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ORIGINAL ARTICLE

Enriching the viral–host interactomes with interactions mediated by SH3 domains

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Abstract Protein–protein interactions play an essential role in the regulation of most cellular processes. The process of viral infection is no exception and many viral pathogenic strategies involve targeting and perturbing host–protein interactions. The characterization of the host protein subnetworks disturbed by invading viruses is a major goal of viral research and may contribute to reveal fundamental biological mechanisms and to identify new therapeutic strategies. To assist in this approach, we have developed a database, VirusMINT, which stores in a structured format most of the published interactions between viral and host proteome. Although SH3 are the most ubiquitous and abundant class of protein binding modules, VirusMINT contains only a few interactions mediated by this domain class. To overcome this limitation, we have applied the whole interactome scanning experiment approach to identify interactions between 15 human SH3 domains and viral proline-rich peptides of two oncogenic viruses, human papillomavirus type 16 and human adenovirus A type 12. This approach identifies 114 new potential interactions between the human SH3 domains and proline-rich regions of the two viral proteomes.

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Introduction

Viruses modify cell physiology and its regulatory networks by manipulating the critical nodes of the underlying cell machinery to gain control of fundamental processes, such as apoptosis, cell cycle, DNA replication and cell proliferation, as a propagation strategy. Understanding how viral proteins interfere with cellular protein interaction networks is therefore essential to explain in detail the molecular mechanism of viral infection and to develop efficient antiviral strategies. In addition, identifying the cellular targets of viral proteins may contribute to reveal new key cell regulators. A few examples of viruses redirecting the cellular metabolism by interfering with key proteins in fundamental cellular processes have already been described with molecular details. For example, human adenovirus E1a and E1b proteins target cell cycle regulator protein pRb (retinoblastoma) and the genome guardian protein p53. The same crucial proteins are targets of HPV (human papilloma virus), polyomavirus, HHV-8 (human herpes virus), HCMV (human cytomegalovirus) and others (Dyson et al. 1992; Lazzi et al. 2002; Lechner and Laimins 1994; Levine et al. 1991).

Our approach aims at studying the network of interactions between viral and host proteins. To this end, we use two independent approaches. On one hand, we extract from literature and deposit in databases all the published information. On the other hand, we use peptide technology to identify, in a high throughput approach, novel interactions

mediated by protein interaction domains. This twofold strategy is based on the hypothesis that subversion of cell physiology mediated by viral infection is mediated by alterations of the network of cellular gene products and metabolites. Such networks are responsible for emergent properties that cannot be understood by analyzing, with a reductionist approach, one protein and one interaction at a time.

Many protein complexes are stabilized by families of relatively small domains that specialize in binding to relatively short extended peptides. Each domain family is characterized by a preference for specific sequence or structural features in the target peptide. Protein domains recognizing proline-rich sequences play a pivotal role in biological processes requiring the coordinated assembly of multi-protein complexes.

The superfamily of proline-rich sequence recognition domains consists of the SH3 (Src homology 3) (Feng et al. 1994; Yu et al. 1994), the WW (Macias et al. 1996), the EVH1/WH1 (Ena/VASP Homology domain 1) (Reinhard et al. 1995), the GYF (Glycine-tYrosine-PHenylalanine) (Nishizawa et al. 1998), the UEV (Ubiquitin E2 Variant) (Pornillos et al. 2002) and profilin (Schutt et al. 1993) domain families. The proline-rich target peptides recognized by these protein interaction modules generally contains several consecutive proline residues, some of which are absolutely necessary for binding. The secondary structure adopted by poly-proline sequences is a left-handed poly-proline II (PPII) helix, a mobile structure often found on protein surfaces (Adzhubei and Sternberg 1993; Stapley and Creamer 1999) or in unstructured protein domains (Williamson 1994).

SH3 domains are independently folding small protein interaction modules of about 60–70 residues found in a large number of proteins involved in several cellular processes (Li 2005; Mayer 2001; Zarrinpar et al. 2003). SH3 domains fold as a β-barrel formed by five or six β-strands, which are arranged into two perpendicular β-sheets. A hydrophobic groove accommodates the PxxP motif present in the ligand (Musacchio et al. 1992; Yu et al. 1992).

