Structure
Review

Transient Protein-Protein Interactions: Structural, Functional, and Network Properties

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Transient interactions, which involve protein interactions that are formed and broken easily, are important in many aspects of cellular function. Here we describe structural and functional properties of transient interactions between globular domains and between globular domains, short peptides, and disordered regions. The importance of posttranslational modifications in transient interactions is also considered. We review techniques used in the detection of the different types of transient protein-protein interactions. We also look at the role of transient interactions within protein-protein interaction networks and consider their contribution to different aspects of these networks.

Introduction

Interactions between proteins play an essential role in the proper functioning of living cells. Different types of protein-protein interactions have been defined in the literature (Nooren and Thornton, 2003a). These definitions include the distinction between obligate and nonobligate complexes, where the former cover complexes of protomers that cannot exist independently, as opposed to nonobligate complexes. Another distinction can be made between permanent and transient complexes. Permanent protein-protein interactions (PPIs) are strong and irreversible, whereas a complex qualifies as transient if it readily undergoes changes in the oligomeric state.

Transient complexes can be further subdivided into weak and strong. Weak transient complexes show a dynamic mixture of different oligomeric states in vivo, whereas strong transient complexes change their quaternary state only when triggered by, for example, ligand binding. Weak transient interactions are characterized by a dissociation constant (K_D) in the micromolar range and lifetimes of seconds. Strong transient interactions, stabilized by binding of an effector molecule, may last longer and have a lower K_D in the nanomolar range (Nooren and Thornton, 2003a). This classification scheme, also summarized in Figure 1, can prove very useful, but these distinctions are not entirely clear cut and it is important to recognize the continuum between the different types of interactions.

Structural Properties of Transient Interactions between Globular Domains

Structural data have been exploited to characterize and distinguish different types of protein interaction (Lo Conte et al., 1999; Nooren and Thornton, 2003a, 2003b; Chakrabarti and Janin, 2002; Mintseris and Weng, 2003; Ansari and Helms, 2005). These indicate that transiently interacting proteins have interfaces that are smaller in size than permanent interfaces and have amino acid compositions that are not drastically different from the rest of the protein surface—the proportion of hydrophobic residues is the same as the rest of the surface but interfaces are slightly richer in neutral polar groups. These transient interfaces also tend to be quite compact, comprising a central core that is fully buried during interaction, surrounded by a rim that presents features more similar to the rest of the surface than the interface core. Transient interfaces also tend to be rich in waters.

Many proteins undergo conformational changes upon transient interaction, with the most extreme cases involving disorder-to-order transitions, as discussed in a recent review (Janin et al., 2008). Furthermore, it has also been observed that strong transient dimers tend to have larger, less planar, and sometimes more hydrophobic interfaces than weak transient dimers, and that they often undergo more extensive conformational changes upon interaction (Nooren and Thornton, 2003a). Finally, a recent study comparing transient and permanent homodimers from the PDB suggests that the interfaces of the weak transient homodimers are loosely packed and that this property may contribute to their lower stability (Dey et al., 2010).

Residues at the interfaces of weak transient complexes are more conserved than residues on the rest of the surface (Nooren and Thornton, 2003b). However, when comparing the sequences of proteins involved in transient and obligate complexes of known structures, it also appears that transient interfaces evolve much faster and therefore show greater sequence plasticity and smaller evidence of correlated mutations across the interface, because the protomers have less time to adjust to changes in the partner's interface (Mintseris and Weng, 2005).

Based on the characterization of transient interfaces and their specific features, a number of structure-based prediction methods are being developed with the aim of predicting interactions and interfaces between proteins, and in some cases, to look specifically for transient complexes. Machine-learning algorithms have been shown to have high potential in discriminating permanent and transient PPIs (Liu et al., 2010). A large number of state-of-the-art approaches to protein-protein interface (not necessarily only transient) prediction have been recently reviewed (Ezkurdia et al., 2009).

Also, a link has been found between the stability of residues in the unbound state and whether they are part of a transient interface or not, thus suggesting possible new approaches for the prediction of interfaces from unbound protein structures (Bonet

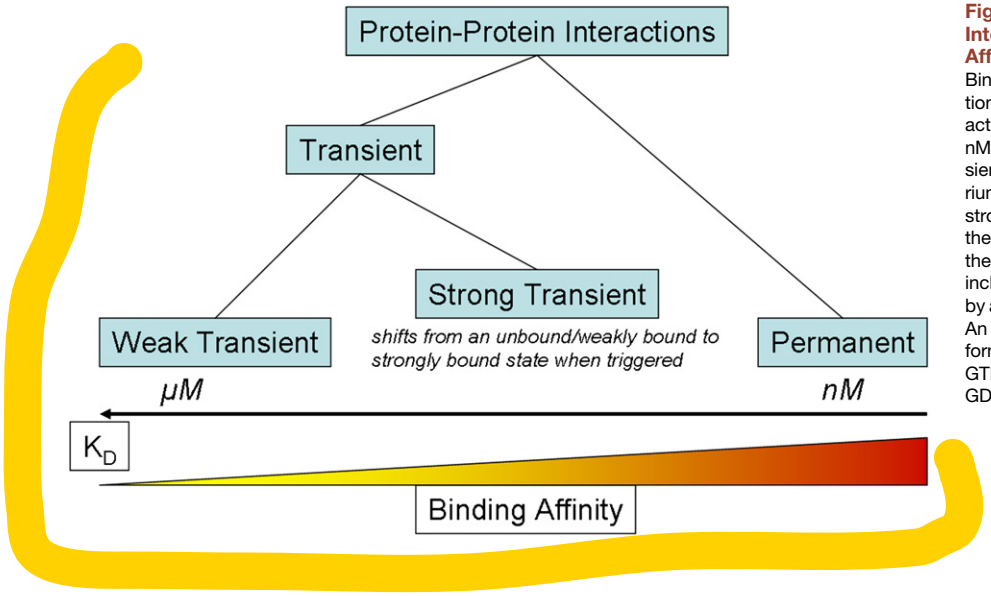

Figure 1. Different Types of Protein Protein Interactions on the Basis of Their Binding Affinities

