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Modification, Development, Application and Prospects of Tandem Affinity Purification Method

Xiaoli Xu[1,2,*], Xueyong Li[1,*],
Hua Zhang[2] and Lizhe An[2]
[1]Department of Burn and Plastic Surgery, Tangdu Hospital,
Fourth Military Medical University, Xi'an,
[2]Key Laboratory of Arid and Grassland Agroecology of Ministry of Education,
School of Life Sciences, Lanzhou University, Lanzhou,
[1,2]China

1. Introduction

Since the completion of genome sequences of several organisms, attention has been focused on the analysis of the function and functional network of proteins. Most cell type-specific functions and phenotypes are mediated and regulated by the activities of multiprotein complexes as well as other types of protein–protein interactions and posttranslational modifications. Accordingly, the formation and function of macromolecular protein complexes support the whole of cell processes. Consequently, analysis of the variations of protein complex composition in different cell and tissue types is essential to understand the relationship between gene products and cellular functions in diverse physiological contexts (Alberts, 1998; Cusick et al., 2005).

With the development of research strategies, many large-scaleprotein-protein interaction studies have been performed in model organisms, especially the budding yeast Saccharomyces cervisiae. Genome-wide yeast two-hybrid screens (Fromont-Racine et al., 1997; Ito et al., 2001; Uetz et al., 2000) and protein chip-based methods (Zhu et al., 2001) allow broader insight into the interaction networks and afford the possibility of high-throughput analysis of function and functional network of proteins. While the former approach provides information relating to interactions between two proteins, typically of binary nature, and has the potential for false-positive and false-negative results, the latter approach is time consuming and labor intensive. These defects may limit their application in large scale protein complex purification.

A novel protein complex purification strategy, named tandem affinity purification (TAP) (Puig et al., 2001; Rigaut et al., 1999), in cooperation with mass spectrometry allows identification of interaction partners and purification of protein complexes. This strategy was originally developed in yeast and has been tested in many cells and organisms.

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[*] These authors contributed equally to this work

2. TAP method: A brief overview

The basic principle of TAP is similar to the epitope tagging strategy but different on the utilization of two sequential tags instead of one. Rigaut et al. (Rigaut et al., 1999) compared several tag combinations aiming at high recovery rates without hampering protein functions and developed the standard TAP tag. The TAP method requires fusing a TAP tag to the target protein. The TAP tag consist of two IgG-binding domains of protein A of *Staphylococcus aureus* (ProtA) and a calmodulin-binding domain (CBP), separated by a cleavage site for the tobacco etch virus (TEV) protease (Rigaut et al., 1999). In addition to the C-terminal TAP tag, an N- terminal TAP tag (Puig et al., 2001), which is a reverse orientation of the C-terminal TAP tag, was also generated (Fig. 1A).
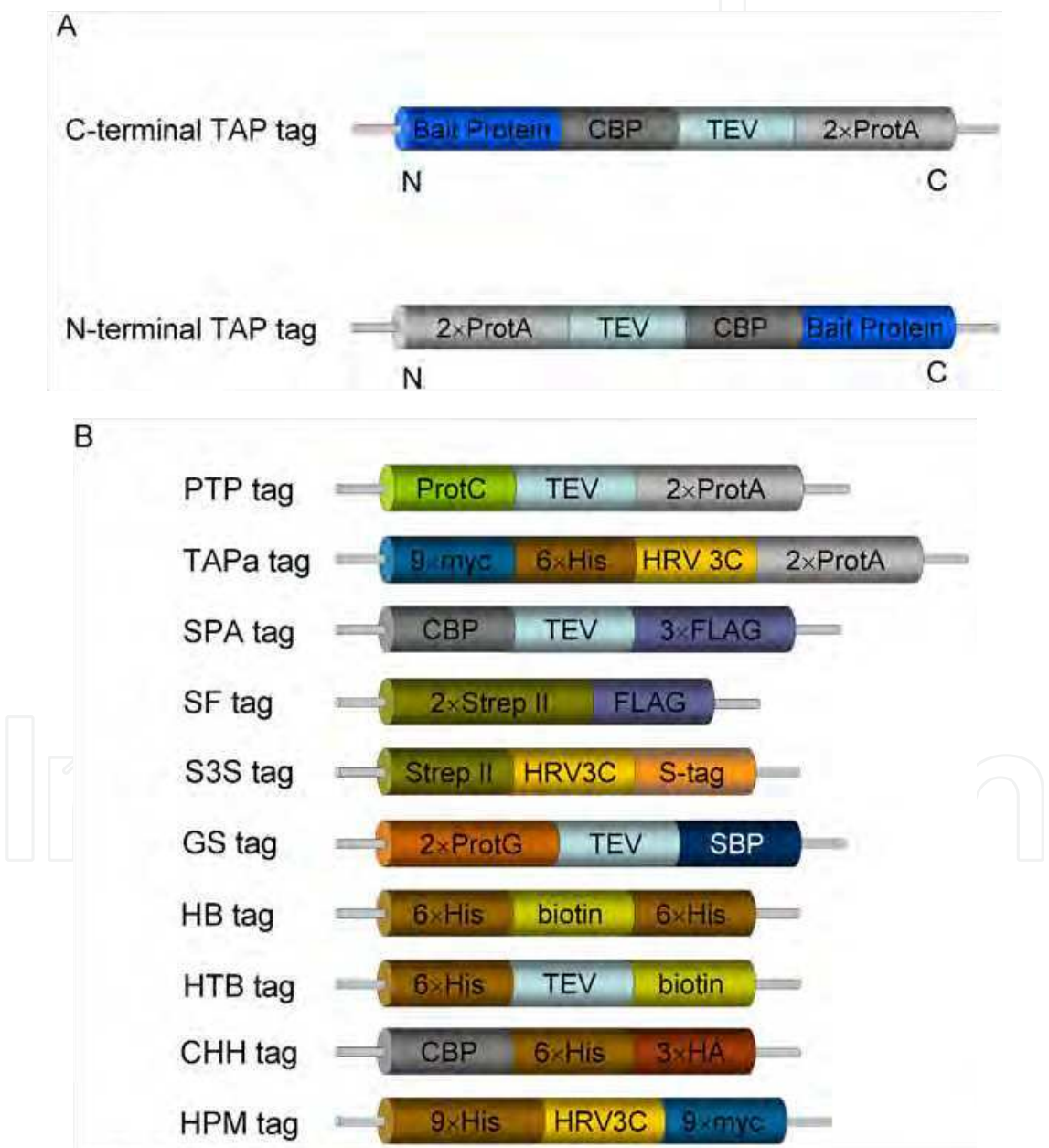

