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Chemical biology on PINs and NeeDLes

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Systematic studies of the organization of biochemical networks that make up the living cell can be defined by studying the organization and dynamics of protein interaction networks (PINs). Here, we describe recent conceptual and experimental advances that can achieve this aim and how chemical perturbations of interactions can be used to define the organization of biochemical networks. Resulting perturbation profiles and subcellular locations of interactions allow us to 'place' each gene product at its relevant point in a network. We discuss how experimental strategies can be used in conjunction with other genome-wide analyses of physical and genetic protein interactions and gene transcription profiles to determine network dynamic linkage (NDL) in the living cell. It is through such dynamic studies that the intricate networks that make up the chemical machinery of the cell will be revealed.

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Introduction

Genome sequencing efforts reveal the high number of genes with unknown function. Consequently, it is important to design new experimental strategies, which could be used to determine gene roles and interrelationships on a large scale. Biochemical pathways are networks of dynamically assembling and disassembling protein complexes. Therefore, a meaningful representation of a biochemical network in a living cell would be first, a step-by-step analysis of individual protein–protein interactions and second, analysis of the dynamics of interactions in response to perturbations that impinge upon the network under study and the time and spatial distribution of these interactions. What we seek are conceptual and experimental approaches that will tell us how, when, where and under what circumstances proteins are processed, modified, activated or inactivated, how they are destroyed and, central to all of these, what other molecules do they interact with.

In this review, we discuss three issues. First, we provide logical and mechanistic context to explain why the measurement of protein–protein interactions on a large-scale has become a crucial component of efforts to define gene function and the organization of biochemical networks. Second, we explain a conceptual basis for using protein interaction data along with other approaches such as genome-wide gene expression analysis and genetics to define the organization of biochemical networks. We specifically discuss how existing experimental techniques and one in particular, protein fragment complementation assays (PCAs), allow us to go beyond the static representations of protein interactions to make inferences on the organization of information flow in biochemical networks. Finally, we provide some perspectives on where protein interaction networks take us, with a discussion of their utility in chemical genetic analyses; the use of organic molecules to probe biochemical processes, and protein interactions as probes for the actions of such molecules on biochemical networks.

Protein–protein interactions and gene function

Even among very well studied organisms such as the budding yeast *Saccharomyces cerevisiae* and enteric bacterium *Escherichia coli*, classical genetic approaches have failed to assign even the most general cellular function to a significant proportion of genes, a far cry from getting specific functional inferences. The solution is to determine on a genomic scale, with assays amenable to that task, gene functions. To this end, the successful yeast two-hybrid system (Y2H) has been used to achieve first draft protein–protein interaction maps for three eukaryotes (yeast, *S. cerevisae*; nematode, *Caenorhabditis elegans*; fruitfly, *Drosophila melanogaster*) [1–4]. On the other hand, *in vitro* strategies to map out protein–protein interactions based on large-scale protein complex purification followed by mass-spectroscopic analysis have also been applied successfully to *S. cerevisae* [5,6]. Protein interaction networks (henceforth 'PINs') obtained in these studies provide a crucial first step towards mapping genes to functions. However, comparison of PINs, specifically for *S. cerevisae*, defined by these various studies and by classic approaches have revealed few of many potential interactions (many false-negatives) along with high false-positive rates [7•]. Although these observations might bring 'grist for the mill' to the usual critics of these methods, it must be recognized that any random screening approach for rare events (i.e. any genetic screen)

suffers from the same problems. More to the point, it must be emphasized that such data, limited as they are, provide a powerful framework for the inference of protein function and biochemical network organization. Below, we present ideas and published work that demonstrate how the static PINs generated by protein–protein interaction screening have been demonstrated to be useful and, further, concepts and methodologies that might help us to transform these static PINs into dynamic models of biochemical networks.

Inference of gene function and networks: combining large-scale data

There have been recent efforts to combine genomic-scale PINs with other information on genes, varying from data as disparate as co-evolution to covariance of gene expression under different conditions. Two particularly interesting and analogous approaches have been described recently. Starting from Bayesian or pseudo-Bayesian frameworks, strategies have been proposed to combine large-scale data to both predict and test the biological validity of protein–protein interactions [8••,9••]. These approaches provide reference test datasets for constructing hypotheses about the linkage between genes and their protein products and should serve as examples of formalisms for evaluating large-scale genomic efforts in the future.

