Protein Interaction-Targeted Drug Discovery: Evaluating Critical Issues

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The goal of drug discovery is to develop novel small-molecule compounds that ameliorate, cure, and (optimally) prevent clinically significant diseases. It has been much asserted that the resources of genome and proteome projects will contribute significantly towards this goal. The volume of information generated through these projects is by any objective standard impressive and overwhelming, providing a great impetus to the development of a bioinformatics community that will be able to make sense of the data onslaught (issues discussed in References 12 and 13). From an intellectual perspective, this work has the potential to add a new level of rigorous mathematical modeling to the common conceptions of cell, tissue, and organism over the next decade or so. However, from a pragmatic perspective focused on drug discovery over the window of the next three to five years, an essential topic of debate is how best to garner information from “-omics” resources as they become available and how to exploit this information to generate useful compounds. In this context, one idea has been to develop novel drugs that have improved specificity of action, and reduced undesirable side effects, by targeting specific proteins or protein-protein interactions (PPIs). The purpose of this review is to discuss what such a strategy entails, how screening for such targeted agents might be practically accomplished, and to raise issues concerning the validation of results.

Defining the Field: What Is a PPI?

As the level of general knowledge of proteins and protein interaction networks advances, the common understanding of a PPI becomes complex. It is currently possible to define this term in a number of different ways, involving different admixtures of physical and functional components.

At the simplest level of definition (Figure 1A), two discrete proteins A and B physically associate with each other in a stable complex, although the number of potential consequences of a two-component interaction make interpretation of the functional significance of an interaction not completely simple. Through an interaction, two proteins may together exert a function not possessed by either

protein in isolation, for example, in an instance in which the association of two transcription factors allows the heterodimer to bind an asymmetric site on DNA. Alternatively, two associating proteins might retain unaltered individual functions, exploiting the interaction for a discrete purpose, such as providing enhanced stability from proteolytic degradation via sequestration of a destruction signal. Another possibility is that a PPI may result in a loss of activity, or destruction for component A or B, or both, as when the interaction of the adenomatous polyposis coli (APC) protein with the β -catenin signaling protein targets the latter for degradation (24). Alternatively, an interaction may serve a regulatory function, by enhancing the docking of the A+B complex to a third component: this third component, C, may be an “upstream” factor controlling the activity of A or B or A+B, or a “downstream” recipient of the activity of A/B/A+B. Alternatively, the interaction of A+B may exclude A or B from interaction with another potential partner molecule, D, as when the association of proteins such as Id helix-loop-helix (HLH) proteins with basic region-HLH transcription factors prevents the transcription factors from forming functional complexes (19). In general, such PPIs may be considered to involve a relatively extensive set of physical contacts between proteins A and B, contributing to both the stability and specificity of the interaction over time, although significant functional consequences may or may not ensue.

In considering the function of signaling networks, it is clear that a definition of a PPI as the stable interaction of two components is inadequate to represent known biological control mechanisms. Many biological responses to stimuli occur over very short time periods and require the ability of key effector proteins to oscillate between different activity states. This flexibility is provided by proteins such as kinases, phosphatases, and guanosine diphosphate exchange factors, which can rapidly modify the physical composition, and hence activity, of a preexisting set of cellular proteins. In contrast to the examples of relatively stable protein interaction discussed above, this class of interactions is likely to be transient and involve weaker associations with higher off rates. Because biological cascades involving kinases, phosphatases, and exchange factors frequently function by amplification of a single initial stimuli to multiple downstream effectors, it is desirable for determinants of specificity to be more limited. Hence, the recognition sites for proteins such as kinases can frequently be defined as a very limited amino acid consensus sequence that tolerates significant degeneracy. In this class of PPI (Figure 1B), although the functional consequence of interaction can be significant, both the physical and temporal extent of interaction can be very limited.

