FEBS Letters 567 (2004) 74–79 FEBS 28391

Minireview

Selectivity and promiscuity in the interaction network mediated by protein recognition modules

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Received 19 March 2004; accepted 22 March 2004

Available online 5 May 2004

Edited by Horst Feldmann

Abstract A substantial fraction of protein interactions in the cell is mediated by families of protein modules binding to relatively short linear peptides. Many of these interactions have a high dissociation constant and are therefore suitable for supporting the formation of dynamic complexes that are assembled and disassembled during signal transduction. Extensive work in the past decade has shown that, although member domains within a family have some degree of intrinsic peptide recognition specificity, the derived interaction networks display substantial promiscuity. We review here recent advances in the methods for deriving the portion of the protein network mediated by these domain families and discuss how specific biological outputs could emerge in vivo despite the observed promiscuity in peptide recognition in vitro.

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Keywords: Protein interaction; SH3; Protein domains; Interaction networks

1. Introduction

The cell is a complex but organized mesh whose principal components are the proteins encoded by the organism's genome. Understanding how this intricate network supports cell physiology and how its disruption may cause a pathological response is one of the primary goals of proteomics and functional genomics. Ideally, we would like to draw a map depicting all the interactions in the cell and trace the functional pathways along the lines connecting the proteins. Recent genome wide analysis of the interactomes of several model organisms has revealed a more intricate picture and has shown our inadequacy to interpret these data [1–6]. The picture is further complicated when we consider that the wiring between the components of the molecular circuit is dynamic: while some complexes are relatively stable, other functional components associate and dissociate quite dynamically with changing environmental conditions. Understanding the rules that govern protein recognition specificity would clearly enhance our ability to infer and interpret the formation of physiological protein complexes.

The biochemical, structural or regulatory functions of the gene products that form the nodes in this network are determined by the polypeptide three-dimensional conformations which in turn impact on their ability to interact with partner proteins.

Structural analysis of functional protein complexes is consistent with the notion that many proteins interact via extended surfaces, including residues that are far apart in the protein primary structure, and only come together upon protein folding. In these cases, it is practically impossible to map the binding determinants to short peptides that match the amino acid sequences of either partners. As a consequence these interactions cannot be inferred on the basis of the partners' primary sequence. Instead, one must rely on solid experimental evidence or sophisticated, computationally intensive and often unreliable docking algorithms.

By contrast, a second class of interactions is asymmetric in nature, with a domain of partner A acting as a receptor for a peptide in partner B. Pioneering work on the Src kinase in the late 1980s and early 1990s demonstrated for the first time the importance of relatively small protein recognition domains in mediating the formation of protein complexes by binding to short linear peptides [7–10]. The Src homology 2 (SH2) [7] and 3 (SH3) [8] domains were originally discovered as homologous regions in proteins involved in signaling. Soon afterward, a number of reports indicated that SH2 domains would mediate the binding to proteins phosphorylated in tyrosines, while SH3 domains showed an affinity for peptides rich in prolines. Most importantly, it was found that both domains bind to their partner proteins even when the partners were denatured on filters implying that the recognized target was a linear determinant on the protein [11].

2. Families of protein modules binding short linear peptides

SH3 and SH2 domains are the prototypes of a growing number of domain families that share the property of binding to relatively short extended peptides. Since their discovery, one of the major themes in the past 15 years has been the realization of the importance of protein interaction domains as distinct modules that determine the interaction partners of various signaling proteins.