Binding affinity is inversely related to the dissociation constant (k_{off}/k_{on}) K_D. While permanent interactions feature strong binding affinities (K_D in the nM range), proteins interacting in a weakly transient manner show a fast bound-unbound equilibrium with K_D values typically in the μM range. The strong transient category of interactions illustrates the continuum that exists between the weak and the more permanent interactions. This category includes interactions that are triggered/stabilized by an effector molecule or conformational change. An example is given by the Ras proteins, which form tight complexes with their partners when GTP-bound and only weak complexes when GDP-bound.

et al., 2006). Furthermore, structural changes have been found to be frequent upon complex formation using an approach based on a structural alphabet that represents possible backbone conformations, and the CAPRI benchmark data set of proteins for which the structures are available both in unbound and bound forms, i.e. generally transient interactions (Martin et al., 2008). This supports the importance of the induced-fit model of protein-protein interaction, according to which proteins undergo conformational changes upon interaction.

Structural Disorder in Transient Interfaces

It has been shown recently that a significant number of interfaces in the PDB (associated both with transient and permanent interactions) can be characterized as protruding or interwound, whereby a segment of the partner protrudes deep inside the other partner (Yura and Hayward, 2009). This phenomenon relates closely to the notion of disorder, because it has been observed that the regions that are interwound in interfaces are more likely to be disordered in the unbound state.

Intrinsic disorder is frequently associated with transient interactions. Disorder-to-order transition upon binding is associated with a decrease in conformational entropy and results in interactions of low affinity (Singh et al., 2007). Interactions involving proteins or regions that remain largely disordered in the bound state feature few molecular contacts (see later discussion on motifs) and may often be transient (Tompa and Fuxreiter, 2008). Furthermore, disorder was found to be enriched among proteins involved in cellular functions that show strong temporal variation in activity and are thus likely to involve transient interactions, supported by the observation that long regions of disorder are found in 66% of signaling proteins (Iakoucheva et al., 2002).

Increased recognition of the importance of structural disorder in proteins has led to the development of a large number of bioinformatics tools to predict disordered regions from protein sequence. Some details on the existing tools for the study of protein disorder are shown in Table 1, and the reader is directed to recent reviews (Dosztanyi et al., 2010; He et al., 2009) for further information. Disorder predictors have been explicitly used to predict protein regions involved in interactions (Oldfield et al., 2005); for instance, the recent web server ANCHOR (Dosztanyi et al., 2009), which combines disorder prediction with prediction of binding regions.

Table 1. Examples of Tools for the Study of Intrinsic Disorder in a Protein

Tool Type	Name	Details	URL
Database	Database of Protein Disorder (Disprot)	Contains details on intrinsically disordered proteins, defined as proteins with at least one experimentally determined region of disorder. Currently contains 1284 disordered regions for 594 proteins, all manually curated. Proteins are searchable and browseable, and each entry contains annotations and further details for each disordered region within the protein	http://www.disprot.org
Prediction Server	Predictor of Naturally Disordered Regions (PONDR)	Takes an amino-acid sequence as input. Uses neural-networks, trained on sequences of known disordered and ordered regions, using properties of the sequences in windows of amino acids to generate inputs values for the predictor	http://www.pondr.com/
Prediction Server	Disopred	Takes an amino acid sequence and uses an combination of PSI-blast, neural networks, and a support vector machine to classify parts of the sequence as ordered or disordered	http://bioinfadmin.cs.ucl.ac.uk/disopred/

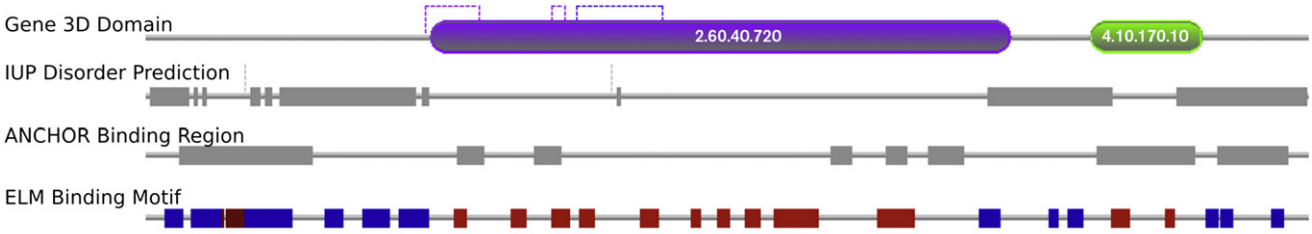

Figure 2. Potential Binding Sites Predicted for the Tumor Suppressor p53
The Gene3D domain prediction shows the position of potential structural domains on the protein. ANCHOR-binding regions, and ELM-binding motifs show potential binding sites for the protein. ELM predictions in red occur in regions covered by a predicted structural domain in Gene3D and are therefore of lower confidence. Using the ELM resource itself might give better predictions since it uses additional filters that were not used here.