Searching VirusMINT (http://mint.bio.uniroma2.it/virusmint/), a database that collects protein interactions between viral and human proteins reported in the literature, we observed that the interactions involving virus proteins and SH3 domain are underrepresented in the database. To fill this gap, we applied the WISE (whole interactome scanning experiment) technique (Landgraf et al. 2004) to identify interactions between 15 human SH3 proteins and viral proline-rich sequences from oncogenic HPV (human papilloma virus type 16) and Ad12 (human adenovirus A type 12). By this approach, we identified 114 new potential interactions between human SH3 domains and the proline-rich regions of the two viral proteomes.

Results and discussion

VirusMINT developments

The VirusMINT database aims at annotating in a structured format all the interactions between human and viral proteins and at integrating this information in the human protein interaction network. Since its first description (Chatr-aryamontri et al. 2009), VirusMINT has acquired an upgraded user interface and improved algorithms have been developed for automatically grouping interactions reported for the same proteins in different viral isolates. In addition, the continuing curation effort has resulted in an increase of the stored interaction information. A screenshot of the human adenovirus interactome, as viewed at the VirusMINT homepage, is shown in Fig. 1.

The VirusMINT database contains interaction data for 418 proteins encoded by 99 different viral strains, corresponding to 1,854 unique interactions supported by 5,140 experimental evidences resulting from more than 1,568 reviewed articles. The initial focus has been on curation of manuscripts describing interactions of viral proteins encoded by the main medically relevant viruses: adenovirus, simian virus 40 (SV40), papillomavirus, Epstein–Barr virus (EBV), hepatitis B virus(HBV), hepatitis C (HCV), herpes virus, human Immunodeficiency virus (HIV), influenza A virus and vaccinia virus. The VirusMINT data set is freely accessible without limitations and can be downloaded in two formats: as tab delimited flat files and in the PSI-2.5 XML format.

SH3-mediated interactions in VirusMINT

More than 200 proteins containing at least one SH3 domain in the human proteome are involved in numerous cellular processes and represent a large and diverse target for viral replication strategies. However, these interactions are underrepresented in VirusMINT (1.8%) when compared with the SH3-mediated interactions (6.9%) in the human interactome that is annotated in the MINT database (MINT, http://mint.bio.uniroma2.it/mint/) (Chatr-aryamontri et al. 2007).

Since very little is known about the viral proteins that have the potential to interact with SH3 domains, we set out to demonstrate the feasibility of using the WISE approach to discover novel putative interactions between human SH3 proteins and viral proline-rich sequences of oncogenic HPV and Ad12.

Identification of potential targets of SH3 domains in viral proteins

The WISE approach consists in the definition of a number of regular expressions covering all the motifs recognized by the members of a given domain family (obtained, for

