Making connections for life: an *in vivo* map of the yeast interactome

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Proteins are the true workhorses of any cell. To carry out specific tasks, they frequently bind other molecules in their surroundings. Due to their structural complexity and flexibility, the most diverse array of interactions is seen with other proteins. The different geometries and affinities available for such interactions typically bestow specific functions on proteins. Having available a map of protein–protein interactions is therefore of enormous importance for any researcher interested in gaining insight into biological systems at the level of cells and organisms. In a recent report, a novel approach has been employed that relies on the spontaneous folding of complementary enzyme fragments fused to two different proteins to test whether these interact in their actual cellular context [Tarassov *et al.*, *Science* 320, 1465–1470 (2008)]. Genome-wide application of this protein-fragment complementation assay has resulted in the first map of the *in vivo* interactome of *Saccharomyces cerevisiae*. The current data show striking similarities but also significant differences to those obtained using other large-scale approaches for the same task. This warrants a general discussion of the current state of affairs of protein–protein interaction studies and foreseeable future trends, highlighting their significance for a variety of applications and their potential to revolutionize our understanding of the architecture and dynamics of biological systems. [DOI: 10.2976/1.2969243]

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To understand what defines a living organism, how it is organized, and how it interacts with its environment is the ultimate *raison d'être* of biology—literally meaning the study of life. It is now generally accepted that a direct link exists between an organism's genetic information and the characteristics central to the definition of life: the ability to grow and reproduce, to respond and adapt to its environment, and to maintain its status by performing chemical reactions via metabolism. As the genome of many organisms and its uniformity across different cells, individuals, and generations is established, we recognize that genomes are largely invariable so information can be passed on for generations without detrimental alteration. In contrast, the mRNA and protein complements of a genome in a cell, tissue, or organism are highly dynamic and continually change in composition. Consequently, research focus in the postgenomic era is shifting back to proteins as the functional gene products and to mRNA as their mediators. Indeed, recent reports on mRNA or protein abundance profiles correlating well with specific phenotypes attest to the biological relevance of this type of information. There is a catch, however, mRNA and protein abundance itself are not accurate measures of the functional state of a cell. A protein's biological activity is more aptly defined by its interactions with other molecules. Moreover, mounting cellular responses requires the coordination of a multitude of interactions that are assembled in protein complexes and functional pathways. Hence, it has been postulated that defining all of the protein–protein interactions that occur in a living organism will provide the most accurate description of its characteristics. Mapping such interactomes and deciphering their structure and dynamics therefore represents a crucial step on our way toward this ultimate goal.

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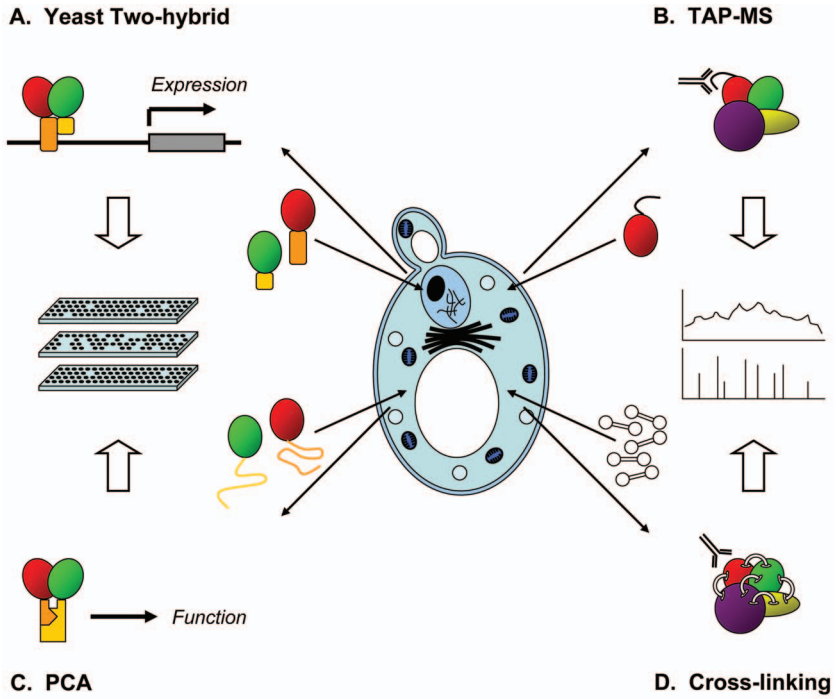