Transient Interactions Mediated by Linear Motifs

Linear motifs (LMs) or SLiMs have attracted a lot of attention in recent years (Ren et al., 2008). LMs are short (generally 2–8 residues in length), conserved amino acid sequences that can interact with globular domains from the same and/or other proteins (Diella et al., 2008). They may account for 15%–40% of interactions between proteins (Petsalaki and Russell, 2008; Neduva et al., 2005). Though usually found in intrinsically disordered regions, LMs can also be found in exposed flexible loops within globular domains, where they do not interfere structurally or functionally with the domain in which they are found (Gould et al., 2010). Figure 2 shows a comparison of the LM(s) obtained from the ELM database (Gould et al., 2010) and ANCHOR binding region predictions for the tumor suppressor protein p53.

Interactions mediated by LMs have very different properties from those interactions between two globular domains because they bind with much smaller interface areas (Stein et al., 2009) and weaker affinities (Diella et al., 2008). Such properties help explain why LM-mediated interactions are frequently involved in signaling and other regulatory transient interactions, where the ability to form and break interactions readily is essential in order to respond quickly to cellular perturbations and changes in environment (Stein et al., 2009).

Although weak in nature, LM-mediated interactions show high specificity with their binding partners. In part, this is conferred by cellular location eliminating certain interactions through the absence of other partners (Stein et al., 2009). Specificity may also be determined by regions adjacent to LMs. Analysis of the relative contributions of LMs and their flanking regions to the interaction-binding energy has shown that flanking regions contribute 21% of the binding energy on average (Stein and Aloy, 2008). The importance of flanking regions is further supported by the observation that they are often structurally conserved (Chica et al., 2009).

While an LM might not form a stable tertiary structure on its own, it will usually fold into a stable structure (along with its flanking regions) once bound to a partner domain. The different types of structures adopted by peptide sequences upon binding have been classified into categories (Mohan et al., 2006): those forming α helices, β strands (see also Remaut and Waksman, [2006]), and a final class forming irregular structure elements (see London et al., [2010] for a more recent analysis). Some example transient peptide-protein interactions are shown in Figure 3. In reality, the complexes these molecules form are usually composed of multiple interactions, and the simultaneous existence of these multiple weak low-affinity interactions gives an inherent stability to the complexes. An example of such cooperativity is given in Figure 4, and more examples can be seen in a recent review by Gibson (2009).

This exploitation of multiple simultaneous low-affinity interactions instead of a series of pair-wise interactions allows a much more deterministic regulation of cell processes, ensuring

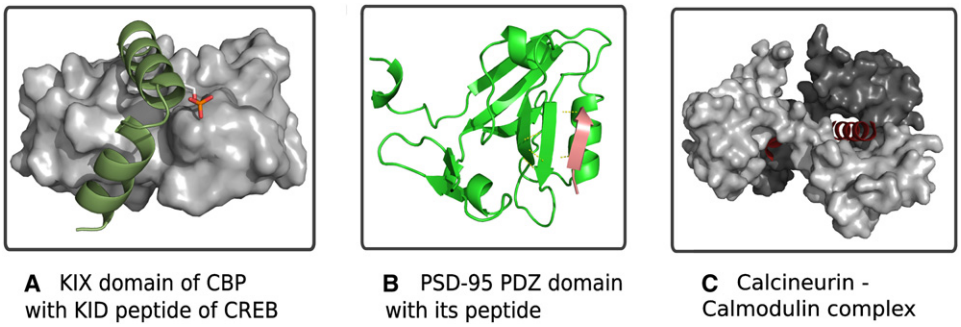

Figure 3. Details of Different Types of Transient Protein-Peptide Interactions
(A) Peptide (green) from the KID domain of CREB binding to a planar part of the surface of the KIX domain of CBP (gray); a phosphate represented as sticks plays an important role in binding (PDB:1KDX).
(B) Cartoon representation of the third PDZ domain from the synaptic protein PSD-95 (green) interacting with a peptide (pink). An extra β strand is added to a β sheet in the domain, in a mechanism coined β-augmentation. H-bonds between strands in the β sheet are represented as dashed yellow lines (1BE9).
(C) α helical peptide (red) from the alpha subunit of calcineurin binding in a deep groove at the interface between two subunits of the calmodulin homodimer (gray and dark gray) (2W73).