A further refinement of the concept of PPIs is derived from the consideration of individual proteins as components of multiprotein complexes or as elements of genetic pathways. To date, many such intracellular multiprotein “machines” have been defined, including among many others the ribosome (3,17), the spliceosome (29), and the cyclosome or anaphase promoting complex (21). Suppose a protein, A, is an important stabilizing component of such

a complex, defined as ABCDEFG (Figure 1C), and associates with the complex based on an interaction with complex components BCDE. It is possible that disruption of the binding of A to the complex may change the stability, interaction properties, and functional activity of complex components F and G, even though A does not at any point form a direct association with F or G. In this more complex class of PPI, although direct physical interaction is absent, indirect transmission of a physical initiating signal through a protein complex has a high functional output.

We note that the similarity of the signaling logic utilized in the different classes of PPI described above to the electronic logic of circuit diagrams has long been noted and is becoming explicitly addressed in bioinformatics efforts to compile database resources to support the modeling of biological systems (5,12,26). Issues related to developing an adequate representational system and community-wide standards to record and display requisite information on the level of a proteome are extremely important but are addressed in multiple recent reviews and will not be further covered here.

Which PPIs Can or Should Be Targeted?

A protein of considerable interest as a drug development target may be involved, with various partners, in all the classes of PPI described above. How does one decide whether it is appropriate to attempt to regulate the interaction profile of this target, and how is this goal accomplished? At present, there is no definitive answer to this question. However, there are clearly some very definite steps that can be taken with available informational and experimental resources that would support such an effort.

Step 1. Identify potential targets based on evidence of critical biological function in the clinical condition to be addressed and based on evidence that the function of the target can be productively manipulated. The combination of sequencing projects, transcriptional profiling, proteome-level interaction mapping, and other components of "discovery science" has promised to identify new targets for drug discovery. While this promise will unquestionably be fulfilled at some point in the future, at present these high-throughput efforts are in the

Figure 1. Different functional consequences of PPIs. (A) Two-component, stable interactions, and potential functional consequences. (B) Consequences of transient interactions (e.g., cyclin-cdk association). (C) Indirect consequences of protein interaction with a complex: association of A with BCDE propagates to FG.

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stage of identifying new genes or proteins whose expression is linked in some way to particular pathological states of interest. The necessary validation steps to prove the targets thus nominated play a critical role in a disease include the generation of suitable *in vivo* animal models, the proof that disruption of the suggested target leads to a disease mimicking the human syndrome of interest, and the demonstration by combined clinical and *in vitro* studies that the function of the suggested target is anomalous in the syndrome being studied. Neither these steps nor the subsequent characterization of the mechanism of function and specificity of action of a confirmed (but highly novel) target are likely to be high throughput. In contrast, literature assembled over decades has yielded a rich trove of preexisting targets directly demonstrated to be the primary lesion in specific defects. As one example, the American Cancer Society and the National Cancer Institute maintain lists of defined oncogenes and tumor suppressors that are known to be predisposing, initiating, or progression factors for a large numbers of different types of cancer. Some of these genes, such as the CHK2 checkpoint control protein, are directly affected in a limited number of cancers, while others, such as the Ras family, are critical to multiple types of frequently occurring cancers.

In evaluating such lists before developing a drug discovery strategy based on regulation of PPIs, there are some critical considerations that might constitute a decision tree for target selection. First, would the mode of function of a drug have to be the disruption or the restoration of protein function to have a desired effect? Clearly, the first category of action would be easier to achieve, making oncogenes preferred targets as a class versus tumor suppressors. Second, how large is the patient base known to have a disease in response to dysregulation of the protein of interest? Of the affected patients, is an identical lesion observed in all cases, or are there multiple classes of mutation that might indicate a complex mode of function for the presumptive target protein? Third, is the protein highly related in sequence and structure to other cellular proteins, allowing effective modeling for targeted drug design? Is the degree of homology to other, possibly essential cellular proteins so great that the lack of specificity of a protein-directed drug is likely to be a concern? Fourth, is the protein of a class that is amenable to PPI analysis? Few technologies are available to conveniently study PPIs of integral membrane proteins, whereas a large number of complementary approaches (e.g., mass spectrometry, two-hybrid technologies, and protein arrays) can be used to study soluble proteins. Such considerations might serve to initially refine the field of target selection.