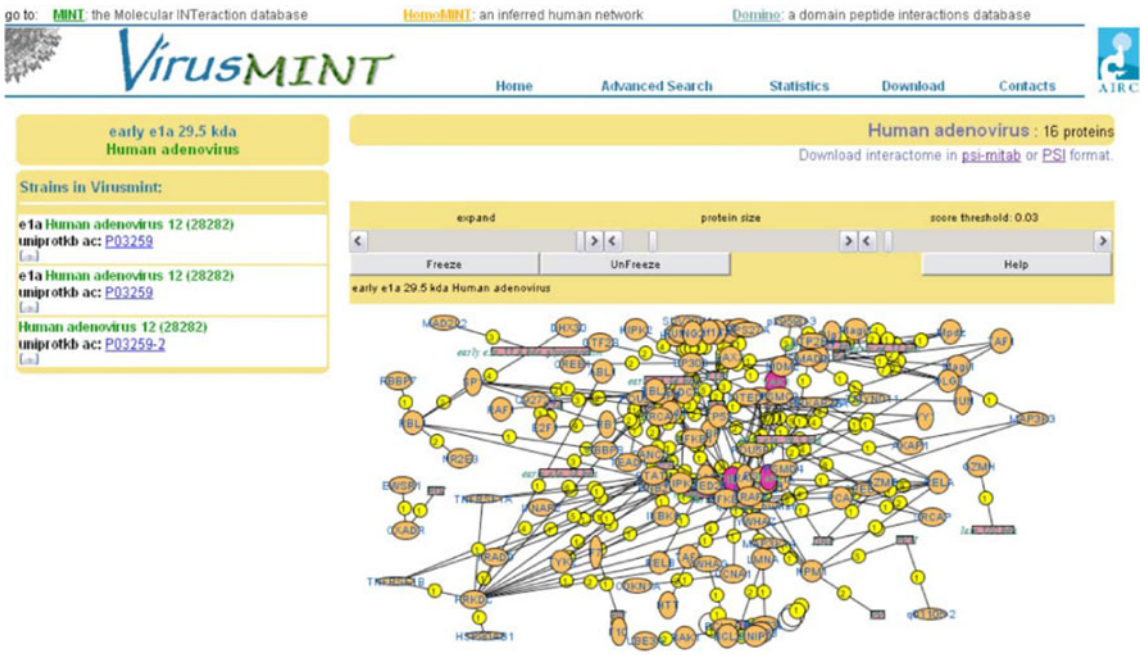

Fig. 1 Screenshot of the human adenovirus interactome. VirusMINT provides an innovative graphic display to visualize a full virus interactome. This function is available both from the Homepage and from the Advanced Search Page. The interactome viewer displays both virus–virus and virus–host interactions

instance, by phage display experiments or from the literature) followed by an informatic scanning of the whole proteome in search of peptides matching the regular expressions. Finally, the putative ligands are experimentally validated by probing peptides synthesized by spatially addressed synthesis on a cellulose membrane.

In particular, we used a list of 21 regular expressions covering most of the SH3 recognition consensus described till now. This list was compiled by combining the results of published and unpublished experiments mostly based on phage display (see Supplementary Table 1). We next searched matching peptides in the HBV, SV40, adenovirus and HPV viral proteomes. Only adenovirus A type 12 and papilloma type 16 viral proteomes were found to contain matching proline-rich peptides (see Supplementary Table 2).

Human adenovirus type 12 proteome has 14 proteins with proline-rich regions: E1A, E1B, maturation proteins and hexon-associated protein (one peptide each); peripentonal hexon-associated protein, a late 100-KD protein, fiber protein and E3 13-kDa protein (two peptides each); E3B 14.5-KD protein, protein VI (three peptides each); penton protein and early E2A DNA-binding protein (four peptides each); DNA polymerase (five partially overlapping peptides); DNA terminal protein (seven partially overlapping peptides). Papilloma type 16 proteome contains only three proteins with proline-rich regions: major capsid protein (two peptides), E4 (three partially overlapping peptides) and the minor capsid protein.

The selected 46 viral proline-rich peptides were synthesized on cellulose membranes by the SPOT synthesis technique and probed with GST fusions to 15 human SH3 domains that, according to our unpublished studies (Cardducci et al. in preparation), were representative of discrete specificity classes. The corresponding proteins have largely different molecular functions: ARHGAP12 is a rho-GTPase activator, ARGBP2a is an adapter protein that plays a role in the assembling of signaling complexes, being a link between ABL kinases and actin cytoskeleton (Soubeyran et al. 2003; Yuan et al. 2005); CAP plays a role in tyrosine phosphorylation of CBL by linking CBL to the insulin receptor (Ribon et al. 1998); DDEF2 is a small GTPase activator (Andreev et al. 1999); Grb2 is an adapter protein that provides a critical link between cell surface growth factor receptors and the Ras signaling pathway (Lowenstein et al. 1992); HCK is a protein tyrosine kinase that may interact (via SH3 domain) with HIV-1 Nef and Vif (Briggs et al. 1997; Hassaine et al. 2001); NCK1 is an adapter protein, which associates with tyrosine-phosphorylated growth factor receptors or their cellular substrates (Latreille and Larose 2006); Nephrocystin-1 (NPHP1) through its SH3 domain binds to Cas and together may play a role in the control of epithelial cell polarity (Donaldson et al. 2000); Pacsin3 (PACN3) binds to endocytic proteins and inhibits endocytosis (Modregger et al. 2000); PLCg1 is a phospholipase involved in host–virus interactions (Korkaya et al. 2001); Rimb1 is a binding partner of the presynaptic active zone proteins RIMs and of voltage-gated

Table 1 List of 15 SH3 domains used in the screening

Protein	Uniprot	Range	SH3
ARGBP2A	O94875	1,041–1,100	3/3
ARHGAP12	Q8IWW6	12–74	1/1
CAP	Q9BX66	867–928	2/3
COOL1	Q14155	184–243	1/1
DDEF2	O43150	944–1,006	1/1
FYN	P06241	82–143	1/1
GRB2	P62993	1–58	1/2
HCK	P08631	78–138	1/1
IRSP53	Q9UQB8	374–437	1/1
NCK1	P16333	1–61	1/3
NCK1	P16333	115–165	2/3
NPHP1	O15259	152–212	1/1
PAC3	Q9UKS6	363–424	1/1
PLCG1	P19174	791–851	1/1
RIMB1	O95153	1,764–1,831	3/3