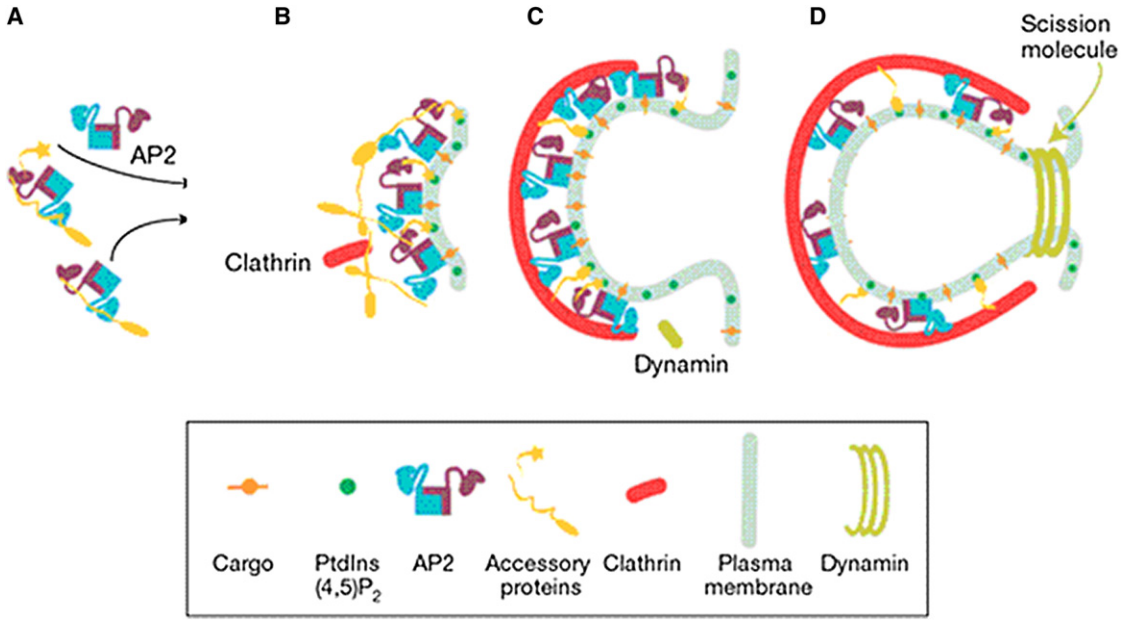

Figure 4. The Step-wise Formation of the Clathrin/AP2 Synaptic Vesicle Complex
An important component of the complex is a set of accessory proteins that, in the initial phase of the vesicle formation, interact simultaneously with many cargo-bound AP2 molecules to help them cluster along the membrane (A and B). These accessory proteins cooperatively stabilize the resulting complex by further binding one another. Only then is Clathrin recruited to the growing complex (B), and many of the accessory proteins change their interaction partner shifting from AP2 to Clathrin, allowing recruitment of plug-in module dynamin that drives vesicle scission (C and D). Importantly, many of the accessory proteins in question exist in low concentrations and mediate only low-affinity interactions, yet they are stabilized within the complex by a mechanism of cooperativity, owing to the synergy between their many interactions with proteins AP2 or Clathrin and between themselves (Schmid and McMahon, 2007).

messenger molecules are guaranteed to reach their intended target, and that a small starting signal is not lost, as might happen if these molecules had to diffuse through the cell unaided, binding only with low affinity when they meet their intended targets (Gibson, 2009; Diella et al., 2008).

Molecular recognition features (MoRFs) are another type of protein-protein interaction-mediating peptide (Oldfield et al., 2005, 2008; Mohan et al., 2006). They are longer than LMs (10-70 residues) and are always located within disordered regions. MoRFs undergo a disorder-to-order transition upon binding (Mohan et al., 2006). They often overlap with LMs and their flanking regions (Fuxreiter et al., 2007; Dunker et al., 2008; Ren et al., 2008).

Multispecificity and the Role of Posttranslational Modifications

Several LMs may coexist together within a given disordered region and it is not unusual for them to overlap. The tumor suppressor protein p53 provides a well-characterized example, for which available structures show that the same disordered region contains multiple overlapping LMs that bind to different interaction partners. Interactions with two such partners, i.e. SIR2 and Cyclin A2, are shown in Figure 5. Both these proteins bind to the same natively unstructured region in the C-terminal region of p53. This region has been predicted to contain multiple LMs and several proteins are predicted to bind to the different LMs within it. These interactions are therefore mutually exclusive, allowing p53 to perform different roles depending on which of its partners are present in its environment, and provides a key mechanism for the cell to switch between different processes (Neduva and Russell, 2005; Oldfield et al., 2008). This "molecular switch" is often modulated by posttranslational modification (PTM).

The p53-MDM2-USP7 pathway provides an interesting biological example of how the binding specificity of a disordered region is modulated by PTMs, leading to switch-like behavior. Certain stress conditions reduce the specificity of the USP7-MDM2 complex via phosphorylation, leading to free USP7 that is then available to bind to and de-ubiquitinate p53 (Figure 5) (Brooks et al., 2007). The scaffolding protein dystroglycan provides a further example of molecular switching, where tyrosine phosphorylation of specific residues can affect its cellular function by changing its binding partners (Moore and Winder, 2010).

The transient, often reversible nature of PTMs, combined with their ability to modulate the binding specificity of LMs, allows them to play a major role as regulators of cellular processes. Computational analysis has shown that the set of protein complexes involved in the cell cycle is similar for all eukaryotes, but that they differ in PTMs and transcriptional control (Jensen et al., 2006). Recent reviews provide more details on the different existing types of PTMs, and on the role of PTMs in PPIs and signaling pathways (Stein et al., 2009; Boehme and Blattner, 2009; Lin et al., 2010).

Transient Interactions in Protein Interaction Networks

Hubs are proteins that are involved in significantly large numbers of interactions within protein interaction networks (PINs). Transient interactions play an important role in determining the behavior of these proteins (Kim et al., 2006; Singh et al., 2007; Higurashi et al., 2008). In a pioneering study, Han et al. (2004) showed that while some hub proteins are highly coexpressed with their partners, implying that they exist as stable complexes,

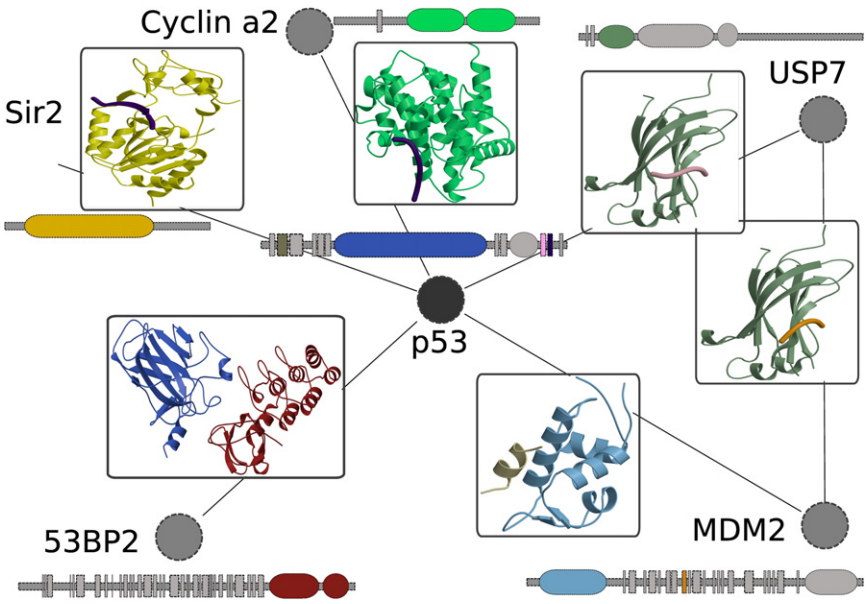

Figure 5. Different Types of Transient Protein-Protein Interactions Involving p53

