hybrid and related technologies. *Current Opinion in Biotechnology* **9**: 90–96.

McNabb DS and Guarente L (1996) Genetic and biochemical probes for protein–protein interactions. *Current Opinion in Biotechnology* **7**: 554–559.