The first column contains the name of the protein of the 14 SH3-containing proteins; the second one their Uniprot accession number; the third one the amino acid range of each SH3 domain; and the last one the numbering N to C-term of SH3 multiple SH3 domains of those proteins, e.g. the NCK1 protein contains 3 SH3 domains and in this study we probed the binding properties of the first two domains, referred to in the table as 1/3 and 2/3

Ca(2+)-channels (Mittelstaedt and Schoch 2007). In Table 1 and Fig. 2, we have listed the SH3 domains used in the experiment with the corresponding amino acid ranges and their multiple sequence alignment, respectively. Some of these proteins contain multiple SH3 domains.

Identification of viral binding partners of SH3 domains

Identification of the domain-binding peptides was obtained by an overlay assays. After binding of the GST–SH3 fusions, the membranes were treated with anti-GST goat antibody and finally with peroxidase-labeled anti-goat antibody. The chemiluminescent signal intensity of each spot was quantified. We considered as positive interactions the spots (peptides) with signal intensity higher than the signal average of each membrane plus one standard deviation. By this approach, we have identified 114 new putative interactions between 15 SH3 domains and the proline-rich regions of two viral proteomes (Fig. 3; Supplementary Table 3). SH3 domains bind with high specificity to few proline-rich sequences, while others recognize several proline-rich sequences, displaying a lower selectivity. Figure 4 shows the VirusMINT protein network including the interactions described here. Only the SH3 of Hck and Fyn were already described in published papers as ligands of viral proteins (Hiyoshi et al. 2008; Shelton and Harris 2008; Trible et al. 2006).

Conclusions

In this work, we demonstrate the feasibility of applying the SPOT synthesis technology, combined with the WISE approach, to the identification of targets of peptide-binding domains in viral proteomes. This strategy may facilitate the identification of critical nodes in the human proteome that are targeted by the viruses. We refrain here from functional speculations because the in vitro data that we have produced need extension and further processing and validation to identify interactions that might be physiologically relevant. Although we have only probed less than 10% of the human SH3 domains, we can easily extend the information presented here to the whole SH3 family by utilizing our classification of the recognition specificity of the entire SH3 domain family (Carducci et al. in preparation). In addition, our data only address the potential of a given SH3 to bind a peptide on a cellulose membrane. There is no guarantee that these partners will ever meet in an infected

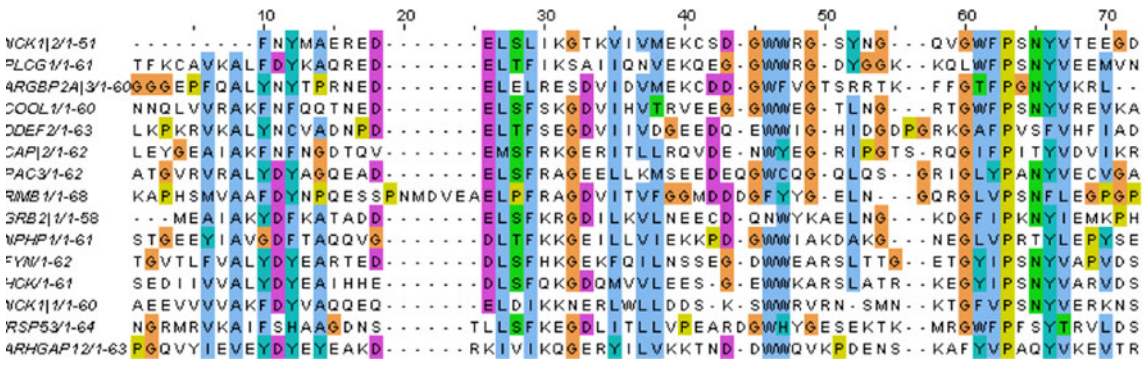

Fig. 2 Multiple alignment of the SH3 domains used in the screening. The 15 domain sequences were used as input for the ClustalW software (Thompson et al. 1994) to generate a multiple sequence alignment. The alignment is visualized by the JalView editor Software (Clamp et al. 2004; Waterhouse et al. 2009) integrated in the ClustalW output web page