Edges represent interactions between p53 and its partners. All proteins are annotated with their names and 2D representations showing putative domains from Gene3D or Pfam (longer, rounded rectangles) and putative ELM-binding regions (shorter rectangles). Each protein is connected to its binding partner. Colors in the structures correspond to those in the sequence, e.g., pink ELMs in the 2D representation of p53 correspond to the pink segment in the structure of the interaction between p53 and USP7. Interactions between p53 (beige peptide) and MDM2 (light-blue domain) (PDB: 1YCQ), p53 (pink peptide) with USP7 (dark green domain) (2FOJ), and USP7 (dark green domain) with MDM2 (orange peptide) (2FOP) represent protein-peptide transient interactions, mediated by linear motifs predicted from ELM. USP7 interacts with peptides from p53 and MDM2 using the same interface. According to one model (Brooks et al., 2007), USP7 interacts with MDM2 in normal conditions, thereby preventing MDM2 self-ubiquitination and ensuring that it is able to ubiquitinate p53, causing degradation of the latter. However, certain stress conditions reduce the affinity of the USP7-MDM2 interaction through phosphorylation, leading to free USP7 that is then able to deubiquitinate p53. The interaction between the core domain of p53 (blue domain) and 53BP2 (red domain) (2YCS) represents a transient domain-domain interaction, where binding of 53BP2 enhances the DNA-binding and transactivation functions of P53 on the promoters of proapoptotic genes. p53 can also bind with either Cyclin a2 (light green) (1H26) or Sir2 (yellow) (1MA3) using the same region, shown in purple.

other hubs have partners that vary in expression, suggesting that they may bind their partners at different times in a transient manner. These two types of hubs were termed "party" and "date" hubs, respectively.

Based on structural data, hubs have also been classified into singlish (with one or two interfaces) and multi-interface (with more than two interfaces). It has been postulated that singlish interface hubs only bind their partners interchangeably in a transient fashion (Kim et al., 2006; Tuncbag et al., 2009). Another study, exploiting the notion of binding states (the number of complexes the protein can be found in) rather than number of interfaces, proposed yet another classification of hubs. This consists of two main categories: transiently interacting proteins that can be found in several different binding states and proteins that are found in a unique binding state. The latter group includes hubs that are part of large stable complexes (Higurashi et al., 2008).

The Role of Disorder in Transient Interactions of Hubs

Several studies have pointed to the importance of disordered regions in the role of hub proteins. Transiently interacting date hubs have been found to be enriched in disordered residues and show a significantly higher number of disordered regions in comparison with stably interacting party hubs (Singh et al., 2007; Ekman et al., 2006). It has also been shown that, unlike the multi-interface stable hubs, the 'singlish interface' (date-like) transient hubs tend to be enriched in disordered residues compared with the whole proteome (Kim et al., 2008).

When looking specifically at interface surfaces, stable hubs also display an abundance of disordered residues (Higurashi et al., 2008), consistent with the established role of disorder in the self assembly of supramolecular stable complexes (Namba, 2001).

Disorder is also a useful property for date hubs because it allows several LMs to coexist within the same stretch of residues, where it binds to different targets at different times (Gould et al., 2010).

Further evidence supporting the role of intrinsic disorder in transient interactions in PINs comes from comparative interactomics, which indicates that the increase in complexity and extensiveness of PINs along the tree of life, from simple prokaryotes to unicellular and finally higher eukaryotes, is concomitant with an increase in disorder content in their proteome (Ward et al., 2004). A higher level of disorder in complex eukaryotic proteomes may be associated with an increase in transient interactions, perhaps in part to mediate functions particular to metazoan organisms such as intercellular signaling, and an increase in regulation of various biological processes (Levy and Pereira-Leal, 2008; Haynes et al., 2006).

The Role of Transient Interactions in the Dynamics of PINs

PINs are intrinsically dynamic; all interactions within a cell do not occur at the same time and in the same location. Proteins often come together to form large complexes (Schmid and McMahon, 2007). Subsets of protein-protein interactions within these complexes may be transient, and this helps explain the dynamic nature of these complexes; different combinations of these transient subsets allow the complex to form different states. These different states, in turn, affect the function of the complex.

Structural mechanisms such as allostery and cooperativity can play an important role in the dynamics of complex machines. The Clathrin/AP2 endocytic vesicle complex provides a good example of cooperativity. It features a sequence of intermediate oligomeric states (illustrated in Figure 4), ultimately leading to the formation of a mature vesicle (Schmid and McMahon, 2007). The assembly of the vesicle results from a combination of cooperativity and structural flexibility and is mediated by a number of accessory proteins that use transient interactions to help recruit

various components of the complex in a timely fashion (Schmid and McMahon, 2007).

As a final note, it has been suggested that hubs that help bridge the functional modules in PINs engage mostly in dynamic (possibly transient) interactions with partners. This supports the idea that transient interactions may act as a mechanism to organize and regulate the functional flow in protein networks (Komurov and White, 2007).