Figure 1. Available strategies for the large-scale analysis of protein–protein interactions in cells and organisms. (A) Yeast two-hybrid (Y2H) screens rely on the coexpression of pairs of proteins that have the binding domain and the activation domain of a transcription factor fused to them. If the two proteins have sufficient affinity to bind each other, the transcription factor will be reconstituted in the nucleus and trigger the expression of a reporter gene, leading to the synthesis of a protein whose presence can be read out on large arrays. (B) Tandem affinity purification and mass spectrometry (TAP-MS) methods involve the expression of a modified form of the endogenous or an exogenous form of a bait protein with a peptide sequence fused to it that can be employed for targeted affinity purification to enrich the bait protein and its interacting proteins from the cell lysate. Purified proteins are enzymatically digested into peptides that are analyzed by mass spectrometry to identify the respective interacting proteins. (C) PCAs are based on the simultaneous presence of two proteins fused to complementary fragments of a reporter protein, e.g., an enzyme, that folds and becomes functional when the two proteins interact in their natural cellular context. Conditional selection based on the restored enzyme activity can be used to highlight spots on large arrays on which colonies grow, indicating positive interaction. (D) Cross-linking strategies require the exposure of intact cells, or cell lysates, to chemical reagents that form covalent bonds between interacting proteins. Affinity purification of the bait protein based on an affinity tags or antibodies binding to the untagged, endogenous form are used to isolate the cross-linked protein assembly, which is then digested into peptides and characterized by mass spectrometry to identify the cross-linked proteins and their respective sites of interaction.

In a recent article in *Science*, Tarassov *et al.* describe the results of a genome-wide screen for protein–protein interactions they have performed in *Saccharomyces cerevisiae* (Tarassov *et al.*, 2008). They are relying on a method called protein-fragment complementation assay (PCA) to obtain a map of the yeast interactome. Exploiting several advantageous traits of this model organism, they incorporate one of two complementary fragments of the mutated murine enzyme dihydrofolate reductase (DHFR) into one gene at a time, thereby establishing two complementary sets of haploid mutant strains. Pairwise mating on a high-density array to produce diploids that express both DHFR fragments on two different proteins and exposing them to the drug methotrexate that inhibits the activity of yeast's own DHFR enzyme then leads to the death of all diploid strains that do not possess DHFR activity. Diploids can survive and grow only if they express two proteins that physically interact with each other, which allows the two complementary fragments of the inhibitor-insensitive murine DHFR to fold and form the functional enzyme. Systematic analysis of colony intensity thus reveals which pairs of yeast proteins interact in their correct cellular context, which can be assembled into an extensive map of the yeast interactome *in vivo*.

While Tarassov *et al.* present the first map of interactions occurring under normal conditions inside cells, the yeast interactome has also been mapped by other large-scale approaches (Fig. 1). The Y2H method employs many of the same steps as PCA to map the yeast interactome (Ito *et al.*, 2001; Ito *et al.*, 2000; Uetz *et al.*, 2000) but relies on the complementation of a transcription factor regulating the expression of an essential gene and conditional survival selection. This requires the screen to take place in the nucleus and eliminates many of the factors regulating protein–protein interactions, most notably protein localization to the same compartment, coregulated expression, possibility of posttranslational modifications, and competition with alternative interacting proteins. Consequently, the results of Y2H screens are often interpreted as two proteins having the po-

tential to interact with each other, information that needs to be confirmed by additional *in vivo* experimentation.

Biological validity is less of a concern for the second alternative technique, as it is generally appreciated that TAP-MS identifies true *in vivo* interactions when applied to yeast (Gavin *et al.*, 2006; Gavin *et al.*, 2002; Ho *et al.*, 2002; Krogan *et al.*, 2006). It does so by extracting and purifying proteins that form interactions inside cells along with their binding partners, followed by protein identification based on mass spectrometry. The need to break up cells to gain access to the bait proteins inevitably removes the cellular context of their interactions and generates dilute protein mixtures in solution, however. This sets off two opposing trends: existing interactions with high dissociation constants are likely to be missed as their yield decreases while additional interactions that did not exist in the cell form in the lysate if proteins possess sufficiently high association rates, i.e., high association constants or high relative concentrations. Consequently, cell lysis decreases true positive and increases false positive identifications over time. The latter is commonly considered more problematic and therefore combated by employing more stringent solution conditions to decrease the association constant and/or by performing two rounds of purification to reduce the concentration of contaminating proteins. As a result, TAP-MS only detects interactions strong enough to withstand the destructive conditions during purification, typically those found in stable protein complexes.