Step 2. Derive from existing data a physical and functional network of interactions for the target. The Ras GTPase is a biologically significant and well-characterized oncoprotein that has been of considerable interest as a target for drug discovery (see Reference 15 for a review). Considering this protein as an exemplar of PPI-targeting strategy development, it is instructive to determine the number of different ways in which Ras-related PPIs can be portrayed.

Logically, the simplest representation would be an indication of the proteins with which Ras can physically and directly associate (Figure 2A). As protein interaction networks become more comprehensive, this analysis identifies a significant number of proteins as Ras interactors, connecting Ras input to a number of different functional pathways. Does Ras interact with these different partners simultaneously or as part of a complex? That is, would disrupting the interaction of Ras with one partner also affect its interactions with other partners? Depending on the answer to this, it may be possible to target Ras so as to interfere with a single Ras-dependent pathway at a time or only possible to interfere with multiple Ras-dependent pathways.

To gain insight into this class of query, it is useful to consider a protein interface diagram, as recently described by Corbett and Alber (Figure 2B) (7). In this approach, structural information about protein-protein interfaces derived from co-crystals of small GTPases with a series of partner proteins is aligned to identify which residues are involved in zero, single, or multiple interactions. This means of display makes it clear that some protein surfaces are interaction-intensive, while others are utilized only by some partners. A structure-targeted drug design effort can be directed accordingly.

Should Ras be considered as a homogenous target? Or does Ras exist in multiple states, such that different intracellular pools of Ras interact with different partners? If so, then determining how the partner selection is regulated may be a critical issue for consideration. Recent studies have emphasized the importance of adapter or bridging proteins that serve to bring signaling proteins together with key partners or to segregate them from others with which they can potentially interact (Figure 2C). Depending on the relative abundance of these modulatory proteins in the cell type relevant to the disease, the actual availability of Ras for its various potential interactions may differ markedly from that in other cell types.

What proteins are functionally dependent on the activity of Ras? Some proteins that are not defined as being directly associated with Ras at this time may nevertheless be at least tenuously associated with a Ras-containing complex and important for mediating Ras-dependent activities in oncogenic transformation. Maintaining a functional map (Figure 2D) of functionally implied interactions could reasonably support the development of validation strategies. Further, detailed knowledge of Ras-intersecting pathways may suggest additional targets of drug screening efforts. The ease of gathering such information increases daily, as online databases such as ProNet and others become more complete and readily screenable.

Finally, as increasing numbers of organismal genomes are sequenced and recorded interaction maps become more detailed for diverse species including *S. cerevisiae* (11,14,18,32), *C. elegans* (4,8,33), *D. melanogaster* (30), and others, it is probably worth the effort, for evolutionarily conserved targets of interest, to develop matching resources for at least some species allowing ready genetic manipulation. Having access to this information for a PPI

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Figure 2. Different images of the oncoprotein Ras. (A) Ras and potential interactions with activators, inactivators, or effectors. (B) Structural representation of Ras surface. Increasing shading represents the degree of utilization of surface in binding interactions (highly simplified from Reference 7). (C) Ras interactions with potential effectors as subject to regulation by scaffolding proteins (adapted from Reference 15). (D) Ras functional interactions with dependent signaling pathway (highly simplified from information at <http://pronet.doubletwist.com/> and in Reference 15).

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profile will not only provide suggestions about which interactions are more or less important (e.g., based on evolutionary depth of conservation of the partners and the interaction interface) but also may in some cases enhance the ability to explore the functional properties of PPI inhibitors by using cross-species experimentation.

Step 3. Determine the previous history of mutational PPI disruption involving the target. In developing a drug to disrupt protein-protein interactions, the goal is to achieve a desirable biological effect and to minimize general toxicity. Particularly in cases in which the target is evolutionarily conserved through genetically manipulable organisms, but also in instances where a target has been well mined by structure-function studies, it can be instructive to analyze results obtained from natural and artificial mutation of the target. Are there defined instances in which a key functional interaction has been shown to be disrupted by mutation or small deletion? Were the consequences acceptable for the cell or organism, or was there a severe outcome? In considering developmental defects, was the defect limited to one or a small number of cell types or generalized? Was the defect restricted to signaling pathways in which the proteins were already known to function, or were there unexplained phenotypic defects? Was there a complete absence of phenotype, suggesting that the functional requirement for the PPI of interest is limited because of redundancy with other proteins or PPIs?