Fig. 1. Diagrammatic sketch of the TAP tag. (A) The original C- and N-terminal TAP tag. (B) Variation of TAP tags developed over the past few years.

The TAP method requires the fusion of the TAP tag to proteins of interest, either at the C- or N-terminus, and the transformation of the construct into appropriate host organisms. The TAP-tagged protein is expressed in host cells at close to physiological concentrations to form a complex with endogenous components. Extracts prepared from cells expressing TAP-tagged proteins are subjected to two sequential purification steps (Fig. 2).

It is well known that the TAP system is very useful for the identification of relatively stable protein complexes, and have helped in the discovery of novel interactions. The TAP method has many advantages: first, the TAP system allows rapid purification of protein complexes without the knowledge of their function or structure. Second, the TAP method enables protein complex purification under native conditions. Third, the tandem purification steps provide highly specific and reduce the high background caused by contaminants substantially. Finally, all protein complex purification can be processed under the same conditions, thus the results are reproducible and comparable, which is significant in large-scale systematic proteome researches. Due to these advantages, the TAP method has been successfully applied in the research of protein-protein interactions in prokaryotic and eukaryotic cells.
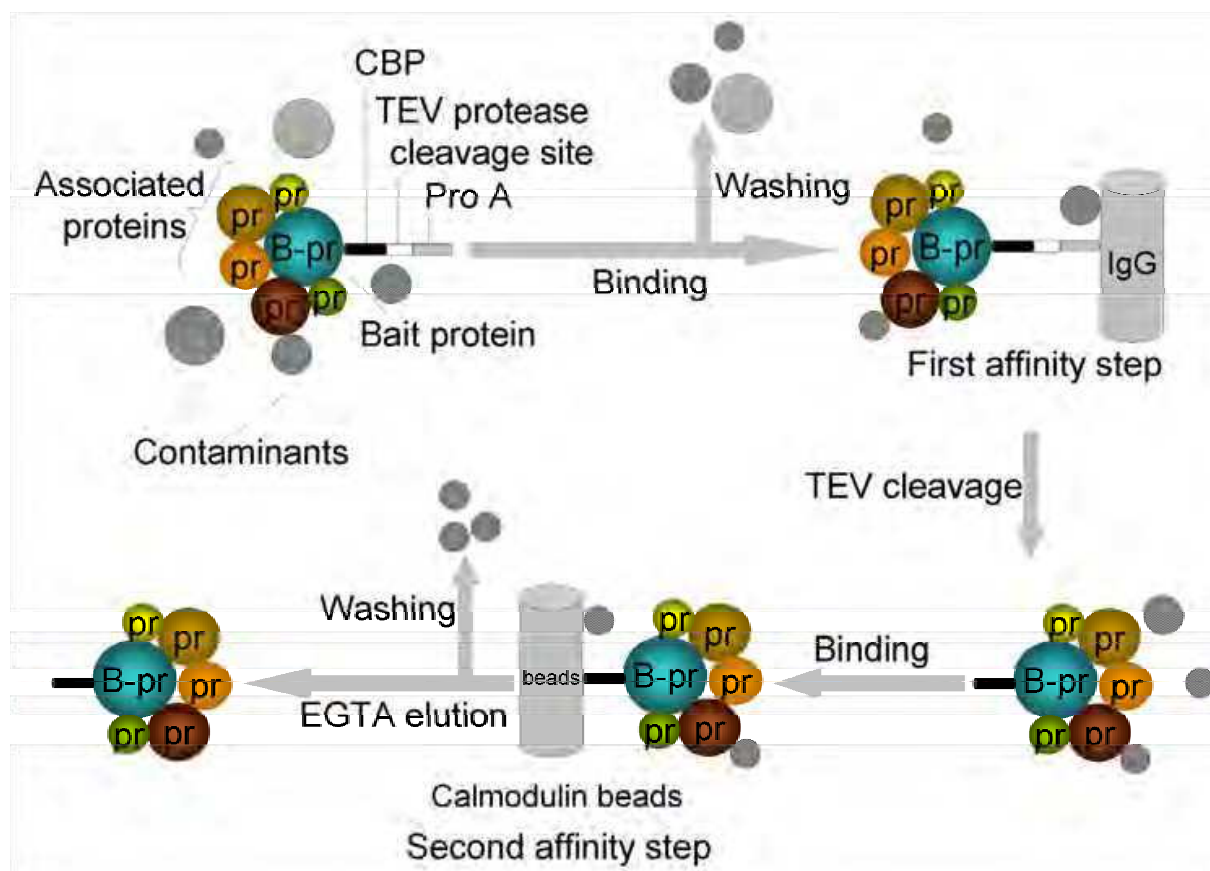

Fig. 2. Schematic of the original TAP method. In the first step, the protein complex, which contains the tagged target protein, combines with an IgG matrix by the ProtA fraction. The protein complex is then eluted using TEV protease under native conditions. In the second step, the elution fraction of the first purification step is incubated with beads coated by calmodulin in the presence of calcium. Subsequently, contaminants and the remainder of TEV protease used in the first step are eliminated through washing. Ultimately, the target protein complex is obtained by elution using EGTA. Adapted from (Xu et al., 2010).