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Abbreviations: SH3, src homology 3; SH2, src homology 2; WISE, whole interactome scanning experiment

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doi:10.1016/j.febslet.2004.03.116

Table 1
Abundance of protein recognition domains in different model proteomes

Domain	*S. cerevisiae*	*C. elegans*	*D. melanogaster*	*M. musculus*	*H. sapiens*	Target motifs[a]
WW	9	47	52	111	125	(L/P)Pp(Y/poY) PPLPp (p/ϕ)P(p/g)PppR (p/ϕ)PP(R/K)gpPp (*po*S/*po*T)P (p/ϕ)PPPPP [13,14]
WH1	1	7	9	15	13	PPxx(F/Y) (D/E)FPxϕP [15]
VHS	4	6	5	14	13	DxxLL [16]
SH3	27	95	202	407	409	(R/K)xϕPxϕP ψPxϕPx(R/K) [17]
SH2	1	76	62	157	139	*po*YxE/N *po*Y(I/V)x(V/I/L/P) *po*YxxQ Yϕ G*po*Y(K/Q)xF [18]
Polo-Box	2	6	2	7	8	(ϕ/P)(ϕ/Q)(T/Q/H/M)S(*po*T)P [19]
BRCT	18	44	49	39	56	*po*Sx(I//V/T)(F/Y) [20,21]
PTB	0	32	23	63	61	Npx(Y/*po*Y) [22]
PDZ	3	127	214	363	356	(S/T)x(V/I/L) (V/Y/F)x(V/I/L)$ (D/E)x(I/V/L)[23]
GYF	3	3	1	4	4	(R)xxPPGxR [24]
FHA	14	15	25	32	29	*po*Txx(I/L/A) [25]
EH	15	16	22	25	29	NPF [26,27]
14-3-3	3	3	5	13	8	R(S/F/Y/W)x *po*SxP Rxxx(*po*S/*po*T)xP [28]
FF	5	33	9	31	29	*po*S[29]
CHROMO	5	30	32	61	47	*Me*K [30]
BROMO	14	29	50	74	99	*Ac*K [31]

[a] Target motifs are represented according to the Seefeld convention [32].

The recent completion of the characterization of the genomes of several model organisms has made it possible to analyze the distribution of these domain families in complete proteomes. In Table 1 we have reported the abundance of the most common and best-characterized protein recognition modules in five proteomes from *Saccharomyces cerevisiae* to *Homo sapiens*. For the *S. cerevisiae*, *Caenorhabditis elegans* and *Drosophila melanogaster* proteomes, these figures were obtained by querying the SwissProt/Trembl database [12]. As a consequence, because of the redundancy of this database, the domain abundances could be slightly overestimated. In the case of the human and mouse proteomes, we made use of the International Protein Index which provides a minimally redundant yet maximally complete set of human, mouse and rat proteins (http://www.ebi.ac.uk/IPI/IPIhelp.html).

As reported in the last column to the right, each domain family binds to relatively short peptides sharing specific sequence motifs. For instance, FHA domains bind to peptides containing phosphorylated threonines, while WW domains form complexes with peptides containing various proline rich motifs. Within each family recognition specificity is modulated by subtle changes in the domain binding surface that result in preference for peptides in which the core recognition motif is embedded in a different sequence context.

3. Experimental methods to derive protein interaction networks

In the past decade, several methods have been developed to help assembling protein interaction networks [33]. A

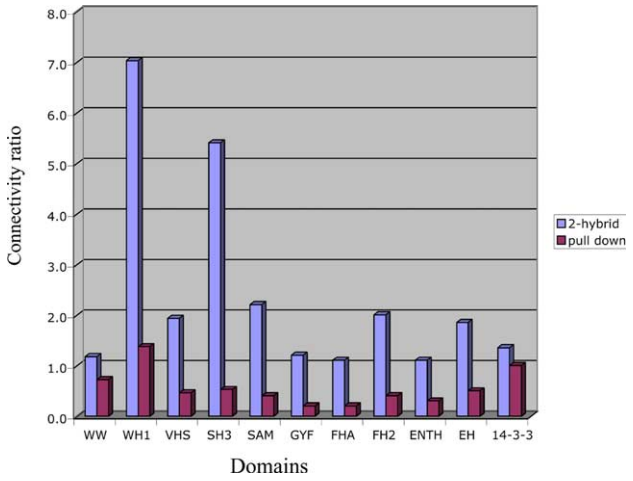

Fig. 1. Comparison of pull-down and yeast two-hybrid methods. The bars represent the ratio between the connectivity of proteins that contain protein interaction domains and the connectivity of all proteins in the yeast proteome. The results obtained with the yeast two-hybrid and the pull-down methods are represented with blue and red bars, respectively.