Where do the mutations map on the structure of the protein? A mutation at the site of contact between protein A and partner B would suggest a direct effect on binding. A mutation at a remote site on B might have a number of different explanations, ranging from induction of an allosteric effect disfavoring binding to A, through disruption of binding with an alternate partner, C, which contributes to the affinity of A for B, to destabilization of B, such that loss of observed AB binding reflects the loss of much of B from the cell. Finally, it is conceivable that a mutation may disrupt PPIs in such a manner as not to be readily detectable by direct physical measurements. In such a situation, A and B may still functionally interact with comparable affinity to wild-type, but propagation of signals from A through B may be reduced or eliminated because of allosteric effects, even though mutations do not target catalytically involved residues on either protein. Each of these possibilities must be considered, as the information gained may provide valuable insights into how A, B, and C function. Each possibility can be adequately evaluated only by merging direct physical analysis with defined functional tests to probe mechanism.

Step 4. Develop a discriminatory strategy to identify specific versus nonspecific inhibition of the selected protein, PPI, and control pathway. The goal may be to validate the mode of action of a compound identified through PPI-based screening or to develop alternative function-based screens to identify drugs affecting a target of interest. For either, it is important early in the screen design process to develop a robust series of complementary approaches that each independently addresses the PPIs and PPI-linked activities of the target.

Numerous reviews have suggested different methodologies for the performance of primary drug screening and validation based on compound-protein docking, assessment of binding to protein arrays, functional screening, microarray-based transcriptional profiling, and others, and a comprehensive overview of these techniques is beyond the scope of this review. Returning to the example of Ras shown above (Figure 2), if the goal were to identify (for example) a drug targeted to disrupt Ras-PI-3-K interactions, there are clearly a number of different assays that it would be of interest to develop. Assay 1 might measure direct binding of the compound to Ras or PI-3-K or both. Assay 2 might directly score the interaction between Ras and PI-3-K and measure the ability of compounds to disrupt it. This assay platform should be designed so as to allow contrast of Ras-PI-3-K binding inhibition to inhibition of Ras interaction with other effectors such as Ral-GDS or Raf1. Assay 3 might measure the compound regulation of activation of PI-3-K kinase activity following Ras-initiated stimulation (again, contrasting this with activation of Raf1 or Ral-GDS effector activity). Assay 4 might assess compound regulation of further downstream effects of Ras-initiated signaling, for example, activation of Akt, which should be dependent on PI-3-K activation. Assay 5 might compare the transcriptional profile of cells treated with a compound thought to target Ras-PI-3-K interactions, versus treated with other compounds known to block other Ras-related signaling cascades. Assay 6 might measure a biological consequence of inhibiting Ras to PI-3-K signaling, such as reversion of anchorage-independent growth of cell lines dependent on this intact signaling axis for transformation. Assay 7 might screen compounds in model organisms for the development of phenotypes reminiscent of those found in organisms mutated for PI-3-K orthologs. Combined application of such assays will allow mechanistic bracketing of the effect of a compound to the signaling point desired. The majority of these assays can serve alternatively as point-of-entry for a primary screen or as a secondary validating screen. Logically, initiating a screen on a target of interest using multiple different approaches appears more likely to yield useful compounds than limiting screening to any single strategy.

Step 5. Be prepared to exploit serendipitous findings. As a final note, it rarely pays to be dogmatic, and committing full effort to a focus on forward, PPI-driven, drug discovery strategy is unlikely to be the exception that proves the rule. PPI-based drug discovery is a relatively new area. Historically, the majority of drugs of desirable clinical use have arisen from phenotypic or biological or otherwise serendipitous screens, and mode of the signaling function has been established after its discovery. If the point of interest is to establish PPI inhibitors for a specific biological pathway of interest, then it would make practical sense to be prepared to retroactively assess preexisting compounds of defined biological activity against a well-developed tool set for that pathway, designed based on the criteria above. As bioinformatics resources become further developed, such reverse-identification of compounds

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of desired activity should be enhanced.