3. The development of TAP tags

Although the TAP system was originally developed in yeast, it has been proven to successfully work in a broad range of organisms. The classic ProtA-TEV-CBP tag may be inefficient to purify all given protein complexes. Therefore several variations of the TAP tag based on other affinity tags have been developed that offer advantages in specific cases (Fig. 1B). The properties of these basic affinity tags (Li, 2010; Lichty et al., 2005; Stevens, 2000; Terpe, 2003) are summarized (Xu et al., 2010) to highlight the advantages and disadvantages of corresponding recombinant tags.

The CBP tag could not always recover protein complexes with high efficient, especially where EGTA may irreversibly interfere with the metal-binding protein function. Consequently, one major type of variation is the replacement of the CBP tag. For example, when purifying protein complexes from mammalian cells growing in monolayer cultures, a biotinylation tag is used as the second affinity tag, taking advantage of the high biotin-avidin binding affinity and resulting in an increased yield of the fusion protein (Drakas et al., 2005). Another example is that the CBP tag has been replaced with a protein C epitope (ProtC) resulting in a new TAP tag, designated PTP (Mani et al., 2011; Schimanski et al., 2005). The advantage of this is that ProtC shows more efficiency and allows the elution either by EGTA or by the ProtC peptide. With respect to isolation of active metal-binding proteins, another replacement of the CBP moiety has been a 9×myc with a 6×His sequence (Rubio et al., 2005). This tag is known as TAPa tag and also contains a human rhinovirus 3C protease cleavage site (HRV 3C) instead of the original TEV site. In contrast to TEV protease, 3C protease still has enzymatic activity at 4℃. These modifications are thought to be beneficial to keep the stabilization of protein complex structures and activities.