comprehensive list is beyond the scope of this short review. For simplicity we distinguish here two broad classes. The first one includes methods that we call analytical. They aim at identifying complexes that can be purified from cell extracts. Typically, a cell culture or a tissue is homogenized and after solubilization of the cell membranes one protein is purified by affinity chromatography. The proteins that form a complex with the bait protein co-purify and are identified either with specific antibodies or by mass spectrometry. Since this method aims at the identification of partners in vivo, in physiological conditions, the complexes that are characterized offer immediate biological insights. The recent success in the characterization of a large fraction of the *S. cerevisiae* interactome [3,4] and in the elucidation of a physical and functional map of the human TNF-α/NF-κB signal transduction pathway [34] has demonstrated the power of this approach when applied in a high throughput fashion. However, the concern is that only relatively abundant proteins forming tight complexes are identified by this methodology. It is conceivable, for instance, that interactions mediated by families of protein modules, which typically have affinity in the 1–50 μM range, are not detected by this technology.

A second class consists of methods (synthetic), including the yeast two-hybrid, phage display, protein/peptide chips, etc., that are usually carried out either in vitro or in a "non-native" in vivo context. Their goal is revealing the binding potential of an entire proteome by enumerating all the possible biochemically significant protein pairs. In essence, each protein is tested against any other element of the proteome either in a library or in an array format. These methods, and more specifically the yeast two-hybrid, have also been shown to be amenable to automation and to a high throughput format [1,2,5,6]. Two-hybrid and related genetic methods have a higher sensitivity and typically interactions in the 10 μM range can easily be detected. On the other hand, they are affected by a relative large number of artifacts (false positives and false negatives) and do not provide information about the biological relevance of the detected interaction.

To ask whether these two classes of methods, analytical and synthetic, could reveal different interactions subsets, we have analyzed a large network of more than 36 000 interactions extracted from the MINT database [35]. Although MINT also contains entries gathered from articles describing low throughput experiments, for this analysis we have only considered high throughput datasets to avoid any bias that might be introduced by the curators in the selection of the experiments to be entered in the database.

In order to obtain a rough estimate of the contribution of proteins containing interaction domains to the establishment of the protein networks, we have calculated the average number of interactions of proteins containing at least one of the domains in Table 1. While the average number of interactions per protein is 4.3 when all the proteins are considered, this figure rises to 5.7 when only proteins containing interaction domains are counted. More strikingly, interactions in which one of the two partners contains at least one of the protein binding modules are more frequent in the two-hybrid than in the co-immunoprecipitation dataset (Fig. 1) implying that the two-hybrid method detects more domain-mediated interactions.

This analysis demonstrates that different methods have different potential and that a complete description of the biologically relevant interactions occurring in a cell will benefit from a combination of approaches that use both analytical and synthetic methods.

4. Combining different approaches

Synthetic methods, being more sensitive, are better suited to revealing potential interactions falling in the 10 μM range that is typical of most protein interactions modules. On the other hand, high sensitivity is often accompanied by a high noise level and, as a consequence any single interaction in datasets obtained by synthetic methods must be considered with caution [36]. However, by combining the information obtained with two "orthogonal" (that is, fundamentally different) approaches it should be possible to lower and eventually to eliminate the noise. We have demonstrated the power of this strategy by applying two different synthetic approaches to the definition of the yeast protein network mediated by the family of SH3 domains and by considering the intersection of the two different interactomes. The first network was derived from screening phage-display peptide libraries to find *consensus* sequences in peptides that bind to SH3 domains. The resulting network connects SH3 domain proteins to those containing the *consensus* peptide. The second network is derived by applying the yeast two-hybrid technique to the search of proteins that have the potential to associate with SH3 domains.