Mapping the biochemical machinery of cells

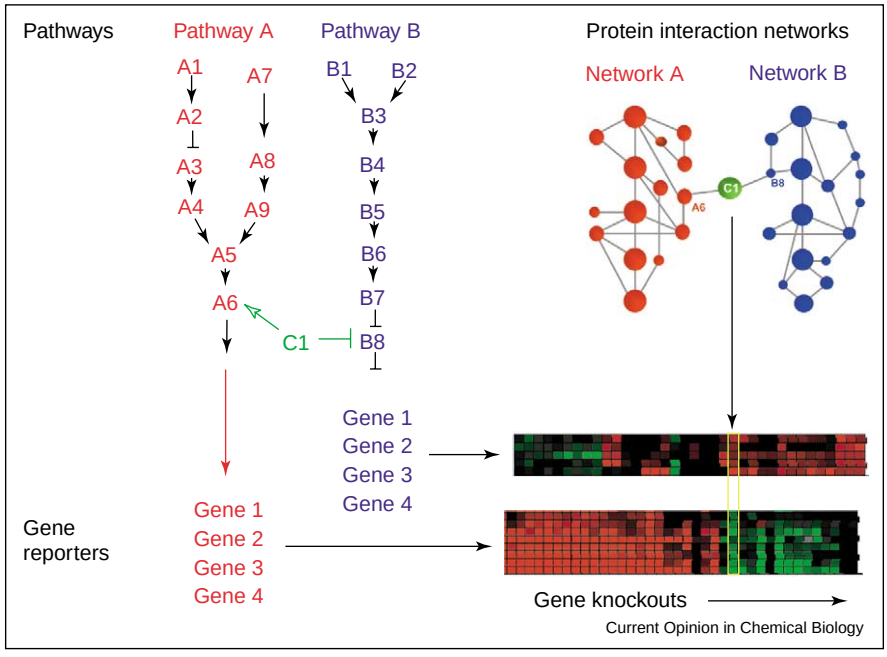
The potential application of genome-wide approaches to cellular biology is becoming a reality. For example, drawing upon systematic genome-wide analyses of *S. cerevisiae*, several groups have suggested that biochemical networks may be organized as modules of physically or genetically linked genes that contribute to a particular cellular function [10,11,12••,13,14•,15,16••], and it has been proposed that such analyses could be used to identify cellular processes to which specific genes are linked and upon which cellular, including chemical perturbations act [17–21,22•–24•,25].

It is first instructive to examine how biochemical networks have been examined at a genome-wide scale by examining patterns of gene transcription. The combination of DNA microarray to monitor gene transcription with ways to systematically perturb a biochemical network allow, in principle, the inference of a network's organization (Figure 1). The most well described analyses of gene expression have been performed for *S. cerevisiae*, for which microarrays representing all predicted genes have been available for some time. The key practical issue is whether a set of specific perturbations is possible. In yeast this is relatively simple, thanks to the availability of a systematic set of gene knockouts, whereas for other organisms small interfering RNAs (siRNAs) are becoming the most common approach to network perturbation [26]. However, this transcriptocentric approach to mapping networks has its pitfalls, in particular that the details of the underlying machinery leading to specific outputs of a system are not known. Thus, any new insights remain as inferences that must still be tested directly. This is where PINs become an important component of a more detailed analysis of biochemical networks. For example, it was Ideker and colleagues who first described how a PIN can be used to guide a systematic analysis of a biochemical network, using a unique metabolic response, the switch from glucose to galactose as carbon source in yeast as an example [27••]. In their strategy, a PIN is constructed for all known interactions for proteins, which are either known to interact or might be inferred to interact on the basis of common changes in expression in response to the glucose–galactose switch. Combining these data with examinations of changes in protein expression and knockouts of individual genes, they were able to both reproduce what was known about the glucose–galactose response, and to identify some proteins whose role in the response had not been shown. Thus, the PIN constructed for the glucose–galactose response genes served as a template for generating and testing hypotheses about the organization of a biochemical network underlying a simple but important biological adaptation. Similarly, Yao and colleagues used PINs and the complete set of single knockout yeast strains as the framework for the detailed analysis of the biochemical networks underlying the vertebrate aryl receptor response [16••]. This study elegantly demonstrated the powerful synergy between protein–protein interactions and computer and genetic approaches to delineate the different genes involved in aryl receptor signaling and to propose specific roles in this receptor function.

PIN-free chemical genetic approaches to mapping molecules to protein targets

Although this review deals mostly with PIN-based analyses of biochemical network organization, important recent genetic and transcription-based studies are worth noting, because of the impact they could have in tackling PIN inference in the future. The combination of genomic data and DNA microarrays now enable, in theory, testing of all yeast genes for interactions with selected drugs to confirm or uncover their mechanism of action (reviewed in this issue by Armour and Lum). A first strategy is to use the exhaustive yeast haploid bank to screen for strains sensitized to drugs or compounds [23•,24•,25]. In one such study, 78 compounds were screened for haploinsufficiency against 3503 heterozygous deletions strains [24•]. Of the 78 compounds tested, 56 resulted in the identification of a small number of potential target genes. In this paper, deeper validations of putative cellular targets for molsidomine, a vasodilator, and 5-fluoro-uracil (5-FU), an anticancer agent, are presented. Results suggest that 5-FU acts on the exosome and disrupts normal maturation of rRNA, in contradiction with the widely held view that it acts as a competitive inhibitor of thymidylate synthase.