Another type of variation is a series TAP tags with smaller size. The original TAP tag is as large as approximately 21 kDa, and this size might impair the function of the tagged protein or interfere with protein complex formation. Because of this, many affinity tags, which range in size from 5–51 amino acids, can be used to replace of CBP or ProtA moiety (Terpe, 2003). One example of a smaller TAP tag is SPA tag, made by substituting 3×FLAG for ProtA (Zeghouf et al., 2004). Replacement of the CBP with a spacer and a single FLAG sequence constitute another smaller tag for TAP (Knuesel et al., 2003). The combination of a streptavidin-binding peptide (SBP) and a CBP has been verified in human cells (Ahmed et al., 2010; Colpitts et al., 2011). Recently, use of another alternative tandem affinity tag, composed of two Strep-tag II and a FLAG-tag (SF), has been published (Gloeckner et al., 2007). This SF tag reduced the size of the TAP tag to 4.6 kDa. This smaller size is less likely to disturb protein activity and structure. Because both tags can be eluted under native conditions, the SF-TAP strategy allows purification of protein complexes in less than 2.5 h. Another similar tandem combination of FLAG-Strep tag II has been developed to purify protein complexes efficiently from *Thiocapsa roseopersicina* (Fodor et al., 2004). And a tandem SBP-FLAG tag has been used to uncover the interacting proteins from HEK293 cells (Zhao et al., 2011). These FLAG-containing combination tags may take advantage of shorter length of the tag and result in higher purity of fusion proteins, while the disadvantage of FLAG tag is the relatively high cost during purification. Lehmann et al. (Lehmann et al., 2009) developed a novel S3S tag comprising a S-tag, a HRV 3C and a Strep-tag II. The S3S tag with a size of 4.2 kDa fulfils the requirements of specificity, high yield and no adverse effects on protein

function. Nevertheless, it is doubtful as to whether large tags actually disturb the function of tagged proteins. It would appear that the majority of proteins tagged with the original protA-TEV-CBP tag remain functional, and even small proteins such as acyl-carrier protein (< 10 kDa) (Gully et al., 2003) and thioredoxin (~12 kDa) (Kumar et al., 2004) can be used as bait to purify protein complexes.

In addition to those described above, there are a variety of other TAP tags that are largely different from the classic TAP tag. Bürckstümmer et al. (Bürckstümmer et al., 2006) designed a new TAP tag, designated as GS tag. This tag comprised two copies of IgG binding units of protein G from *Streptococcus* sp. (ProtG) and a SBP. The GS tag was able to purify recombinant proteins with high efficiency and purity, however, the size of the GS tag, at approximately 19 kDa, might be the obvious disadvantage. In a recently published paper, a new tandem affinity tag, the HB tag (Guerrero et al., 2006), consisting of two 6×His motifs and a biotinylation signal peptide has been developed. The HB tag is compatible with *in vivo* cross-linking to purify protein complexes under fully denaturing conditions, which may be beneficial to detect transient and weak protein-protein interactions. A useful derivative of the HB tag is the HTB tag, which includes a TEV cleavage site allowing for protease-driven elution from streptavidin resins (Tagwerker et al., 2006). A CHH tag consisting of a CBP, 6×His residues and three copies of the hemagglutinin (3×HA) has been designed (Honey et al., 2001). However, in fact, the 3×HA peptide is usually used to detect the expression levels of tagged proteins rather than act as the third purification step. In practice, the elution buffer for the calmodulin resin is incompatible with binding to the Ni^{2+} resin. Although buffer exchange may solve this problem, it results in a significant loss of yield. For this reason, the combination of CBP and His tags is generally not recommended. As for purification of associated proteins from *Drosophila* tissues, the 3×FLAG-6×His tag provided significant higher yields than the tranditional tag (Yang et al., 2006). At the same time, a similar combination of His and FLAG epitope was constructed to isolate protein complexes from pathogenic fungus (Kaneko et al., 2004). The HPM tag, another bipartite affinity tag, consisting of 9×His, 9×myc epitope and two copies of HRV 3C inserted was successfully applied in yeast (Graumann et al., 2004).

4. Application of the TAP method

With the development of the TAP approach over the past decade, this method has been employed in the analysis of protein-protein interactions and protein complexes in many different organisms, including yeast, mammals, plants, *Drosophila* and bacteria (Table 1) (Chang, 2006; Xu et al., 2010).