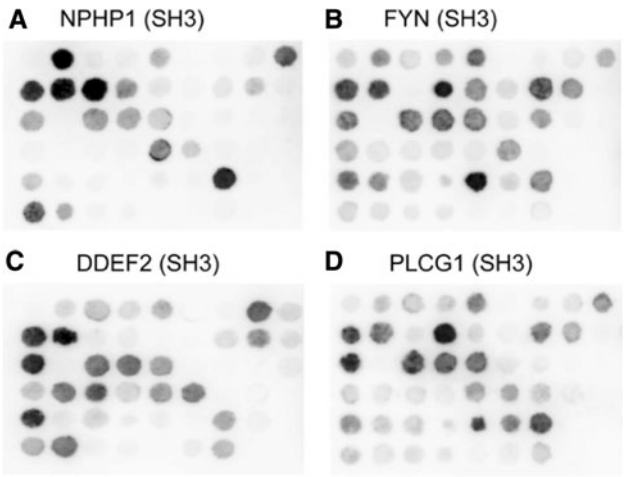

Fig. 3 Identification of SH3 viral binding partner by the Spot method. The viral peptides were synthesized on cellulose membranes and then probed with different GST–SH3 fusion proteins. The binding was revealed by anti-GST antibodies as described; the binding intensity was evaluated in arbitrary chemiluminescence units. Each array (9 × 6 spots) contains 46 viral Pro-rich peptides (Supplementary Table 2), a peptide (LAKDLIVPRRPEWNA) recognized by anti-GST antibody (column 9 row 1) and four peptides of the viral adenovirus A type 12 DNA polymerase protein, lacking any proline residue and used as negative controls. Their sequences are: QDFKYQYLK (column 8 row 5), DKEEYLLNQ (column 8 row 6), SHELYDYVR (column 9 lane 2), ELYDYVRAS (column 9 lane 3). Four empty spots (column 9 row 3, 4, 5, 6) were included for evaluation of the signal background intensity. The membranes were probed with the SH3 domains of the NPHP1 (**a**) FYN, (**b**) DDEF2 (**c**) and PLCG1 (**d**) proteins, respectively

cell. Gene expression and protein concentration and localization data in different cell types are likely to confer a physiological dimension to these in vitro data.

As an example, the biological significance of which would be worth investigating is the inferred interaction between the Cool1 SH3 domain and some viral proteins. Cool1, a Rac/Rho GEF, is found in a multiprotein complex with the p21-dependent kinase PAK that has already been described as HIV Nef target (Renkema et al. 2001). This mechanism could offer an alternative viral strategy of disturbing the COOL1-PAK protein complex that is involved in focal adhesion assembly and control of growth and apoptosis.

It has been reported that viral proteins have a tendency to interact with hubs, i.e., proteins that display a high connectivity in the protein interaction network, and bottlenecks, proteins that are key connectors of many pathways in the network (Yu et al. 2007).

For these reasons, we evaluated the enrichment, if any, of human hub proteins in the VirusMINT database. We considered as hubs the proteins, the connectivity of which in the human MINT network exceeds the connectivity of 95% of the nodes. By this definition, 310 of the 5,736 proteins in MINT are hubs. 72 of these are also present in VirusMINT, i.e., they are partners of viral proteins. Since the VirusMINT interactome contains a total of 508 proteins, this means that the hubs are 14% of the total human proteins in VirusMINT corresponding to threefold enrichment.

The MINT database, for each interaction, provides a score, ranging from 0 to 1, and representing the experimental evidence supporting the interaction. By establishing a threshold of 0.3, one can obtain a smaller but higher confidence interactome. A hub enrichment analysis carried out on such a network yields similar results confirming that viral proteins have a preference to target human hubs.