Realizing that each of these large-scale approaches has unique characteristics, one should not expect them to generate the same data. Indeed, Tarassov *et al.* demonstrate that the PCA method confirms between 16 and 41% of the interactions reported in previous screens. In contrast, about 80% of their interactions are new, and only 10% of their interactions match the two available TAP-MS maps completely, while for 36% of them only one, and for 38% neither one of the two interacting proteins are covered. This agrees with previous reports showing limited overlap already between the two TAP-MS data sets (Collins *et al.*, 2007) and demonstrates that this new map indeed expands our coverage of the yeast interactome. Moreover, the calculated predictive positive values (PPV) and true positive/false positive ratios reveal that the PCA and TAP-MS data sets are of similar high quality while Y2H approaches fair significantly worse (Tarassov *et al.*, 2008). This correlates well with TAP-MS also mapping interactions formed in their natural context in cells and indicates the leap forward that the PCA approach represents in the area of high-density array screens.

For the PCA approach to reach this level of data quality, several filtering steps were necessary. Over 15×10^6 matings in 3,247 screens were initially performed and led to the discovery of 2,770 interactions among 1,124 proteins, representing just below 20% of the 5,756 consensus sequences that had initially been cloned. In this process, 344 proteins were eliminated as potential false positives based on additional screens against the reporter fragments alone, and an additional 83 proteins were removed as their promiscuous interaction patterns resembled those of other highly expressed proteins contained in the previous set of 344 proteins. The final reported PCA data set is significantly smaller than those of the two TAP-MS approaches, which encompassed 2,760 proteins that partition into 491 complexes (Gavin *et al.*, 2006), and 2,708 proteins forming 7,123 interactions in 547 protein complexes and 429 additional interactions between complexes (Krogan *et al.*, 2006), respectively. The stringent data filtering may have removed many potentially valid interactions falling below the defined threshold, which would also explain the limited overlap. Additional comparison and integration of the PCA data set with those of Y2H and TAP-MS approaches could potentially restore connections within the network that may have been broken unintentionally in this process, which will help further refine the yeast interactome map.

Concerning the overall organization of the interactome, the PCA-based map confirms the results of the previous TAP-MS analyses suggesting the existence of a hierarchy of several interaction layers *in vivo*. In addition to clusters that correspond to well-characterized complexes, the authors observe subclusters that correlate with previously reported affinity-purified subcomplexes. As previous reports suggested the presence of multiple complexes of similar composition but distinct function (Gavin *et al.*, 2006), the detection of interactions present in all of them may be favored due to their higher abundance. Alternatively, this may be a reflection of the differences in binding strengths between subunits in a complex: core components typically show high affinity to each other, which will result in a stronger signal in TAP-MS as well as PCA, making their detection more likely than those loosely associated with the complex. Consistent with this notion, statistical analysis reveals a considerable enrichment of proteins that share cellular compartments, biological processes, or molecular functions, which may assist in the functional annotation of the 278 uncharacterized proteins and their 286 interactions.

Additionally, links are also observed between complexes that involve different biological processes, cellular compartments, and molecular functions (64, 56, and 63%). Semantic analysis of the gene ontology hierarchy of the proteins forming these interactions indicates that they are nonrandom. They must therefore represent connections between different processes and compartments, integrating them into higher-order networks that reflect a level of cellular organization beyond complexes and pathways. This observation is indeed significant and will provide a useful tool for the discovery of additional high-level regulation, which is demonstrated by the identification of new proteins and interactions that play a role in processes associated with the budneck and with autophagy. In comparison to the TAP-MS studies (Gavin *et al.*, 2006; Krogan *et al.*, 2006), these features are on average