4.1 TAP in yeast

The TAP method was originally developed for analysis of protein complexes in yeast at near-physiological conditions. Gavin et al. (Gavin et al., 2006; Gavin et al., 2002) and Krogan er al. (Krogan et al., 2006) utilized TAP in the large-scale analysis of multi-protein complexes in *Saccharomyces cerevisiae*, in which hundreds to thousands tagged proteins were successfully purified and the associated proteins and involved protein-protein interactions were identified. These results give the possibility to intensive study the functional and organizational network of proteins in yeast.

As to a given protein, the TAP system could also provide opportunity to investigate protein interaction (Graumann et al., 2004; Guerrero et al., 2006; Honey et al., 2001; Krogan et al., 2002). For example, TAP analysis revealed more than one hundred previously known and possible interacting proteins for 21 tagged proteins, which are involved in transcription and progression during mitosis (Graumann et al., 2004). In addition, an active Clb2-Cdc28 kinase complex was purified from yeast cell lysate by TAP (Honey et al., 2001), and four proteins were identified by mass spectrometry to be associated with this complex.

The application of TAP protocol was successful not only in *S. cerevisiae*, but also in *Schizosaccharomyces pombe* and *Candida albicans*. A large number of researches have carry out the TAP strategy to isolate protein complexes and associated partners (Cipak et al., 2009; Gould et al., 2004; Kaneko et al., 2004; Tasto et al., 2001).

Organism	Protein complex	Bait protein	Tandem tag	Proteins identified	Functional pathway
Yeast	RNA polymerase II elongation factors	15 proteins	Protein A, CBP	25	Transcriptional elongation
	Cyclin-dependent kinase complex	Cdc2p	Protein A, CBP	3	Orchestrating cell cycle
	Arp2/3 complex	Arp2p	Protein A, CBP	6	Nucleation
	Swi 5 contaning complex	Swi 5	Protein A, CBP	4	Mating-type switching and homologous recombination regulation
	Spetin complex	CaCDC11	His, FLAG	4	Cytokinesis, virulence
Human cells	DNA-dependent protein kinase	Ku70, Ku80	Protein G, SBP	Many	Multiple
	Human cytomegalovirus protein kinase	UL97	Protein A, Protein C	1	Human cytomegalovirus replication
	ADAP	ADAP	S tag, Strep II	Many	Integrin adhesion regulation
	Many	Spartin	Protein A, CBP	94	Multiple
	Parkin-associated complex	Parkin	streptavidin-binding peptide, CBP	14	Regulation mitochondrial activity
	Many	32 proteins	Protein A, CBP	Many	mRNA formation
	MCM complex	MCM-BP	Protein A, CBP	5	Initiation of DNA replication
	IRF-4 containing complex	IRF-4	Protein A, CBP	1	Activiates IL-2 and IL-4 promoters
	Flavivirus capsid containing complex	Flavivirus capsid	SBP, CBP	4	Disruption of nucleosome formation

Organism	Protein complex	Bait protein	Tandem tag	Proteins identified	Functional pathway
	HDGF	HDGF	SBP, FLAG	106	Multiple
	HP1 containing complex	HP1α, β, γ	Protein A, CBP	Many	Multiple
	Gβγ-Rap1-Radil complex	Gβ$_2$, Gγ$_2$	Strep, CBP	2	Cell-matrix adhesion
Nicotiana benthamiana	Resistance protein N containing complex	Resistance protein N	ProteinA, Myc, His	1	Plant defense
Arabidopsis	Many	6 proteins	Protein A, CBP	42	Cell division signaling
Rice	Many	45 proteins	Protein A, CBP	Many	Multiple
Drosophila	Notch signaling complex	4 proteins	Protein A, CBP	400	Signaling pathway
Escherichia coli	Thioredoxin associated complex	Thioredoxin	Protein A, CBP	80	Multiple
	RecQ complex	RecQ	Protein A, CBP	3	DNA unwinding
	RNP	SrmB	Protein A, CBP	2	Ribosome assembly
Dictyostelium	Microtubule-associated protein	DdEB1, DdCP224	Protein A, CBP	Many	Multiple
	Arp2/3 complex	pArc-34	Protein A, CBP	6	Nucleation
Trypanosome brucei	RNA polymerase I	RPB6z	Protein A, Protein C	3	Transcription
	Alba containing complex	Alba1, 2, 3, 4	Protein A, Protein C	Many	Regulation of translation
Plasmodium falciparum	Translation elongation factor	PfEF-1β	Protein A, Protein C	4	Translation elongation

Table 1. Representative applications of the TAP method.