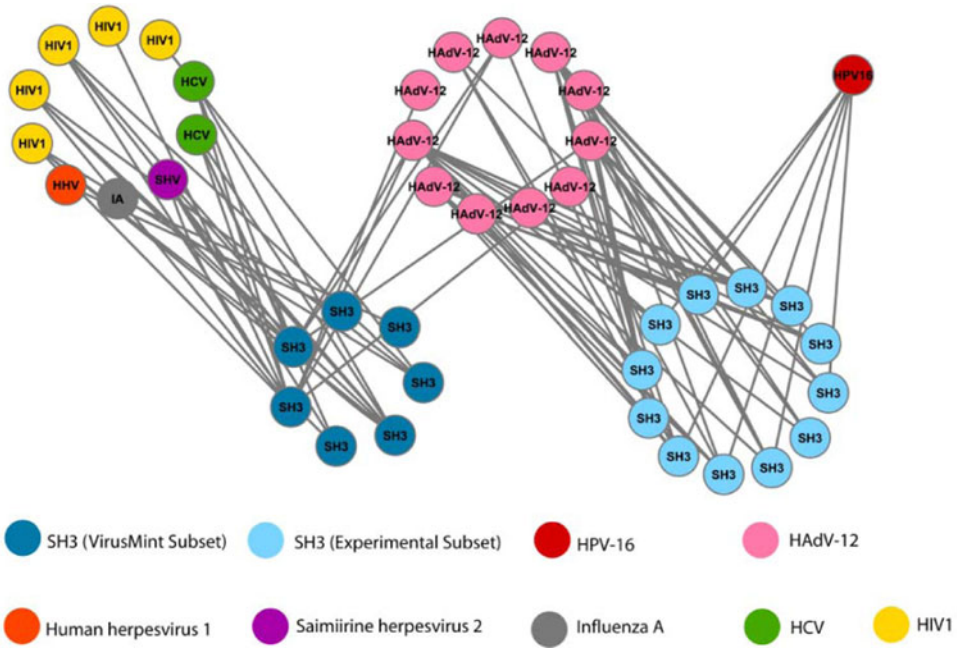

Fig. 4 Enrichment of SH3-mediated interactions in the VirusMINT database obtained by SPOT synthesis experiments. The virus strains and proteins containing SH3 domains present in the network are indicated. The *lower nodes on the left* are the SH3 domain containing proteins that were already present in the VirusMINT database, while the *lower nodes on the right* represents the SH3 used as probes in our screening. Two of the SH3-containing proteins (HCK and FYN) are shared by the two subsets. The network was visualized by the Cytoscape software (Shannon et al. 2003)

Interestingly, most of the SH3 proteins interacting with virus proteins detected in this study, such as GRB2, FYN and NCK1, and HCK and ARHG7 are indeed highly connected cell proteins, confirming the crucial role of these proteins in the cell-regulatory circuits and, as a consequence, in the host–virus relationship.

Experimental procedures

Recombinant protein production and overlay protocol

Escherichia coli BL21 Rosetta cells were transformed with pGEX-4TK recombinant plasmids directing the expression of specific GST–SH3 proteins. A single colony was grown in 25 ml of LB with ampicillin (100 μg/ml) and chloramphenicol (30 μg/ml) at 37°C until an OD of 0.6 was reached. Protein production was performed at 30°C for 5 h after the addition of IPTG (isopropyl-beta-D-thiogalactopyranoside) at a final concentration of 0.5 mM. The bacteria were pelleted and resuspended in lysis buffer (50 mM Tris, pH 8.0, 5 mM EDTA, 0.1% Triton X-100, 150 mM NaCl) containing a proteinase inhibitor mixture (Roche Applied Science) and lysed by a treatment with lysozyme (200 μg/ml final) for 1 h on ice followed by three rounds of sonication. The lysate supernatant was incubated for 1 h with 100 μl of a 50% solution of glutathione–Sepharose beads (Amersham Biosciences) at 4°C. Finally, the beads were extensively washed in PBS, and the SH3 domains were eluted in 50 mM Tris, pH 8.0, with 10 mM glutathione. The amount of proteins produced was determined with a Bio-Rad protein assay. Proteins were dialyzed in PBS overnight.

Spot synthesis

Cellulose membrane-bound peptides were automatically synthesized according to standard SPOT synthesis protocols (Kramer et al. 1997) using a Spot synthesizer MultiPep Spotter (Intavis AG, Germany). To limit background signals, all cysteines were replaced by serines. The generated peptide arrays were synthesized on Amino-PEG500-UC540 Sheet (acid hardened) (Intavis AG, Germany). The membrane was activated in ethanol (3 times for 10 min), washed three times in PBS, blocked in PBS/BSA 5% and then incubated with glutathione *S*-transferase (GST) fusion proteins (10 μg/ml) in PBS/BSA 5%. Incubation with GST alone at the same concentration in the same buffer was used as negative control. The membrane was then treated with anti-GST antibody and finally with peroxidase-labeled anti-goat antibody. Chemiluminescent signals were acquired with a LAS3000 instrument (FujiFilm) and analyzed with the software AIDA.