The Importance of Transient Interactions in Proposed Models of PIN Evolution

Our knowledge of the different types of PPIs has important implications for existing theories on the evolution of PINs. The duplication-divergence model (Ispolatov et al., 2005) proposes that a duplicate protein inherits some or all of the associations from its ancestor. This model may not make sense for obligate complexes as the duplication of a subunit participating in an obligate complex is not desirable as it would lead to a stoichiometric imbalance in the concentration of the individual constituents of the complex (Veitia, 2003). Such a constraint is not observed with transient homomer complexes that were shown to have evolved in higher organisms to incorporate additional copies of their constituent protomers (Nooren and Thornton, 2003a).

Duplication in networks was further examined by Kim et al. (2006), who found that paralogs frequently use the same interface to bind a common partner (i.e., a date hub protein) and rarely interact at different sites, as this would have required coevolution of the hub and the duplicate protein to form a new interface.

The duplication-divergence model (applied to both transient and permanent complexes) draws support from structural observations that suggest that a significant proportion of protein complexes evolve via duplications of proteins involved in homomeric interactions (Pereira-Leal et al., 2007). Such duplications, followed by divergence of one or the other duplicated copy, result in heteromeric interactions of paralogous proteins. More recent studies exploiting additional experimental interaction data and protein family information confirmed this trend but failed to detect the trend in *E. coli*, suggesting specific constraints might be operating in prokaryotic organisms (Reid et al., 2010).

Experimental Detection of Transient PPIs

Owing to their unstable nature, weak PPIs can be technically difficult to study and harder to detect than more stable interactions. The high-throughput yeast two-hybrid (Y2H) screen is able, in principle, to detect binary transient interactions (Shoemaker and Panchenko, 2007a). However, as with other experimental PPI-detection methods, Y2H has its own weaknesses, including a high false-positive rate and attempts to deal with this problem often result in reducing the overall ability of the method to detect transient interactions (Bruckner et al., 2009).

Despite this, Y2H remains the most popular method for large-scale detection of PPIs, owing to its scalable and accessible nature (Bruckner et al., 2009). Recent technological developments of the approach have enabled detection of additional types of interactions, such as those involving cytosolic, membrane-bound, or extracellular proteins (Bruckner et al., 2009). Other research efforts have focused on counteracting the problem of false negatives. For instance, a recent study advised on the benefit of pooling interaction data readouts from repeated Y2H screens in order to achieve higher detection coverage (Venkatesan et al., 2009). This approach was shown to have the additional benefit of highlighting the transient interactions, since these are often only detected by single screens in contrast to the more permanent type of interactions whose stable nature allows them to be detected repeatedly in multiple screens (Vinayagam et al., 2010).

Another recognized high-throughput PPI detection method is tandem affinity purification run in conjunction with mass spectroscopy (TAP-MS) (Collins and Choudhary, 2008). The method has been traditionally unable to detect transient interactions, which are often lost during the washing steps necessary to remove nonspecific binding. However, through the use of chemical crosslinking, it has become possible to freeze transiently formed complexes by inducing covalent-bond formation between interacting partners in vivo (Worthington et al., 2006); further technological developments then allow preservation of the crosslink during the washing phase of TAP (Tagwerker et al., 2006; Stingl et al., 2008).

The application of TAP-MS with crosslinking has been limited to small-scale detection of transiently formed complexes (Tagwerker et al., 2006; Stingl et al., 2008); its large-scale efficacy remains to be demonstrated. Scalability also remains an issue with other technical advances for detecting transient interactions; for instance, the protocol applying single-step affinity purification combined with SILAC (stable isotope-labeled amino acids), which has been recently shown to detect the dynamic components of protein complexes (Mousson et al., 2008).

A functionally important subset of transient interactions is dependent on PTM events. These interactions are often missed when PPI screening is performed in yeast. This limitation has been tackled in different ways, including the use of a mammalian cell culture system, an approach that remains constrained by the troublesome nature of mammalian cell transfection (Bruckner et al., 2009). Among other methods that feature the use of endogenous cell systems for PPI screening, the bimolecular fluorescence complementation (BiFC) assay is worth mentioning, owing to its ability to detect transient (as well as permanent) PPIs in intact cells, eliminating the need for purification (Morell et al., 2007) at a potentially high-throughput rate (Kerppola, 2006). The ability of various experimental techniques to identify transient PPIs is summarized in Figure 6.

Computational Prediction of Transient PPIs

A rigorous assessment of high-throughput as well as literature-curated PPI data has shown that experimental data can be prone to error and are not completely comprehensive (Venkatesan et al., 2009; Braun et al., 2009; Cusick et al., 2009). Therefore, computational methods can be applied to increase confidence and predict interactions currently hidden from the experimental techniques.

There are now many types of computational tools to predict interactions between proteins, including methods that rely on structural data, genomic context information, experimental data, and methods that combine these data sources. Interaction prediction methods have been the subject of several recent reviews (Shoemaker and Panchenko, 2007b; Skrabanek et al., 2008; Valencia and Pazos, 2008). We do not aim to review the