Figure 1



Combining gene expression profiles and protein interaction networks to determine the organization of biochemical pathways. Two related pathways (A and B) are thought to be organized as depicted (upper left). The role of each protein as an activator (arrows) or inhibitor (T-bars) of the pathway is indicated. Interactions of the component proteins of these pathways (upper right) suggest a modular organization in which an additional protein (C1) has been identified, linking the two pathways. To confirm the organization of these pathways and role of each protein, the expression of reporter genes that are activated by the individual pathways is monitored (lower right). Relative expression levels are determined for wild type *versus* cells in which individual pathway component proteins have been knocked out. The position in the pathway and role of the novel pathway integrator C1 are determined. First, the position of C1 in the pathways is determined by which proteins in the two pathways C1 interacts with (A6 and B8). Second, the role of C1 is determined by comparing reporter gene expression in wild type and cells in which C1 is knocked out (lower right, yellow rectangle). Knocking out C1 results in an increase in expression of pathway B reporter genes (red squares) and a decrease in expression of pathway A reporter genes (green squares). Expression profile results are consistent with an inhibitor role of C1 on pathway B and an activating role on pathway A as depicted.

This conclusion is supported by an independent study using similar methods [23•].

Despite the caveats mentioned above, DNA microarray analyses can still validate the mechanisms of action of small-molecule inhibitors. For example, a recent paper presents the discovery of a molecule, dubbed Uretupamine, found by screening an arrayed combinatorial chemical library for binding to the yeast protein Ure2p [22•]. Strikingly, these authors found that treatment with this small molecule leads to Ure2p-dependant increase in the expression of a subset of glucose-responsive genes under its control, but not of non-glucose-dependant genes. Similarly, small-molecule libraries were screened in yeast for their antagonizing potency toward rapamycin, to identify novel components of Tor signaling pathway [28•]. Two compounds were discovered and were shown to target, respectively, the yeast homologue of PTEN (PtdIns(3,4)P_2 phosphatase) and a protein of unknown function, also apparently involved in phosphoinositide signaling. Interestingly, we had previously reported a potential cross-talk between Tor and receptor tyrosine kinase pathways through PI3K signaling in mammalian cells, as determined from chemical perturbation of this PIN [29••]. These results highlight the fact that small-molecule modulators can potentially go one step further than regular genetic methods in network inferences (reviewed in this issue by Butcher and Schreiber).

Because the methods described above require no *a priori* knowledge of a drug mechanism, they could be useful for determining protein targets, but also to eliminate compounds with pleiotropic effects or to directly screen chemical libraries for novel or improved drug properties. Another positive consequence of these studies is that as more 'old' drugs get attributed a defined mechanism, they become useful tools for interaction network inference as described below.

Other PIN perturbation strategies

Treatments of cells with growth-factors or hormones, or a switch in growth media culture composition are classic means of changing cell state and thus, perturbing PINs. In mammalian cell models, the overexpression of genes and siRNA technology are currently the main strategies amenable to large-scale-induced perturbation of PINs

[30]. In a recent study, Plavec *et al.* used an ELISA assay to detect the change in expression of seven inflammatory response genes induced by treatment with three cytokines (e.g. comparison between no treatment and treatment with IL-1β, TNF-α and IFN-γ) in the context of primary endothelial cells overexpressing either of 24 proteins (such as TNF-α receptor type I, constitutive active form of RAS, dominant negative of SHP2, etc) potentially implicated in pathways regulating the inflammation process [31]. The 28 data points harvested for each overexpressed proteins were concatenated, clustered and used to link proteins displaying similar response patterns. Results confirmed known links for genes involved in the same pathway, but also novel relationships between pathways.

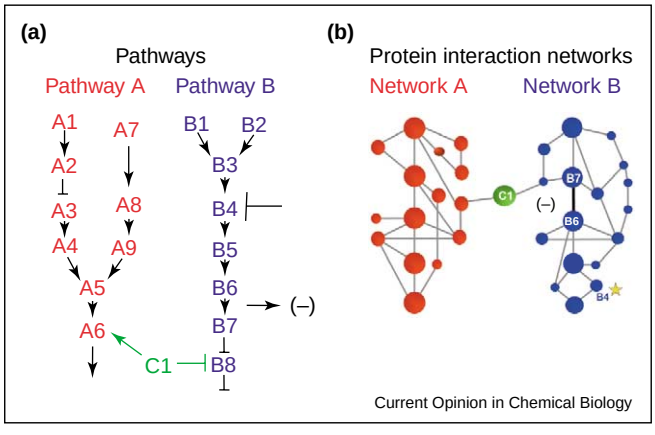
Another strategy with promising possibilities relies on exogenous homodimerizing and heterodimerizing domains based on FK506 binding protein that had been designed to induce a pharmacologically controlled dimerizing event in cellular pathways [32,33]. For example, the consequences of agonist-independent recruitment of β-arrestin to a G-protein-coupled receptor on MAPK signaling and receptor recycling have been recently reported using this system [34].