The proposed strategy involved four steps:

(i) Screen random peptide libraries by phage display to define the *consensus* sequences for preferred ligands that bind to each peptide recognition module.

(ii) Develop a predictive algorithm to identify domain binding-partners in the proteome, on the basis of these *consensus* sequences, or the list of selected ligands. This leads to a protein–protein interaction network obtained by linking each protein containing an SH3 domain to partner proteins

containing a peptide that match the sequence of the *consensus* ligand.

(iii) Use the yeast two-hybrid system to experimentally derive a protein–protein interaction network by testing each peptide recognition module for association to each protein of the proteome.

(iv) Determine computationally the intersection of the inferred and experimental networks and test the biological relevance of key interactions within this set.

Since phage display and two-hybrid are two "orthogonal" experimental approaches, the noise created by the detection of false positives originates from different technical artifacts. Thus, by considering only the intersection of the two networks, that is the interactions inferred by both methods, the noise is considerably reduced. In fact Tong et al. were able to show that nine out of nine interactions, centered on the protein Las17, in the resultant network can be proved to occur in living yeast cells and may therefore be physiologically relevant.

One of the conclusions of this approach is that the inferred number of ligands for each SH3 domain is larger than anticipated. This finding is somewhat disturbing since it is difficult to reconcile with the view that specific cell responses are the result of the precise wiring of the proteins in the cell.

It should be pointed out that phage display and two-hybrid only address the problem of identifying proteins with the potential for binding to any given recognition domain. Although we use this information to infer the formation of protein complexes in vivo, there are a number of reasons why this inference could turn out to be incorrect. For instance, a potential peptide target could be unavailable for interaction in the native protein structural context. Alternatively, the two inferred partners could be located in different cell compartments or expressed in different tissues or at different times during development. In other words in vivo spatial and temporal segregation mechanisms could serve to increase the specificity of the network.

These caveats considered the observation that nine out of nine tested interactions were found to be correctly predicted still suggests that, at least in *S. cerevisiae*, a large fraction of pairs of proteins with the in vitro "biochemical potential" to form a complex can be found associated in vivo.

5. Specificity and promiscuity

We usually represent the results of our experiments aimed at deciphering biological pathways with cartoons containing interaction diagrams that are typically linear or, at most, contain few ramifications. This is convenient and helps us to formulate simple hypotheses, although we are aware that this simplification might be unrealistic. On the other hand, graphic representations of protein interaction networks, derived from high throughput experiments, are highly intricate and pathways can hardly be discerned.

One of the central problems is whether our interpretation of biological phenomena should necessarily rely on a cell model involving specific pair-wise protein interactions or whether an alternative model based on a dynamic equilibrium between equivalent, relatively low specificity and low affinity, interactions could eventually explain the observed specific biological outputs. The apparent paradox of protein recognition specificity in interactions mediated by protein recognition modules was discussed by Ladbury and Arold [37] and by Mayer [38] in two interesting reviews. According to one view, domains of the same family have diverged sufficiently to support protein interaction network of high specificity. However, as we have discussed, the characterization of a large number of interactions mediated by families of protein interaction modules has revealed that most domains bind to a large number of targets with comparable affinities. Furthermore, the regions of peptide sequence-space recognized by different domains of the same family often overlap.

This insufficient inherent specificity seems to require different mechanisms to account for specific biological outputs. As already mentioned, selectivity could be achieved by compartmentalization of the prospective partners or by the cooperative effect of multiple interactions.