Reciprocally, even though the idea of developing a drug targeted to a single PPI of interest may be attractive as a means of achieving a “clean”, well-understood mode of action for a compound, too much specificity of targeting may be suboptimal in practice. There is no reason to exclude further analysis of a PPI-targeted agent that has desirable biological properties simply because it subsequently is found to have binding properties or activities unrestricted to the original target.

Counterexample

Although there is little doubt that small-molecule interference with critical PPIs will eventually develop into a major research and development initiative, there is currently a limited number of published examples of such agents (6,9,16,27,31,35). One provocative example to consider is the results obtained with conjugates, analogues, and peptidomimetics based on the tripeptide glutathione (GSH), which have proven to be useful agents in the disruption of important cellular protein complexes. Through exploitation of a serendipitous finding, this example demonstrates the complexity of studying protein and PPI-targeted drugs in practice and how studies performed with small-molecule PPI inhibitors can, conversely, illuminate the requisite mechanisms of protein and signaling function.

GSH derivatives were initially created as tools to regulate the function of glutathione-S-transferases (GST), proteins important in cellular detoxification, multidrug resistance, and other processes (Reference 20 and as described below), in work guided by detailed structure-function analysis of the GSH-GST interface. Intriguingly, in studies of a photoaffinity ligand of a GSH conjugate (^{35}S -azidophenacyl glutathione, APA-SG), this agent was shown to bind with high affinity to the DNA-PKcs protein. DNA-dependent protein kinase (DNA-PK) forms a holoenzyme complex composed of a catalytic subunit (DNA-PKcs) and two autoantigens, Ku70 and Ku80. DNA-PK is an essential enzyme in the repair of DNA double-stranded breaks, such that modulation of its function would affect cellular processes including V(D)J recombination, response to ionizing radiation or chemotherapeutic drugs, and maintenance of telomeres (10). Hence, drugs affecting DNA-PK might have applications in immune system function, cancer biology, and aging. Therefore, the mechanism of this inhibition was additionally investigated. The APA-SG ligand inhibited the ability of the reconstituted DNA-PK enzyme complex to phosphorylate an *in vitro* peptide substrate (28). Further, when whole cell fractions were immunoprecipitated with a polyclonal antibody to DNA-PKcs, both the Ku70 and Ku80 subunits were coprecipitated and could be detected through standard immunoblot technology, but in the presence of APA-SG, the catalytic subunit disassociated from the Ku autoantigen complex and prevented the immunoprecipitation of the full trimeric protein complex. The interference with protein interactions was thus strongly nominated as a direct

cause of the APA-SG inhibition of kinase activity.

In further study of the DNA-PK-directed APA-SG activity, a series of competition assays confirmed that the binding of APA-SG to the DNA-PKcs was specific and could be prevented by other agents that incorporated the glutathione peptide motif as an integral structural component. This observation led to the analysis of other GSH analogues as plausible pharmacological inhibitors of this important enzyme system. Effective members of this set included a GSH peptidomimetic agent, γ -glutamyl-S-(benzyl) cysteinyl-R(-)-phenyl glycine diethyl ester (TLK199), a drug initially designed as an inhibitor of the cytosolic phase II detoxification enzyme GSTP1-1. A further indication of the critical importance of the GSH tripeptide motif (gly-cys-arg) was provided by the nanomolar inhibition constant for DNA-PK of a GSTP1-1-activatable prodrug, γ -glutamyl- α -amino- β -(2-ethyl-N,N,N'-tetrakis(2-chloroethyl) phosphorodiamidate)-sulfonyl-propionyl-R(-)-phenylglycine (TLK286). This drug also caused a disassociation of the trimeric DNA-PK holoenzyme complex. The implication from these combined results was that a modified component of the GSH tripeptide is critical to the non-covalent binding of these agents to the DNA-PKcs at a site that destabilizes its interaction with the Ku subunits, resulting in a loss of kinase activity. However, at present, the structural basis of this effect remains unknown. Beyond determination of this point, an interesting aspect of future analyses of the function of these agents will be the study of their impact on the interaction of DNA-PK with other cellular proteins and protein complexes. As a component of the DNA repair machinery, DNA-PK is involved in diverse interactions; the recent report (1) of association of the DNA damage sentinel p53 with DNA-PK provides additional value to DNA-PK as an intriguing target for drug intervention in human cancer.