From static to dynamic PINS

The holy grail of chemical biology is to develop a small-molecule modulator for every protein function. More modestly, and in the perspective of network inferences, combinations of genetic and chemical approaches and careful selection of prioritized targets could provide a more efficient way to understand how these affect not just their primary target, but individual or multiple biochemical pathways. In the examples described above, PINs are either not used or are treated as static entities. Therefore, it would be of tremendous value if the interactions themselves could be studied as dynamic entities, what we will call network dynamic linkages (NDLs). Specifically, if we could understand where, when and how interactions are occurring in a living cell, we could use these as sources of information not merely as a framework for constructing a static map of biochemical networks, but a dynamic description of information flow through networks. The development of assays to follow protein–protein interactions dynamically *in vivo* in their normal cellular context (e.g. sub-cellular localization) has been spectacular in the past decade (reviewed in [35,36]). Our laboratory has developed a general strategy for monitoring the dynamics of protein–protein interactions *in vivo* and in real-time called protein fragment complementation assays (PCA) [29••,37-46].

In a PCA-based biochemical network mapping, specific protein–protein interactions, between proteins that interact at various strategic points in a network serve as 'sentinels' for the state of the network under different conditions (Figure 2). First, cells containing PCA sentinels are treated with agents (chemical inhibitors, siRNAs, hormones, etc.) that would be thought to perturb the biochemical network under study. A change in the PCA sentinels' reporter signal would then reveal what the relationship is between the point of action of the perturbing agent (say some enzyme in a subnetwork or 'module') and the sentinels. So for instance, if an enzyme were inhibited with a small molecule and the sentinel signal decreased, we could hypothesize that this enzyme must

Figure 2



Using dynamic perturbations of protein–protein interactions to infer the organization of pathways. **(a)** The actions of an inhibitory perturbing agent (e.g. a small-molecule inhibitor, classic genetic approach or siRNA) that acts on protein B4 (T-bar), is detected downstream by a change in the interaction of proteins B6 and B7 with each other (arrow). In this case, the effect of the perturbation is a decrease in the number of interacting proteins (–) as detected by a reporter of that interaction (output signal of the interaction as detected by PCA sentinel, for instance). However, the effect could equally be positive, depending on the consequences of inhibiting the upstream protein. **(b)** Within the PIN for Pathway B, a perturbation of protein B4 (star) somehow propagates through the network to somehow affect the link (wide bar) between proteins B6 and B7. This does not imply that protein B4 physically interacts with either B6 or B7; the propagation of an effect through the PIN may be due to direct physical links or to enzymatic processes not obvious in the PIN.

be somehow positively coupled to the function of the sentinel proteins. A series of perturbations within individual modules in the network would result in a pattern of responses or 'pharmacological profile', as detected by PCA, which should be consistent with the response of the network under study. Second, interactions of protein components of a network should take place in specific sub-cellular compartments or locations consistent with their function. The combined pharmacological profiles and sub-cellular interaction patterns serve then to describe a biochemical network [29••,47,48•].

Having described the network mapping process, there are immediate applications of this approach to expanding network structure by adding novel components. The cellular targets of small-molecules and cross-talks between signaling pathways can be revealed by using a PCA sentinel approach [29••,49]. Recently, we used this method to show that insulin and TGF-β signaling pathways are linked to each other through interaction of PKB and SMAD3, which was perturbed by hormones, small molecules and siRNAs that activate or inhibit one pathway or the other [48••]. A much more ambitious effort is underway to map the actions of a large number of drugs and siRNAs to multiple cellular biochemical networks, as revealed by sentinel PCAs (JK Westwick, personal communication). This study reveals how both predicted and novel actions of small molecules and gene knockdowns emanate from their sites of action to exert either very specific effects on individual or several pathways as well as surprising pleiotropic effects of what are thought to be specifically acting molecules.

New generation of biosensors

In addition to the detection of protein–protein interactions, the study of PIN dynamics would benefit from using biosensors that detect enzymatic activity or activation/inactivation state, cofactor or second-messenger level in a spatio-temporal manner. The classic *in vivo* biosensors are based on intramolecular fluorescence resonance energy transfer (FRET) between genetically encoded reporters based on green fluorescent protein (reviewed in [50]). First examples were the Cameleon calcium-sensing reporters [51]. Using the same principle, sensors of kinase activity were designed for protein kinase A, tyrosine kinase and protein kinase C [52–54]. These sensors enable high spatial and temporal detection of kinase activities in non-disrupted cells.