4.2 TAP in mammalian systems

The application of TAP method has made considerable progress in mammalian systems (Bürckstümmer et al., 2006; Davison et al., 2009; Drakas et al., 2005; Gottlieb and Jackson, 1993; Holowaty et al., 2003; Jeronimo et al., 2007; Kamil and Coen, 2007; Knuesel et al., 2003; Lehmann et al., 2009; Milewska et al., 2009; Sakwe et al., 2007). For instance, human active SMAD3 protein complex was purified from cell lysates through TAP method, and HSP70 was identified as a novel combination partner of SMAD3 (Knuesel et al., 2003). However, in this research the TAP system took a FLAG tag for the second purification step, the elution conditions of which was incompatible with the liquid chromatography-MS/MS sequence application. Therefore, an additional purification step might be reqired to resolve this trouble, which would be time consuming and lead to more sample loss. Sakwe et al. identified a new form of the minichromosome maintenance (MCM) complex in human cells (Sakwe et al., 2007). In another study using the TAP process, Holowaty et al. (Holowaty et al., 2003) expressed Epstein-Barr nuclear antigen-1 (EBNA1) protein in fusion with a TAP tag at the C-terminus in human 293T cells. Several specific cellular

protein interactions and some important regulating proteins were discovered. The TAP method could also be used in analysis nuclear protein interaction. The specific association of interferon regulatory factor (IRF)-4 with c-Rel was revealed from human HUT102 cells (Shindo et al., 2011). A directed proteomic analysis of heterochromatin protein 1 (HP1) isotypes interacting partners were identified by the TAP approach (Rosnoblet et al., 2011). Using TAP strategy, more than one hundred proteins were found to interact with hepatoma-derived growth factor (HDGF) in human HEK293 cells (Zhao et al., 2011). The relationship between HDGF and associated proteins suggests that DHGF as a multifunctional molecular might be involved in many cellular activities. In human HEK293 cells, Gβγ subunits of heterotrimeric G proteins were identified to enter in a protein complex with Rap1a and its effector Radil (Ahmed et al., 2010). This result sggested that the Gβγ -Rap1-Radil complex played an important role in cell adhesion.Besides, TAP also allows for purification of protein complexes from mouse fibroblast cells growing in monolayer cultures and mouse embryonic stem cells (Drakas et al., 2005; Mak et al., 2010). It is meaningful that the TAP system could be used to analysis the interaction of virus and host cells during the infection procedure (Colpitts et al., 2011).

4.3 TAP in plants

Recent studies have shown that the TAP strategy is useful in plant protein complex analysis. The first report of the purification of protein complexes from plant tissue by the TAP method was published in 2004 by Rohila et al (Rohila et al., 2004). By using a TAP-tagged hybrid transcription factor as bait, HSP70 and HSP60 were co-purified. This result was verified by former reports (Dittmar et al., 1997; Stancato et al., 1996). Through the TAP strategy, Liu et al. demonstrated that Hsp90 associated with the plant resistance protein N (Liu et al., 2004), which meant that Hsp90 plays an important role in plant defense (Kanzaki et al., 2003; Takahashi et al., 2003). In another study, the Cf-9 protein function in initiating defense signaling was also investigated by TAP (Rivas et al., 2002). The TAP applications described above were all carried out in a transient expression system of *Nicotiana benthamiana*.

On the other hand, the TAP system was utilized to purify a protein complex in stable expression system of *Arabidopsis thaliana*, for the first time in 2005 (Rubio et al., 2005). The components of the target protein complex were all co-purified with the tagged bait. The superiority of this TAP strategy is based on a constitutive promoter, which allowed for over-expression of TAP fusion proteins. The strength of this method is that over-expression increases incorporation of the tagged protein into a protein complex, when the tagged protein is the core component of a complex or a mutant and suppressed expression for the target protein is harmful to cells. In 2006, Brown et al. (Brown et al., 2006) utilized TAP tagged fatty acid synthase components to investigate protein interactions *in vivo* from stably transfected *A. thaliana*. In addition to the application of TAP to a whole, *A. thaliana* cell suspension culture is ideal for investigating protein-protein interactions involved in cell cycle (Van Leene et al., 2007).

Purification of protein complexes by TAP was demonstrated to be effective in rice (Rohila et al., 2006; Rohila et al., 2009), suggesting that the TAP method could be utilized in cereal crops.