Additionally complicating the analysis, however, it is clear that despite the nanomolar inhibition constant (K_i) for the inhibition of DNA-PK activity by TLK199, the biological effects of this drug are not restricted to this enzyme, nor are they limited to inhibition of the GSTP1-1 catalytic activity. Insight into an additional activity of TLK199 and GSH derivatives was provided by the recent report (2) of an unexpected PPI of GSTP1-1 with the c-Jun NH_2 -terminal kinase (JNK1). JNK1 is generally recognized as a gatekeeper of cellular stress response (23). In an unstressed cell, JNK1 must be maintained in an inactive state. Strikingly, this quiescence has been shown to be subject to a negative regulatory function provided by direct JNK1 binding by GSTP1-1. Based on fluorescent resonance energy transfer (FRET) analysis of the binding (34), the apparent dissociation constant of binding (K_d) for full-length JNK1 binding to GSTP1-1 was determined to be 188 nM, with the binding domain localized to a C-terminal fragment of JNK1. Tetracycline-regulated expression of this C-terminal fragment acted in a dominant-negative manner to induce endogenous JNK1 activity, while a series of co-immunoprecipitation assays confirmed that the fragment competed with full-length JNK1

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for binding to GSTP1-1, releasing the endogenous enzyme and initiating kinase activity (34). These data provided a solid rationale for defining GSTP1-1 as a critical ligand-binding protein with a role in regulating stress kinase pathways. Notably, under conditions of “environmental stress” that either directly or indirectly produce reactive oxygen species (e.g., UV light, hydrogen peroxide, and alkylating agents) and lead to ligand binding by GSTP1-1, GSTP1-1 is disassociated from JNK1 and the resultant kinase cascade is activated (2). Hence, the drug-mediated regulation of ligand binding on GSTP1-1 may regulate cell survival under stressed conditions through a JNK1, versus a DNA-PK, induced mechanism. A final level of intricacy in understanding the PPI modulatory behavior is provided by the recent suggestion that DNA-PK and JNK1 may themselves conditionally interact (22).

From these observations, there are important implications with respect to drug design and discovery. Of particular note, while TLK199 was designed in concept to bind to the GSH site (G-site) of the GSTP1-1 isozyme and to inhibit its catalytic glutathionylation properties, at least some biological activities of the drug appears to depend on an allosteric effect independent of the detoxification reaction, as when mutations are induced within the cat-

alytic domain of the GSTP1-1 G-site, the ligand binding properties of the protein are not altered (25). Conversely, TLK199 and other GSH derivatives appear to bind quite specifically to other, initially unintended targets and also regulate their PPIs. A key lesson to take from this example is the need to thoroughly investigate the possibility that alternative binding targets, and alternative mechanisms, may contribute partly or entirely to desirable biological phenotypes induced by a “targeted” drug.

Summary

Employment of the decision strategies outlined in this general discussion should help to pinpoint mode of activity in drug development and validation. Overall, as a paradigm for drug development, a search for small molecules that can interfere with PPIs would seem to have significant long term potential. At present, the level of structural knowledge in databases is not sufficient to predict in toto the protein binding properties of a modeled drug, but as databases improve, this may become generally feasible. A major point that remains to be determined is how much specificity of protein binding can be incorporated into molecules of generally less than 500 Da. Fi-

nally, integration of PPI-targeting strategies with other approaches towards drug design will enhance the number of signaling pathways that can effectively be targeted. These points will be particularly pertinent as technologies permit a systematic identification of encoded protein interactions that govern the proteomic complement of cells.