The development of fluorescent dyes reacting with cysteine residues has also led to the development of versatile sensors. The FlAsH and ReAsH compounds are arsenic derivatives that react with the motif -Cys-Cys-Xaa-Xaa-Cys-Cys-, which confer apparently sufficient specificity to be used directly on cells expressing proteins tethered to the tetracysteine tag. Using both dyes in pulse labeling experiments in live cells, Gaietta *et al.* proposed a model for assembly of gap junction in which new molecules are added at the periphery and old ones are removed at the centre [55]. Another reactant based on coumarin fluorescence, which conjugates on two cysteine (located 10 Å apart on a helical peptide) through two maleimide moieties, has been recently reported [56]. This dye is less toxic and does not necessitate, in principle, special care to protect cells. However, it has not yet been applied *in vivo*.

A new fluorescent dye with interesting properties for studying protein–protein interactions has been reported. The molecule I-SO reacts with a single cysteine and was designed to have an increased quantum yield in a non-polar environment, such as a buried protein–protein interface, a rare property of fluorescent dye [57]. Residues 201 to 321 of N-WASP that included the GBD domain, which interacts with GTP-loaded CDC42, were mutated to leave only one I-SO reactive cysteine at position 271. This residue is buried in a hydrophobic pocket formed by CDC42-GTP and N-WASP. As predicted, the fluorescence intensity of the biosensor increased threefold in the presence of active CDC42. Injection of this biosensor in live cells enables following and localizing endogenous CDC42 activity [58•]. This dye is versatile and could be used to develop several useful biosensors. Limitations are that the reporter polypeptide has to be purified and that it should have only one exposed cysteine to avoid spurious reaction with the dye.

Similarly to PCA, biosensors could be used in principle as reporters of signaling pathways or cell state. However, it is not clear if the aforementioned biosensors are all amenable to large-scale study. In the future, it should be a criterion to consider in the design of these types of technologies.

Conclusions and perspectives

We have attempted here to provide an overview of how the construction of PINs can and will continue to guide efforts to provide the basis for gene function assignment and for studying biochemical networks. Static PINs as provided by two-hybrid and mass-spectroscopic approaches will have an enduring place in these efforts, while PCA-based approaches will allow us to 'place' each gene product at its relevant point in a network and define the dynamic organization of the network. Use of small-molecule modulators and other perturbations (e.g. overexpression of wild type, dominant-negative or constitutively active forms of enzymes, receptor treatment with agonist or antagonist, artificially triggered dimerizing event, siRNAs or classic genetic approach) could be used to generate a functional profile. In addition, the development of biosensors that detect the level of specific enzymatic activities, second-messenger or cofactor concentration in real time is also important to improve our capacity to model PIN dynamics. In conclusion, the capacity to form inferences about the

dynamic organization of PINs is a first step toward proposing models of cellular biochemical networks.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Uetz P, Giot L, Cagney G, Mansfield TA, Judson RS, Knight JR, Lockshon D, Narayan V, Srinivasan M, Pochart P *et al.*: **A comprehensive analysis of protein-protein interactions in *Saccharomyces cerevisiae***. *Nature* 2000, **403**:623-627.

2. Ito T, Chiba T, Ozawa R, Yoshida M, Hattori M, Sakaki Y: **A comprehensive two-hybrid analysis to explore the yeast protein interactome**. *Proc Natl Acad Sci USA* 2001, **98**:4569-4574.

3. Li S, Armstrong CM, Bertin N, Ge H, Milstein S, Boxem M, Vidalain PO, Han JD, Chesneau A, Hao T *et al.*: **A map of the interactome network of the metazoan *C. elegans***. *Science* 2004, **303**:540-543.

4. Giot L, Bader JS, Brouwer C, Chaudhuri A, Kuang B, Li Y, Hao YL, Ooi CE, Godwin B, Vitols E *et al.*: **A protein interaction map of *Drosophila melanogaster***. *Science* 2003, **302**:1727-1736.

5. Gavin AC, Bosche M, Krause R, Grandi P, Marzioch M, Bauer A, Schultz J, Rick JM, Michon AM, Cruciat CM *et al.*: **Functional organization of the yeast proteome by systematic analysis of protein complexes**. *Nature* 2002, **415**:141-147.

6. Ho Y, Gruhler A, Heilbut A, Bader GD, Moore L, Adams SL, Millar A, Taylor P, Bennett K, Boutilier K *et al.*: **Systematic identification of protein complexes in Saccharomyces cerevisiae by mass spectrometry**. *Nature* 2002, **415**:180-183.

7. • von Mering C, Krause R, Snel B, Cornell M, Oliver SG, Fields S, Bork P: **Comparative assessment of large-scale data sets of protein-protein interactions**. *Nature* 2002, **417**:399-403.