Lim and co-workers [39] have recently suggested that interaction specificity could be accomplished by a combination of positive and negative selection acting on the sequence of a physiological peptide ligand. They have analyzed in detail the interaction between the SH3 domain of Sho1 and the protein Pbs2, interaction that is required for the high-osmolarity glycerol pathway in baker's yeast. By testing hybrid molecules in which the Sho1 SH3 was substituted by any of the remaining yeast SH3 domains, they found that the sequence of the Pbs2 proline-rich peptide target is absolutely selective for the Sho1 SH3 domain. The authors suggest that the amino acids flanking the PxxP core motif have evolved to be recognized specifically by the Sho1 SH3 domain and not by the other yeast SH3 domains. Moreover, experiments with mutant domains indicated that this peptide is not optimized for affinity but rather for specificity. In other words, although Sho1 SH3 has a recognition profile similar to the one of the remaining yeast SH3 domains, the high specificity of the Sho1–Pbs2 interaction is achieved by the ligand exploiting niches of the sequence space that are not covered by other competing SH3 domains.

The generality of this concept and its extension to other SH3 domains or to domains of different families remains to be established. More generally, we would like to know all the potential partners of each protein interaction domain and quantitatively analyze the overlap between the different sets of targets.

6. Peptide scanning of the proteome

A couple of recent reports have shown that questions of this type can now be answered [40,41]. Utilizing slightly different strategies, these two approaches exploit the power of parallel peptide synthesis to identify all the peptides in a proteome that can bind to an SH3 or a WW domain, respectively.

Langraft et al. have developed a new strategy, named WISE (whole interactome scanning experiment), that permits rapid and reliable identification of the partners of any peptide recognition module by peptide scanning of a proteome. This was achieved by a combination of phage display and Spot synthesis [42] and was applied to the discovery of all the peptides in the yeast proteome that have the potential to bind to eight SH3 domains. They first identified the peptides in the proteome that match a relaxed consensus, as deduced from peptides selected by phage display experiments. Next, they synthesized all the matching peptides at high density on a cellulose membrane and

probed them directly with the SH3 domains fused to glutathione *S*-transferase. The Spot synthesis approach is semi-quantitative and spot intensities correlate with the dissociation constants of the several thousands interactions that are simultaneously analyzed in an array format. Therefore, the edges of the derived interaction networks can be associated to a figure that enables us to estimate the likelihood of each interaction occurring in any given physiological settings. Most importantly it was possible to show that, among the six inferred ligands of the Abp1 SH3 domain having a dissociation constant lower than 50 μM, two were peptides of well established physiological partners of Abp1. The remaining four could also be shown to be part of proteins that associate with Abp1 in vivo. These findings lend further support to the observation that a large fraction of natural peptides with the biochemical potential to bind to any given SH3 domain is actually used in vivo to mediate the formation of a complex.

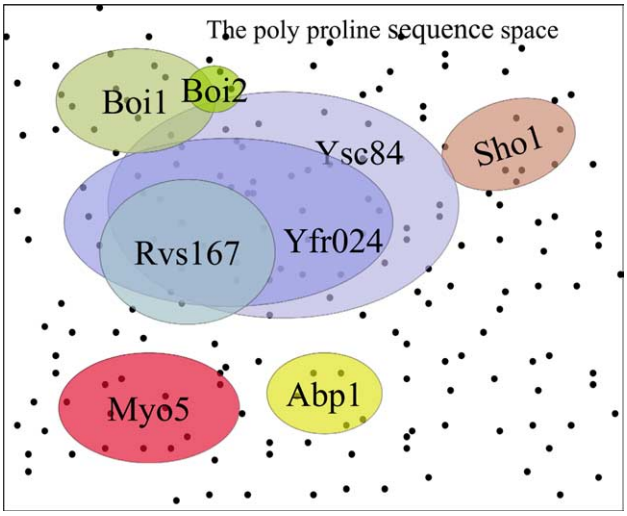

Fig. 2. Schematic representation of the polyproline peptide space. Each black dot represents a proline rich peptide that has the potential to bind an SH3 domain. The ensemble of peptides that bind to a specific SH3 domain with an affinity above an arbitrary threshold of 50 μM is enclosed in an oval. Data are from Langraft et al. [40].