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REFERENCES

- Achanta, G., H. Pelicano, L. Feng, W. Plunkett, and P. Huang. 2001. Interaction of p53 and DNA-PK in response to nucleoside analogues: potential role as a sensor complex for DNA damage. *Cancer Res.* 61:8723-8729.
- Adler, V., Z. Yin, S.Y. Fuchs, M. Benezra, L. Rosario, K.D. Tew, M.R. Pincus, M. Sardana et al. 1999. Regulation of JNK signaling by GSTp. *EMBO J.* 18:1321-1334.
- Benjamin, D.R., C.V. Robinson, J.P. Hendrick, F.U. Hartl, and C.M. Dobson. 1998. Mass spectrometry of ribosomes and ribosomal subunits. *Proc. Natl. Acad. Sci. USA* 95:7391-7395.
- Boulton, S.J., A. Gartner, J. Reboul, P. Vaglio, N. Dyson, D.E. Hill, and M. Vidal. 2002. Combined functional genomic maps of the *C. elegans* DNA damage response. *Science* 295:127-131.
- Brent, R. 2000. Genomic biology. *Cell* 100:169-183.
- Clackson, T., W. Yang, L.W. Rozamus, M. Hatada, J.F. Amara, C.T. Rollins, L.F. Stevenson, S.R. Magari et al. 1998. Redesigning an FKBP-ligand interface to generate chemical dimerizers with novel specificity. *Proc. Natl. Acad. Sci. USA* 95:10437-10442.
- Corbett, K.D. and T. Alber. 2001. The many faces of Ras: recognition of small GTP-binding proteins. *Trends Biochem. Sci.* 26:710-716.
- Davy, A., P. Bello, N. Thierry-Mieg, P. Vaglio, J. Hitti, L. Doucette-Stamm, D. Thierry-Mieg, J. Reboul et al. 2001. A protein-protein interaction map of the *Caenorhabditis elegans* 26S proteasome. *EMBO Rep.* 2:821-828.
- Degterev, A., A. Lugovskoy, M. Cardone, B. Mulley, G. Wagner, T. Mitchison, and J. Yuan. 2001. Identification of small-molecule inhibitors of interaction between the BH3 domain and Bcl-xL. *Nat. Cell Biol.* 3:173-182.
- Featherstone, C. and S.P. Jackson. 1999. Ku, a DNA repair protein with multiple cellular functions? *Mutat. Res.* 434:3-15.
- Gavin, A.C., M. Bosche, R. Krause, P. Grandi, M. Marzioch, A. Bauer, J. Schultz, J.M. Rick et al. 2002. Functional organization of the yeast proteome by systematic analysis of protein complexes. *Nature* 415:141-147.
- Ideker, T., T. Galitski, and L. Hood. 2001. A new approach to decoding life: systems biology. *Annu. Rev. Genomics Hum. Genet.* 2:343-372.
- Ideker, T., V. Thorsson, J.A. Ranish, R. Christmas, J. Buhler, J.K. Eng, R. Bumgarner, D.R. Goodlett et al. 2001. Integrated genomic and proteomic analyses of a systematically perturbed metabolic network. *Science* 292:929-934.
- Ito, T., T. Chiba, R. Ozawa, M. Yoshida, M. Hattori, and Y. Saka-ki. 2001. A comprehensive two-hybrid analysis to explore the yeast protein interactome. *Proc. Natl. Acad. Sci. USA* 98:4569-4574.
- Kolch, W. 2000. Meaningful relationships: the regulation of the Ras/Raf/MEK/ERK pathway by protein interactions. *Biochem. J.* 351:289-305.
- Kopytek, S.J., R.F. Standaert, J.C. Dyer, and J.C. Hu. 2000. Chemically induced dimerization of dihydrofolate reductase by a homobifunctional dimer of methotrexate. *Chem. Biol.* 7:313-321.
- Link, A.J., J. Eng, D.M. Schieltz, E. Carmack, G.J. Mize, D.R. Morris, B.M. Garvik, and J.R. Yates, III. 1999. Direct analysis of protein complexes using mass spectrometry. *Nat. Biotechnol.* 17:676-682.