This study analyses and compares the results of genomic-scale protein–protein interaction studies in yeast, including discussion of false-negatives and false positives.

8. •• Jansen R, Yu H, Greenbaum D, Kluger Y, Krogan NJ, Chung S, Emili A, Snyder M, Greenblatt JF, Gerstein M: **A Bayesian networks approach for predicting protein-protein interactions from genomic data**. *Science* 2003, **302**:449-453.

This study describes how different large-scale datasets can be combined in a Bayesian statistical framework to predict protein–protein interactions.

9. •• Lee I, Date SV, Adai AT, Marcotte EM: **A probabilistic functional network of yeast genes**. *Science* 2004, **306**:1555-1558.

An important example of how different large-scale datasets can be combined in a Bayesian statistical framework to test the biological validity of protein functional links.

10. Hartwell LH, Hopfield JJ, Leibler S, Murray AW: **From molecular to modular cell biology**. *Nature* 1999, **402**:C47-C52.

11. Winzeler EA, Shoemaker DD, Astromoff A, Liang H, Anderson K, Andre B, Bangham R, Benito R, Boeke JD, Bussey H *et al.*: **Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis**. *Science* 1999, **285**:901-906.

12. •• Ihmels J, Friedlander G, Bergmann S, Sarig O, Ziv Y, Barkai N: **Revealing modular organization in the yeast transcriptional network**. *Nat Genet* 2002, **31**:370-377.

A particularly thoughtful study, which reveals the pitfalls of hierarchical clustering in the analysis of transcriptional networks and describes an improved method for classifying individual genes into multiple gene network modules.

13. Snel B, Bork P, Huynen MA: **The identification of functional modules from the genomic association of genes**. *Proc Natl Acad Sci USA* 2002, **99**:5890-5895.

14. • Rives AW, Galitski T: **Modular organization of cellular networks**. *Proc Natl Acad Sci USA* 2003, **100**:1128-1133.

This works describes construction of PINs for yeast signal transduction pathways.

15. Tong AH, Lesage G, Bader GD, Ding H, Xu H, Xin X, Young J, Berriz GF, Brost RL, Chang M *et al.*: **Global mapping of the yeast genetic interaction network**. *Science* 2004, **303**:808-813.

16. •• Yao G, Craven M, Drinkwater N, Bradfield CA: **Interaction networks in yeast define and enumerate the signaling steps of the vertebrate aryl hydrocarbon receptor**. *PLoS Biol* 2004, **2**:E65.

This study identifies proteins that affect aryl receptor signaling by screening a bank of haploid yeast strains. Using previously determined yeast PINs, genes involved are found to constitute a connected network of protein interactions. Combination of simple perturbation experiment with chimeric forms of the receptor and computer tools delineates sub-groups in the PIN and their specific roles in the aryl receptor signaling.

17. Hartwell LH, Szankasi P, Roberts CJ, Murray AW, Friend SH: **Integrating genetic approaches into the discovery of anticancer drugs**. *Science* 1997, **278**:1064-1068.

18. Marton MJ, DeRisi JL, Bennett HA, Iyer VR, Meyer MR, Roberts CJ, Stoughton R, Burchard J, Slade D, Dai H *et al.*: **Drug target validation and identification of secondary drug target effects using DNA microarrays**. *Nat Med* 1998, **4**:1293-1301.

19. Giaever G, Shoemaker DD, Jones TW, Liang H, Winzeler EA, Astromoff A, Davis RW: **Genomic profiling of drug sensitivities via induced haploinsufficiency**. *Nat Genet* 1999, **21**:278-283.

20. Hardwick JS, Kuruvilla FG, Tong JK, Shamji AF, Schreiber SL: **Rapamycin-modulated transcription defines the subset of nutrient-sensitive signaling pathways directly controlled by the Tor proteins**. *Proc Natl Acad Sci USA* 1999, **96**:14866-14870.

21. Hughes TR, Marton MJ, Jones AR, Roberts CJ, Stoughton R, Armour CD, Bennett HA, Coffey E, Dai H, He YD *et al.*: **Functional discovery via a compendium of expression profiles**. *Cell* 2000, **102**:109-126.

22. • Kuruvilla FG, Shamji AF, Sternson SM, Hergenrother PJ, Schreiber SL: **Dissecting glucose signalling with diversity-oriented synthesis and small-molecule microarrays**. *Nature* 2002, **416**:653-657.

This study demonstrates the utilization of arrayed combinatorial chemical libraries for finding a small-molecule modulator for a regulator of glucose metabolism in yeast, Ure2p. Interestingly, this modulator represses only a subset of genes under the control of Ure2p.