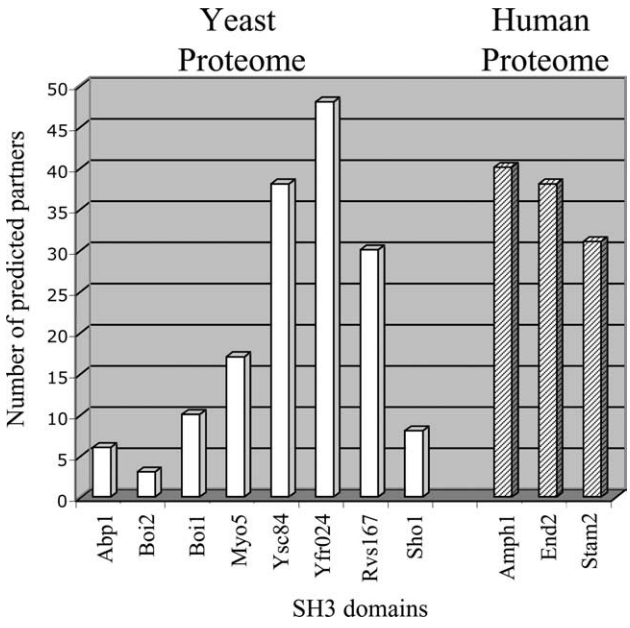

Fig. 3. SH3 peptide targets. The bars represent the number of "proteome peptides" that bind to 11 SH3 domains with an estimated dissociation constant lower that 100 μM. The yeast SH3 domains (left) were tested against approximately 1500 peptides from the yeast proteome, while the human SH3 domains were tested with approximately 3000 peptides extracted from the human proteome.

The domains that were tested in this study could be grouped by this approach into five classes with partially overlapping specificity. Within one class, however, the domains (Ysc84, Rvs167 and Yfr024c) display high promiscuity and bind to a large number of common targets with comparable affinity. These results are schematically represented in Fig. 2. In order to have a global picture of promiscuity and specificity in the yeast network interweaved by the SH3 domain family, we must await the complete characterization of the full set of SH3 domains.

WISE can also be used to estimate the number of peptides that bind with affinity above any given threshold to protein binding modules (Fig. 3). We estimate that the yeast proteome contains as few as six peptides that bind to the Abp1 SH3 domain with a dissociation constant lower than 100 μM, while it contains as many as 50–80 peptides with corresponding affinity for the SH3 domain of Yfr024c. Similar figures were obtained in the experiment that aimed at characterizing the map of the WW domain family interaction [41].

7. Conclusions

Peptide recognition modules are functionally independent domains found widespread in eukaryotic proteomes. Interactions mediated by these domains have been implicated in important functions in practically all domains of cell biology. Although a variety of approaches have contributed to revealing some degree of intrinsic specificity of different members within a domain family, it has become apparent that this is not sufficient to support on its own a cell model where the observed specific biological outputs are guaranteed by a highly specific wiring of the protein interaction network.

Spatial and temporal compartmentalization, additive effects of multiple separate interactions and the selection for target/domain pairs representing a specific combination within the context of the sequence space contained in a proteome are often invoked as mechanisms that could ensure a further level of selectivity in vivo. Alternatively, we should be ready to accept that, at least in some cases, some degree of promiscuity, appropriately directed by natural selection, could confer robustness on some cell organizing mechanism.

The recent development of strategies to scan an entire proteome in search of binding peptides for SH3 and WW domains is likely to produce over the next few years a wealth of binding data. WISE and related approaches can be easily extended to a variety of protein interaction domains, including those binding to modified peptides, thereby offering a new powerful proteomic tool to help in completing a full description of the cell interactome. This combined with results from global analyses of protein expression and localization [43,44] should soon put us in the position to answer very general questions about specificity and promiscuity in protein networks.

Acknowledgements: This work was supported by FP6 contract No. 505520 (Interaction Proteome), AIRC (Associazione Italiana per la Ricerca sul Cancro) and MIUR (Italian Ministry for University and Research).

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