- Mrowka, R., A. Patzak, and H. Herzog. 2001. Is there a bias in proteome research? *Genome Res.* 11:1971-1973.
- Norton, J.D. 2000. ID helix-loop-helix proteins in cell growth, differentiation, and tumorigenesis. *J. Cell Sci.* 113:3897-3905.
- O'Brien, M.L. and K.D. Tew. 1996. Glutathione and related enzymes in multidrug resistance. *Eur. J. Cancer* 32A:967-978.
- Page, A.M. and P. Hieter. 1999. The anaphase-promoting complex: new subunits and regulators. *Annu. Rev. Biochem.* 68:583-609.
- Park, S.J., E.J. Oh, M.A. Yoo, and S.H. Lee. 2001. Involvement of DNA-dependent protein kinase in regulation of stress-induced JNK activation. *DNA Cell Biol.* 20:637-645.
- Pearce, A.K. and T.C. Humphrey. 2001. Integrating stress-response and cell-cycle checkpoint pathways. *Trends Cell Biol.* 11:426-433.
- Peifer, M. and P. Polakis. 2000. Wnt signaling in oncogenesis and embryogenesis—a look outside the nucleus. *Science* 287:1606-1609.
- Ruscoe, J.E., L.A. Rosario, T. Wang, L. Gate, P. Arifoglu, C.R. Wolf, C.J. Henderson, Z. Ronai, and K.D. Tew. 2001. Pharmacologic or genetic manipulation of glutathione S-transferase P1-1 (GSTp1) influences cell proliferation pathways. *J. Pharmacol. Exp. Ther.* 298:339-345.
- Schwikowski, B., T. Ideker, and P. Uetz. 2002. Visualization and integration of protein-protein interactions. In E.A. Golemis (Ed.), *Protein Interactions*. CSH Laboratory Press, Cold Spring Harbor, NY.
- Sharma, S.V., C. Oneyama, Y. Yamashita, H. Nakano, K. Sugawara, M. Hamada, N. Kosaka, and T. Tamaoki. 2001. UCS15A, a non-kinase inhibitor of Src signal transduction. *Oncogene* 20:2068-2079.
- Shen, H., M.P. Schultz, and K.D. Tew. 1999. Glutathione conjugate interactions with DNA-dependent protein kinase. *J. Pharmacol. Exp. Ther.* 290:1101-1106.
- Staley, J.P. and C. Guthrie. 1998. Mechanical devices of the spliceosome: motors, clocks, springs, and things. *Cell* 92:315-326.
- Stanyon, C.A. and R.L. Finley, Jr. 2000. Progress and potential of Drosophila protein interaction maps. *Pharmacogenomics* 1:417-431.
- Tilley, J.W., G. Kaplan, K. Rowan, V. Schwinge, and B. Wolitzky. 2001. Imide and lactam derivatives of N-benzylpyroglutamate-L-phenylalanine as VCAM/VLA-4 antagonists. *Bioorg. Med. Chem. Lett.* 11:1-4.
- Uetz, P. and R.E. Hughes. 2000. Systematic and large-scale two-hybrid screens. *Curr. Opin. Microbiol.* 3:303-308.
- Walhout, A.J., R. Sordella, X. Lu, J.L. Hartley, G.F. Temple, M.A. Brasch, N. Thierry-Mieg, and M. Vidal. 2000. Protein interaction mapping in *C. elegans* using proteins involved in vulval development. *Science* 287:116-122.
- Wang, T., P. Arifoglu, Z. Ronai, and K.D. Tew. 2001. Glutathione S-transferase P1-1 (GSTP1-1) inhibits c-Jun N-terminal kinase (JNK1) signaling through interaction with the C terminus. *J. Biol. Chem.* 276:20999-21003.
- Young, K., S. Lin, L. Sun, E. Lee, M. Modi, S. Hellings, M. Husbands, B. Ozenberger, and R. Franco. 1998. Identification of a calcium channel modulator using a high throughput yeast two-hybrid screen. *Nat. Biotechnol.* 16:946-950.

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