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References

Adzhubei AA, Sternberg MJ (1993) Left-handed polyproline II helices commonly occur in globular proteins. J Mol Biol 229:472–493

Andreev J, Simon JP, Sabatini DD, Kam J, Plowman G, Randazzo PA, Schlessinger J (1999) Identification of a new Pyk2 target protein with Arf-GAP activity. Mol Cell Biol 19:2338–2350

Briggs SD, Sharkey M, Stevenson M, Smithgall TE (1997) SH3-mediated Hck tyrosine kinase activation and fibroblast transformation by the Nef protein of HIV-1. J Biol Chem 272:17899–17902

Chatr-aryamontri A, Ceol A, Palazzi LM, Nardelli G, Schneider MV, Castagnoli L, Cesareni G (2007) MINT: the Molecular INTeraction database. Nucleic Acids Res 35:D572–D574

Chatr-aryamontri A, Ceol A, Peluso D, Nardozza A, Panni S, Sacco F, Tinti M, Smolyar A, Castagnoli L, Vidal M, Cusick ME, Cesareni G (2009) VirusMINT: a viral protein interaction database. Nucleic Acids Res 37:D669–D673

Clamp M, Cuff J, Searle SM, Barton GJ (2004) The Jalview Java alignment editor. Bioinformatics 20:426–427

Donaldson JC, Dempsey PJ, Reddy S, Bouton AH, Coffey RJ, Hanks SK (2000) Crk-associated substrate p130(Cas) interacts with nephrocystin and both proteins localize to cell–cell contacts of polarized epithelial cells. Exp Cell Res 256:168–178

Dyson N, Guida P, Munger K, Harlow E (1992) Homologous sequences in adenovirus E1A and human papillomavirus E7 proteins mediate interaction with the same set of cellular proteins. J Virol 66:6893–6902

Feng S, Chen JK, Yu H, Simon JA, Schreiber SL (1994) Two binding orientations for peptides to the Src SH3 domain: development of a general model for SH3–ligand interactions. Science 266:1241–1247

Hassaine G, Courcoul M, Bessou G, Barthalay Y, Picard C, Olive D, Collette Y, Vigne R, Decroly E (2001) The tyrosine kinase Hck is an inhibitor of HIV-1 replication counteracted by the viral vif protein. J Biol Chem 276:16885–16893

Hiyoshi M, Suzu S, Yoshidomi Y, Hassan R, Harada H, Sakashita N, Akari H, Motoyoshi K, Okada S (2008) Interaction between Hck and HIV-1 Nef negatively regulates cell surface expression of M-CSF receptor. Blood 111:243–250

Korkaya H, Jameel S, Gupta D, Tyagi S, Kumar R, Zafrullah M, Mazumdar M, Lal SK, Xiaofang L, Sehgal D, Das SR, Sahal D (2001) The ORF3 protein of hepatitis E virus binds to Src homology 3 domains and activates MAPK. J Biol Chem 276:42389–42400

Kramer A, Keitel T, Winkler K, Stocklein W, Hohne W, Schneider-Mergener J (1997) Molecular basis for the binding promiscuity of an anti-p24 (HIV-1) monoclonal antibody. Cell 91:799–809

Landgraf C, Panni S, Montecchi-Palazzi L, Castagnoli L, Schneider-Mergener J, Volkmer-Engert R, Cesareni G (2004) Protein interaction networks by proteome peptide scanning. PLoS Biol 2:E14

Latreille M, Larose L (2006) Nck in a complex containing the catalytic subunit of protein phosphatase 1 regulates eukaryotic initiation factor 2 alpha signaling and cell survival to endoplasmic reticulum stress. J Biol Chem 281:26633–26644

Lazzi S, Bellan C, De Falco G, Cinti C, Ferrari F, Nyongo A, Claudio PP, Tosi GM, Vatti R, Gloghini A, Carbone A, Giordano A, Leoncini L, Tosi P (2002) Expression of RB2/

p130 tumor-suppressor gene in AIDS-related non-Hodgkin's lymphomas: implications for disease pathogenesis. Hum Pathol 33:723–731

Lechner MS, Laimins LA (1994) Inhibition of p53 DNA binding by human papillomavirus E6 proteins. J Virol 68:4262–4273

Levine AJ, Momand J, Finlay CA (1991) The p53 tumour suppressor gene. Nature 351:453–456

Li SS (2005) Specificity and versatility of SH3 and other proline-recognition domains: structural basis and implications for cellular signal transduction. Biochem J 390:641–653

Lowenstein EJ, Daly RJ, Batzer AG, Li W, Margolis B, Lammers R, Ullrich A, Skolnik EY, Bar-Sagi D, Schlessinger J (1992) The SH2 and SH3 domain-containing protein GRB2 links receptor tyrosine kinases to ras signaling. Cell 70:431–442