4.4 TAP in Drosophila

In 2003 Forler et al. (Forler et al., 2003) successfully expressed TAP-tagged human proteins and purified their *Drosophila melanogaster* (Dm) binding partners in Dm Schneider cells. The critical advantage in this system is the introduction of RNA interference (RNAi), which can suppress the expression of the corresponding endogenous proteins, thereby avoiding competition from them during protein complex assembly. But the complexes purified through this system consisted of two different source proteins, human bait protein and Dm binding partners, therefore the reliability of the interaction needed validation with other experimental strategies. Both in *Drosophila* cultured cells and embryos, several components of the Notch signaling pathway were tagged with a TAP tag and many novel interactions were uncovered (Veraksa et al., 2005). Throughout the TAP progress, Hsc70 and Hsp83 were validated as cofactors of the *Drosophila* nuclear receptor protein for the first time (Yang et al., 2006).

4.5 TAP in bacteria

In recent years, with the development of the TAP procedure, the application of TAP was extended to purification of protein complexes from bacteria. Gully et al. (Gully et al., 2003) first used the TAP protocol in *E. coli* to isolate native protein complexes. Kumar et al. (Kumar et al., 2004) have identified 80 proteins associated with thioredoxin in *E. coli* suggesting multifunction of thioredoxin. Shereda et al. (Shereda et al., 2007) employed the TAP approach to purify the RecQ complexes, and three heterologous proteins were identified. On the basis of the amount of these three binding proteins, these interactions were classed as direct or indirect. This may imply a new application aspect of TAP in interaction identification. SrmB, one of the five *E. coli* DEAD-box proteins was discovered to form a specific ribonucleoprotein (RNP) complex with r-proteins L4, L24 and the 5′ region of 23S rRNA using the TAP procedure (Trubetskoy et al., 2009). Similar to the application of the TAP method in global protein complexes analysis in yeast, a large-scale analysis of protein complexes, which revealed a novel protein interaction network in *E. coli*, was reported (Butland et al., 2005). Besides the application of the TAP method in *E. coli*, TAP was also carried out in *Thiocapsa roseopersicina* (Fodor et al., 2004) and *Bacillus subtilis* (Yang et al., 2008).

4.6 TAP in other organisms

The efficiency of the TAP method in purification of protein complexes and identification of interactions was also tested in other organisms, including *Dictyostelium* (Koch et al., 2006; Meima et al., 2007), *Trypanosoma bruce* (Mani et al., 2011; Nguyen et al., 2007; Palfi et al., 2005; Schimanski et al., 2005; Walgraffe et al., 2005) and *Plasmodium falciparum* (Takebe et al., 2007).

5. Problems and future prospects

The TAP method has been successfully used for purification and identification of protein complexes and interacting components both in prokaryotic and eukaryotic organisms. However, in practice, the application effects of the method may have been influenced by its inherentvice. Gavin et al. (Gavin et al., 2002) found that in their large-scale analysis of yeast proteome, not all of the tagged proteins could be purified and not all of the purified tagged proteins could interact with other proteins. They ascribed this failure to the intrinsic quality

of the TAP tag. The TAP tag fused to a target protein may interfere with protein function, location and interactions (Mak et al., 2010). One of the possible solutions is to add the tag at the other terminus of the ORFs or to replace the original tag with another one. The CBP affinity step has been proved to be problematic in that case where many endogenous proteins of mammalian cells interact with calmodulin in a calcium-dependent manner (Agell et al., 2002; Head, 1992). A simple alternative solution is replacing the CBP tag with other affinity tags, such as the FLAG sequence (Gloeckner et al., 2007; Knuesel et al., 2003), ProtC (Schimanski et al., 2005) and biotinylation tag (Drakas et al., 2005). The main challenge of the TAP strategy comes from the competition of endogenous proteins with the tagged protein in protein complex assembly. This can be resolved by using RNAi to reduce the endogenous expression level (Forler et al., 2003).In some cases, when the target protein is essential and a mutant of it might be harmful and lethal, the over-expression strategy is a perfect strategy to obtain a protein complex containing the tagged target protein (Ho et al., 2002; Rohila et al., 2006; Rubio et al., 2005). However, bait overexpression possibly cause the formation of nonbiological interactions. In addition, overesexpression may affect cell viability or cellular activity (e.g. negative regulators of cell metabolism). On this occasion, an inducible promoter is a viable choice, which allows experimental modulation of target protein expression, both in terms of amount and timing.