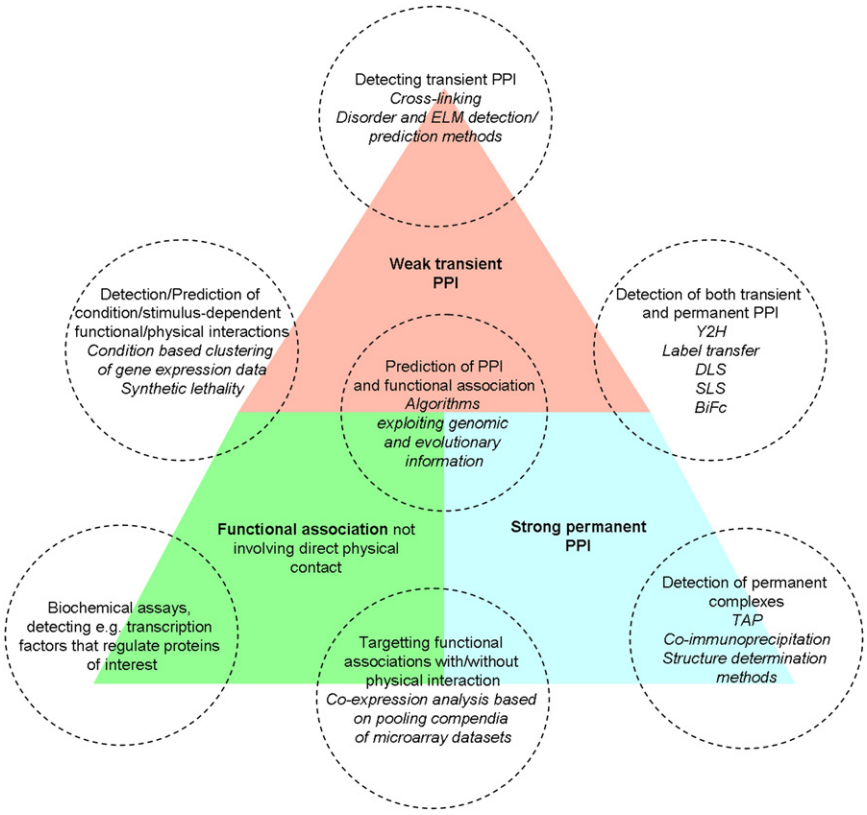

Figure 6. The Different Types of Data Captured by Various PPI Detection and Prediction Methods

Single approaches can be biased and limited, but when taken together they can provide a more comprehensive view of biological networks. BiFC, bimolecular fluorescence complementation; TAP, tandem affinity purification; DLS, dynamic light scattering; SLS, static light scattering.

different approaches here, but briefly consider how these methods can be enhanced by combining them and by including predictions based on experimental data. We will then briefly review methods aimed at predicting peptide-mediated interactions, as these are often involved in transient interactions and have been less extensively covered in the literature.

Computational tools that exploit microarray data can be used for predicting protein interactions. The underlying idea for these approaches is to look for gene coexpression across different conditions, assuming proteins that display similar patterns of expression are more likely to be associated functionally. Although the coexpression-based approach is more suited to predicting strongly correlated stable complexes (Figure 6), the development of statistical frameworks that attempts to find temporal coexpression of genes in a subset of conditions has broadened the applicability of the method to predicting transient interactions (Adler et al., 2009). However, this approach aims to predict functionally associated proteins, not necessarily those that are physically interacting (Figure 6).

Integrating different data sources can deliver significantly increased performance (Jansen et al., 2003; Hwang et al., 2005; Myers and Troyanskaya, 2007; McDowall et al., 2009). These integrated approaches are identifying protein associations not detected by the experimental methods (Brown and Jurisica, 2005). In a recent study, less than 1% of the protein associations predicted using an integrated computational approach were matched by experimental data. The predicted associations not found by the experimental studies included sets of highly connected proteins likely to be involved in signaling and regulation, and therefore likely to be transient (Ranea et al., 2010).

Prediction of Transient Peptide-Mediated PPIs

Current high-throughput experimental techniques for the discovery of protein-protein interactions can show significant underrepresentation of LM mediated peptide-protein interactions. For example, they account for as little as 1% of all putative interactions in some Y2H screens, much lower than the number of interactions they are estimated to account for in vivo (Neduva and Russell, 2006).

Experimental detection of peptide-mediated PPIs is difficult for several reasons. The fact that multiple partners might bind to many regions of the protein being analyzed makes it difficult to determine which peptide is responsible for an interaction with a given partner. In addition, the bound complexes can be difficult to capture because they are involved in transient processes with low affinity interfaces (Diella et al., 2008). Finally, a general problem with any transient interaction is that the conditions under which the interaction is being detected are critical. This is especially true for some peptide-mediated interactions where PTMs of the peptide are important for binding to occur. In order to guide such experiments, a number of computational methods have been developed to predict peptide-protein interactions, the domains involved, and the LMs they interact with.

Computational methods to predict LMs generally search for conserved stretches of residues that may be responsible for binding globular domains. However, this process is very difficult for LMs, as their short length, with sometimes only a few key residues being conserved, means that there can be large numbers of false positives. Biological information, such as cellular compartmentalization of the query protein and whether or not the part of the protein in which the motif is found is ordered, must be taken into account to address this problem. The reader is directed to the recent review by Diella et al. (2008) for a more in-depth description of LM prediction methods.

Structure-based methods to predict potential interactions between LMs and globular domains also exist (see Table 2 for further details and examples). Of particular interest to the structural biologist is PepSite (Petsalaki et al., 2009), which, given a protein-peptide interaction, predicts further details, for example, binding position and orientation.

Table 2. A Selection of Computational Tools for the Prediction and Analysis of Interactions Involving Disordered Regions