Macias MJ, Hyvonen M, Baraldi E, Schultz J, Sudol M, Saraste M, Oschkinat H (1996) Structure of the WW domain of a kinase-associated protein complexed with a proline-rich peptide. Nature 382:646–649

Mayer BJ (2001) SH3 domains: complexity in moderation. J Cell Sci 114:1253–1263

Mittelstaedt T, Schoch S (2007) Structure and evolution of RIM-BP genes: identification of a novel family member. Gene 403:70–79

Modregger J, Ritter B, Witter B, Paulsson M, Plomann M (2000) All three PACSIN isoforms bind to endocytic proteins and inhibit endocytosis. J Cell Sci 113 Pt 24:4511–4521

Musacchio A, Noble M, Pauptit R, Wierenga R, Saraste M (1992) Crystal structure of a Src-homology 3 (SH3) domain. Nature 359:851–855

Nishizawa K, Freund C, Li J, Wagner G, Reinherz EL (1998) Identification of a proline-binding motif regulating CD2-triggered T lymphocyte activation. Proc Natl Acad Sci USA 95:14897–14902

Pornillos O, Alam SL, Rich RL, Myszka DG, Davis DR, Sundquist WI (2002) Structure and functional interactions of the Tsg101 UEV domain. EMBO J 21:2397–2406

Reinhard M, Giehl K, Abel K, Haffner C, Jarchau T, Hoppe V, Jockusch BM, Walter U (1995) The proline-rich focal adhesion and microfilament protein VASP is a ligand for profilins. EMBO J 14:1583–1589

Renkema GH, Manninen A, Saksela K (2001) Human immunodeficiency virus type 1 Nef selectively associates with a catalytically active subpopulation of p21-activated kinase 2 (PAK2) independently of PAK2 binding to Nck or beta-PIX. J Virol 75:2154–2160

Ribon V, Printen JA, Hoffman NG, Kay BK, Saltiel AR (1998) A novel, multifunctional c-Cbl binding protein in insulin receptor signaling in 3T3–L1 adipocytes. Mol Cell Biol 18:872–879

Schutt CE, Myslik JC, Rozycki MD, Goonesekere NC, Lindberg U (1993) The structure of crystalline profilin-beta-actin. Nature 365:810–816

Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 13:2498–2504

Shelton H, Harris M (2008) Hepatitis C virus NS5A protein binds the SH3 domain of the Fyn tyrosine kinase with high-affinity: mutagenic analysis of residues within the SH3 domain that contribute to the interaction. Virol J 5:24

Soubeyran P, Barac A, Szymkiewicz I, Dikic I (2003) Cbl-ArgBP2 complex mediates ubiquitination and degradation of c-Abl. Biochem J 370:29–34

Stapley BJ, Creamer TP (1999) A survey of left-handed polyproline II helices. Protein Sci 8:587–595

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Trible RP, Emert-Sedlak L, Smithgall TE (2006) HIV-1 Nef selectively activates Src family kinases Hck, Lyn, and c-Src through direct SH3 domain interaction. J Biol Chem 281:27029–27038

Waterhouse AM, Procter JB, Martin DM, Clamp M, Barton GJ (2009) Jalview Version 2—a multiple sequence alignment editor and analysis workbench. Bioinformatics 25:1189–1191

Williamson MP (1994) The structure and function of proline-rich regions in proteins. Biochem J 297(Pt 2):249–260

Yu H, Rosen MK, Shin TB, Seidel-Dugan C, Brugge JS, Schreiber SL (1992) Solution structure of the SH3 domain of Src and identification of its ligand-binding site. Science 258:1665–1668

Yu H, Chen JK, Feng S, Dalgarno DC, Brauer AW, Schreiber SL (1994) Structural basis for the binding of proline-rich peptides to SH3 domains. Cell 76:933–945

Yu H, Kim PM, Sprecher E, Trifonov V, Gerstein M (2007) The importance of bottlenecks in protein networks: correlation with gene essentiality and expression dynamics. PLoS Comput Biol 3:e59

Yuan ZQ, Kim D, Kaneko S, Sussman M, Bokoch GM, Kruh GD, Nicosia SV, Testa JR, Cheng JQ (2005) ArgBP2gamma interacts with Akt and p21-activated kinase-1 and promotes cell survival. J Biol Chem 280:21483–21490

Zarrinpar A, Bhattacharyya RP, Lim WA (2003) The structure and function of proline recognition domains. Sci STKE 2003:RE8