It is thought that the TAP approach is not a powerful tool to detect transient interactions. Therefore, an *in vivo* cross-linking step is added to freeze both weak and transient interactions taking place in intact cells before lysis (Guerrero et al., 2006; Rohila et al., 2004). The cross-linking method has been widely used in the investigation of protein-DNA and protein-protein interactions (Hall and Struhl, 2002; Jackson, 1999; Kuo and Allis, 1999; Orlando et al., 1997; Otsu et al., 1994; Schmitt-Ulms et al., 2004; Schmitt-Ulms et al., 2001; Vasilescu et al., 2004).

Although the two sequential purification steps of the TAP method largely reduce the background resulting from non-specific protein binding compared to a single purification step, these contaminants cannot be removed completely. Collins et al. (Collins et al., 2007) have compared the results from the two large-scale studies of protein complexes in yeast (Gavin et al., 2006; Krogan et al., 2006) and found the two datasets shared very low degrees of overlap. The major difference between the two datasets was mainly caused by non-specific interactions. The problem of non-specifically interacting proteins can be overcome by comparing several interaction datasets (Ewing et al., 2007), using stable-isotope labelling by amino acids in cell culture (Blagoev et al., 2003; Mann, 2006) or isotope-coded affinity tag (Ranish et al., 2003), thereby completely eliminating false-positive interactions.

The TAP system is considered to be inefficient in identifying interactions occurring only in special physiological states or those which occur for a short period. Whether the TAP tag impairs protein function and complex assembly also remains largely unknown and speculative. These disadvantages may affect its application in such instances.

6. Conclusion

Understanding protein function is a major goal in biology. Although the TAP method has some inherent shortcomings, it is undoubtedly a reasonable system for use in purification of

protein complexes and identification of protein-protein interactions. In addition to identifying interactions between proteins, the TAP method could be used to characterize and verify interactions between protein and DNA or between protein and RNA (Hogg and Collins, 2007; Zhao et al., 2011). For protein-DNA/RNA interaction analysis, the use of benzonase must be avoided, and RNase inhibitors should be added to protect RNA intact. At the same time, the TAP method can also be used to analyze the effect of mutants on protein interaction and association, possibly resulting in the discovery of binding sites. Protein purification under near-physiological conditions through the TAP strategy is compatible with functional studies and this advantage allows for mapping of large-scale functional interaction networks. As the procedures and conditions used during the TAP process do not vary greatly among different proteins, the results that are generated by this method should be compiled in a database in order to provide comparable and detailed information on the potential and confirmed functions of proteins, as well as the composition of protein complexes and even the structure and activity of protein complexes.

7. Abbreviations used

TAP, tandem affinity purification; ProtA, IgG-binding units of protein A of *Staphylococcus aureus*; CBP, calmodulin-binding domain; TEV, tobacco etch virus; ProtC, protein C epitope; HRV 3C, human rhinovirus 3C protease cleavage site; SBP, streptavidin-binding peptide; ProtG, IgG binding units of protein G from *Streptococcus* sp.; HA, hemagglutinin; ADAP, adhesion and degranulation promoting adaptor protein; MCM, minichromosome maintenance; EBNA1, Epstein-Barr nuclear antigen-1; IRF, interferon regulatory factor; HP1, heterochromatin protein 1; HDGF, hepatoma-derived growth factor; Dm, *Drosophila melanogaster*; RNAi, RNA interference; RNP, ribonucleoprotein.

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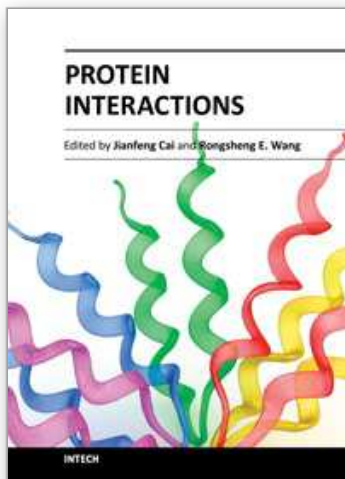
Protein Interactions

Edited by Dr. Jianfeng Cai

Protein interactions, which include interactions between proteins and other biomolecules, are essential to all aspects of biological processes, such as cell growth, differentiation, and apoptosis. Therefore, investigation and modulation of protein interactions are of significance as it not only reveals the mechanism governing cellular activity, but also leads to potential agents for the treatment of various diseases. The objective of this book is to highlight some of the latest approaches in the study of protein interactions, including modulation of protein interactions, development of analytical techniques, etc. Collectively they demonstrate the importance and the possibility for the further investigation and modulation of protein interactions as technology is evolving.

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