23. • Giaever G, Flaherty P, Kumm J, Proctor M, Nislow C, Jaramillo DF, Chu AM, Jordan MI, Arkin AP, Davis RW: **Chemogenomic profiling: identifying the functional interactions of small molecules in yeast**. *Proc Natl Acad Sci USA* 2004, **101**:793-798.

Use of pooled culture of almost exhaustive heterozygous yeast strain for screening 10 drugs and compounds for haploinsufficiency to determine their modes of action.

24. • Lum PY, Armour CD, Stepaniants SB, Cavet G, Wolf MK, Butler JS, Hinshaw JC, Garnier P, Prestwich GD, Leonardson A *et al.*: **Discovering modes of action for therapeutic compounds using a genome-wide screen of yeast heterozygotes**. *Cell* 2004, **116**:121-137.

Use of pooled culture of a very large set of heterozygous yeast strain for screening 78 drugs and compounds for haploinsufficiency to determine their modes of action.

25. Parsons AB, Brost RL, Ding H, Li Z, Zhang C, Sheikh B, Brown GW, Kane PM, Hughes TR, Boone C: **Integration of chemical-genetic and genetic interaction data links bioactive compounds to cellular target pathways**. *Nat Biotechnol* 2004, **22**:62-69.

26. McManus MT, Sharp PA: **Gene silencing in mammals by small interfering RNAs**. *Nat Rev Genet* 2002, **3**:737-747.

27. •• Ideker T, Thorsson V, Ranish JA, Christmas R, Buhler J, Eng JK, Bumgarner R, Goodlett DR, Aebersold R, Hood L: **Integrated**

genomic and proteomic analyses of a systematically perturbed metabolic network. *Science* 2001, **292**:929-934.
A pioneering example of integrating different sources of data such as direct protein–protein interaction, gene knockout effect and coordination in expression level to reproduce and expand the PIN for the glucose–galactose switch pathway in yeast.

28. Huang J, Zhu H, Haggarty SJ, Spring DR, Hwang H, Jin F, Snyder M, Schreiber SL: **Finding new components of the target of rapamycin (TOR) signaling network through chemical genetics and proteome chips**. *Proc Natl Acad Sci USA* 2004, **101**:16594-16599.

29. •• Remy I, Michnick SW: **Visualization of biochemical networks in living cells**. *Proc Natl Acad Sci USA* 2001, **98**:7678-7683.
First example of how PCAs combined with chemical perturbations can be used to infer and identify novel links in biochemical networks.

30. Paddison PJ, Silva JM, Conklin DS, Schlabach M, Li M, Aruleba S, Balija V, O'Shaughnessy A, Gnoj L, Scobie K *et al.*: **A resource for large-scale RNA-interference-based screens in mammals**. *Nature* 2004, **428**:427-431.

31. Plavec I, Sirenko O, Privat S, Wang Y, Dajee M, Melrose J, Nakao B, Hytopoulos E, Berg EL, Butcher EC: **Method for analyzing signaling networks in complex cellular systems**. *Proc Natl Acad Sci USA* 2004, **101**:1223-1228.

32. Clackson T, Yang W, Rozamus LW, Hatada M, Amara JF, Rollins CT, Stevenson LF, Magari SR, Wood SA, Courage NL *et al.*: **Redesigning an FKBP-ligand interface to generate chemical dimerizers with novel specificity**. *Proc Natl Acad Sci USA* 1998, **95**:10437-10442.

33. Rollins CT, Rivera VM, Woolfson DN, Keenan T, Hatada M, Adams SE, Andrade LJ, Yaeger D, van Schravendijk MR, Holt DA *et al.*: **A ligand-reversible dimerization system for controlling protein-protein interactions**. *Proc Natl Acad Sci USA* 2000, **97**:7096-7101.

34. Terrillon S, Bouvier M: **Receptor activity-independent recruitment of betaarrestin2 reveals specific signalling modes**. *EMBO J* 2004, **23**:3950-3961.

35. Tsien RY: **Imagining imaging's future**. *Nat Rev Mol Cell Biol* 2003, **(Suppl)**:SS16-SS21.

36. Michnick SW, Remy I, Campbell-Valois FX, Vallee-Belisle A, Pelletier JN: **Detection of protein-protein interactions by protein fragment complementation strategies**. *Methods Enzymol* 2000, **328**:208-230.

37. Pelletier JN, Campbell-Valois FX, Michnick SW: **Oligomerization domain-directed reassembly of active dihydrofolate reductase from rationally designed fragments**. *Proc Natl Acad Sci USA* 1998, **95**:12141-12146.

38. Remy I, Michnick SW: **Clonal selection and *in vivo* quantitation of protein interactions with protein-fragment complementation assays**. *Proc Natl Acad Sci USA* 1999, **96**:5394-5399.