Focus	Name	Details	URL
Predict interactions between peptide and domain	ADAN	Database, accessible online. Contains functionally annotated protein domain structures involved in interactions with linear motifs, where possible alongside their peptide ligands. In addition, it performs a structural prediction of the putative ligands for a given domain using a domain if available, or homology modeling if not, and then the FOLDX algorithm, and stores the predictions.	http://adan-embl.ibmc.umh.es
Predict interactions between peptides and domains	PepSite	Software, accessible online. Predicts potential binding areas in peptides that bind to a known, and predicts the orientation and position of the binding sites on the protein.	http://pepsite.embl.de/
Predict interactions between peptides and domains	SMALI	Software, accessible online. Takes as input a peptide, and looks for potential domain binding partners, or takes an SH2 domain as input and looks for potential binding domains, from a user-specified list.	http://lilab.uwo.ca/SMALI.htm
Predict interactions between peptides and domains	FOLDX	Software. Has been used to predict peptide interaction partners for SH2 domains, and also is employed by ADAN to predict partner domains.	http://foldx.crg.es/
Viewing peptide-protein interactions	3DID	Database, accessible online. Contains high resolution solved structures of domain-domain protein interactions, and currently (as of September 2010) 2345 structure of domain-motif interactions. The interactions were taken from the protein databank by taking all PDB entries containing at least two interacting proteins and filtering these entries using information from the protein families database (Pfam) and data on known protein binding linear motifs from the Eukaryotic Linear Motif database.	http://3did.irbbarcelona.org/
Viewing peptide-protein interactions	PEPX	Database, accessible online. Contains protein-peptide complexes. Browseable by several methods including CATH domain superfamily. To populate the database, the PDB was mined to find 1431 protein-peptide complexes with peptides of length 35 residues or less, which were clustered using three-dimensional similarity of the protein-peptide interface.	http://pepx.switchlab.org/
Detection of disordered regions involved in interactions	ANCHOR	Server uses disorder prediction methods (specifically IUPred) and identifies potential binding sites where there is a dip in predicted disorder	http://anchor.enzim.hu/
Novel motif detection	DiLiMot	Server. Takes a set of protein sequence that carry a common feature and looks for consensus motifs between the sequences.	http://dilimot.embl.de/
Novel motif detection	D-motif/D-star	Software (downloadable). Uses interaction data alongside sequence information to improve results and reduce false positives	http://www.comp.nus.edu.sg/~bioinfo/hugowill/DSTAR.html
Find already known motif	ELM	Database and server. An ELM motif is a regular expression that describes an LM. The database allows the different instances of the motifs to be browsed, and links to experimental methods used to determine the instance, and also gives a quality score. When searching the database with a query set of proteins in order to predict LMs in a query protein, ELM uses a number of filtering steps to reduce false positives and ensure only biologically plausible instances are returned	http://elm.eu.org/
Find already known motif	Scansite	Database and server, for a number of domains, predicts potential interactors with domains involved in signaling, important for identifying potential transient interactors. User can search with own motif or use predetermined ones within Scansite.	http://scansite.mit.edu/

While much progress has been made on the prediction of interactions mediated by disordered regions, it must be stressed that most prediction methods suffer from a high rate of false positives. Some increased confidence might be gained by combining different methods, but the predictions should be validated experimentally. Computational predictions confirmed experimentally have been demonstrated in the detection of peptides that modulate platelet function (Edwards et al., 2007), as well as peptides that interact specifically with different basic-region leucine zipper transcription factors (Grigoryan et al., 2009).

Conclusion

Transient protein-protein interactions perform essential functional roles in biological systems, notably in regulating the dynamics of biological networks. However they are not easy to detect experimentally. Obtaining high-resolution three-dimensional structures of transient complexes is challenging. The efficacy of X-ray crystallography for this purpose is limited, owing to the difficulty in crystallizing weak interactions, and NMR is therefore the best candidate for characterizing structures of transient complexes (Vaynberg and Qin, 2006). NMR is constrained in the size of the complexes it can deal with, but a number of technological advances have reduced that limitation (Bonvin et al., 2005) and more and more structures of transient protein-protein interactions are being solved (Vaynberg and Qin, 2006). NMR has certainly been used successfully for solving the structures of several domain-peptide interactions (Shi and Wu, 2007), and the fact that many transient interactions are mediated by such interactions between domains and small peptides suggests that NMR has the potential to provide very useful structural insights into transient PPIs in general. Stabilization of weak interactions by chemical crosslinking has also been used in combination with other approaches such as mass spectrometry to obtain insights into the structures of transient PPIs (Singh et al., 2010).

Structural information can also be inherited between proteins exploiting the same interface peptide region on a common partner. For example, if domain A is able to interact with two different domains, B and C, but is interacting with both of them via the same peptide on their surface, solving the structure of the complex of domain A with the interacting peptide from B might be sufficient to give insights into the interaction mechanism between both A-B and A-C (see also Bravo and Aloy, [2006]).

Computational approaches for predicting protein interactions can prove very helpful in extending the known repertoire of transient interactions. Several recent discoveries and approaches have emerged that have boosted our ability to predict transient PPI. For example, with regards to characterizing the protein surfaces involved in interactions, the increasing numbers of structures of protein complexes in the PDB have helped identify distinguishing properties of interfaces in transient interactions between globular domains (Nooren and Thornton, 2003b) and have allowed the development of automated methods to predict transient interactions from structural data with some success (see for example Block et al., [2006]).

It is becoming clear that interactions involving disordered regions or LMs play an extremely important role in PINs, and that these interactions are very often transient and involved in regulation of cell processes. These types of interactions are particularly important in higher organisms, and understanding them better may have important implications, notably for our understanding of disease and for the discovery of new drug targets (Russell and Gibson, 2008; Neduva and Russell, 2006). Computational and experimental approaches are already being developed to recognize these motifs.

The emerging picture of the cell is one in which weak interactions between biomolecules are made possible by the existence of molecular scaffolds close to which biomolecules can be found in high concentrations, thereby promoting interactions despite their weak affinity (Gibson, 2009). In this context, it is likely that transient interactions will be found to play an even more important role than is currently understood.

Methods to identify transient interactions between proteins can only be improved if we continue to refine our understanding of the properties of such interactions at the structural, sequential, and systems biology levels. This process will be facilitated by the accumulation of interaction data from systems biology and structural biology. Continuous development of technologies that are fine-tuned for the detection of weak protein interactions will help us gain further knowledge about the structural basis of transient interactions and will improve our ability to computationally predict them.

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