39. Galarneau A, Primeau M, Trudeau LE, Michnick SW: **Beta-lactamase protein fragment complementation assays as *in vivo* and *in vitro* sensors of protein protein interactions**. *Nat Biotechnol* 2002, **20**:619-622.

40. Ghosh I, Hamilton AD, Regan L: **Antiparallel leucine zipper-directed protein reassembly: application to the green fluorescent protein**. *J Am Chem Soc* 2004, **122**:5658-5659.

41. Paulmurugan R, Umezawa Y, Gambhir SS: **Noninvasive imaging of protein-protein interactions in living subjects by using reporter protein complementation and reconstitution strategies**. *Proc Natl Acad Sci USA* 2002, **99**:15608-15613.

42. Hu CD, Chinenov Y, Kerppola TK: **Visualization of interactions among bZIP and Rel family proteins in living cells using bimolecular fluorescence complementation**. *Mol Cell* 2002, **9**:789-798.

43. Wehrman T, Kleaveland B, Her JH, Balint RF, Blau HM: **Protein-protein interactions monitored in mammalian cells via complementation of beta-lactamase enzyme fragments**. *Proc Natl Acad Sci USA* 2002, **99**:3469-3474.

44. Spotts JM, Dolmetsch RE, Greenberg ME: **Time-lapse imaging of a dynamic phosphorylation-dependent protein-protein interaction in mammalian cells**. *Proc Natl Acad Sci USA* 2002, **99**:15142-15147.

45. Paulmurugan R, Gambhir SS: **Monitoring protein-protein interactions using split synthetic renilla luciferase protein-fragment-assisted complementation**. *Anal Chem* 2003, **75**:1584-1589.

46. Luker KE, Smith MC, Luker GD, Gammon ST, Piwnica-Worms H, Piwnica-Worms D: **Kinetics of regulated protein-protein interactions revealed with firefly luciferase complementation imaging in cells and living animals**. *Proc Natl Acad Sci USA* 2004, **101**:12288-12293.

47. Remy I, Michnick SW: **Regulation of apoptosis by the Ft1 protein, a new modulator of protein kinase B/Akt**. *Mol Cell Biol* 2004, **24**:1493-1504.

48. •• Remy I, Montmarquette A, Michnick SW: **PKB/Akt modulates TGF-beta signalling through a direct interaction with Smad3**. *Nat Cell Biol* 2004, **6**:358-365.
Describes how physical interactions between two proteins and perturbations of multiple upstream pathways led to the discovery of a simple mechanism, integrating two competing signal transduction pathways.

49. Remy I, Michnick SW: **Dynamic visualization of expressed gene networks**. *J Cell Physiol* 2003, **196**:419-429.

50. Zhang J, Campbell RE, Ting AY, Tsien RY: **Creating new fluorescent probes for cell biology**. *Nat Rev Mol Cell Biol* 2002, **3**:906-918.

51. Miyawaki A, Llopis J, Heim R, McCaffery JM, Adams JA, Ikura M, Tsien RY: **Fluorescent indicators for Ca^{2+} based on green fluorescent proteins and calmodulin**. *Nature* 1997, **388**:882-887.

52. Zhang J, Ma Y, Taylor SS, Tsien RY: **Genetically encoded reporters of protein kinase A activity reveal impact of substrate tethering**. *Proc Natl Acad Sci USA* 2001, **98**:14997-15002.

53. Ting AY, Kain KH, Klemke RL, Tsien RY: **Genetically encoded fluorescent reporters of protein tyrosine kinase activities in living cells**. *Proc Natl Acad Sci USA* 2001, **98**:15003-15008.

54. Violin JD, Zhang J, Tsien RY, Newton AC: **A genetically encoded fluorescent reporter reveals oscillatory phosphorylation by protein kinase C**. *J Cell Biol* 2003, **161**:899-909.

55. Gaietta G, Deerinck TJ, Adams SR, Bouwer J, Tour O, Laird DW, Sosinsky GE, Tsien RY, Ellisman MH: **Multicolor and electron microscopic imaging of connexin trafficking**. *Science* 2002, **296**:503-507.

56. Girouard S, Houle MH, Grandbois A, Keillor JW, Michnick S: **Synthesis and characterization of dimaleimide fluorogens designed for specific labeling of proteins**. *J Am Chem Soc*, in press.

57. Toutchkine A, Kraynov V, Hahn K: **Solvent-sensitive dyes to report protein conformational changes in living cells**. *J Am Chem Soc* 2003, **125**:4132-4145.

58. • Nalbant P, Hodgson L, Kraynov V, Toutchkine A, Hahn KM: **Activation of endogenous Cdc42 visualized in living cells**. *Science* 2004, **305**:1615-1619.
A fluorescent dye whose signal is sensitive to the polar environment is described. Application of this dye to follow spatially and temporally the activation level of CDC42 in intact cells is also described.