5%–10% more prominent in the PCA data. A possible explanation for this observation may be the design of the PCA approach: diploid survival only requires reconstitution of minimal DHFR activity. This results in high sensitivity, allowing the detection of interactions formed by only 25–100 molecules per cell (Remy and Michnick, 1999). Indeed, statistical analysis shows that the proteins contained in the PCA map are only slightly more expressed than the whole yeast proteome and span the entire range of protein abundance, confirming this added benefit. While this validates the high quality of the final filtered data and demonstrates little bias toward abundant proteins, it also implies that survival may not require the formation of a strong and sustained interaction. The affinity of an interaction, its relative yield, and its duration of existence are likely contributing factors to the observed signal, i.e., the colony intensity. Normalizing the relative intensity of each colony to the abundance of the two interacting proteins may therefore give a better indication of the strength of an interaction. This may help distinguish permanent and high affinity interactions from more transient and lower affinity ones and provide an additional means of comparing PCA and TAP-MS data sets in the future.

The importance of adding qualifiers such as affinity, yield, and duration to individual protein–protein interactions becomes even more apparent when interaction maps are interpreted. Essentially, the experimental data is encoded in units of two nodes (proteins) being connected by an edge (interaction). This holds true for all interaction studies, regardless of whether they are using a one-to-one format (PCA, Y2H) or testing one-to-n associations (TAP-MS), as each experiment only connects bait and other protein nodes via individual interactions. In the TAP-MS approach, higher-order structures such as protein complexes are highlighted when groups of proteins copurify with several members of the complex serving as baits. Likewise, the PCA and Y2H approaches often identify clusters of high edge density that usually coincide with complexes.

For the visualization of higher-order structures, the assembly of individual interactions into a network is therefore essential. Nonetheless, the resulting map only represents a two-dimensional projection of an *n*-dimensional space, with two edges involving the same node demonstrating that one protein, or more precisely the product of one gene, interacts with two other proteins. Nothing is known about their spatial and temporal coexistence, their functional relationship, their interplay, or whether they bind a gene product with the same protein sequence or structure. Indeed, many different scenarios can be envisioned that cannot be distinguished in the interaction map, as they would result in the same two-dimensional projection (Fig. 2). Consequently, these biologically significant features need to be explored by other means, for example, by selecting a small area of the map and performing targeted biochemical studies. Such small-scale experiments have been carried out for a long time, and their results form the content of the Munich Information Center for Protein Sequences (MIPS) database that the authors have utilized to filter and benchmark their own data set. The lack of global context in these targeted studies makes interactome maps an important resource that provides a much needed framework for them.

Beyond providing a top-level view of a cell's organization, are there additional applications for such an interactome map? As it only provides a snapshot of a particular state, it is comparable to mapping mRNA levels, expression patterns of proteins, protein distributions in cells, or the presence of post-translational modifications, to name the most prominent examples. In each of these cases, only the generation and subsequent comparison of two or more states at varying conditions can reveal the dynamics of these systems and shed light onto the underlying regulatory mechanisms. This can sometimes be straightforward, as, for example, the processes underlying the yeast cell cycle are known in considerable detail. Analyzing the corresponding dynamics of the protein interaction network should therefore be an excellent test case. Indeed, Tarassov *et al.* have demonstrated that such information is accessible, as they have mapped interactions that involve processes at the budneck (Tarassov *et al.*, 2008). Applying external stimuli such as specific ligands that result in signaling through well-known pathways should be equally feasible. In both cases, sufficient information is available from small-scale experimentation to evaluate and optimize these approaches. The next step could involve testing the cellular responses of less well-characterized stimuli. For such studies to succeed, we will need to know the inherent flexibility of an interactome that forms the baseline dynamics, however. So far, we do not even know how many of the mapped interactions are actually indispensable for the survival of a cell or for maintaining a healthy status. If gene knockouts are any indication, there will be a range from "unnecessary" to "essential," likely correlating with the global organization of the cellular network and the availability of alternative pathways or with the involvement in a particular process or state. The classification into "party" and "date" hubs is only a first attempt at defining these features (Aloy and Russell, 2006).

The generation of dynamic protein interactome maps could also entail the identification of changes that occur as cells go through different phases of their life cycle, e.g., during maturation of stem cells or even the progression from a healthy state through various stages of disease development. This might reveal key players involved in these processes that could become targets of drug intervention. Indeed, information on the status of protein–protein interactions is highly desirable for drug development purposes in general. Drugs themselves often interact with proteins, e.g., by simulating natural receptor ligands as agonists or by preventing them from binding to their receptors as antagonists. Alternatively, drugs can act as enzyme inhibitors that block or alter an en-

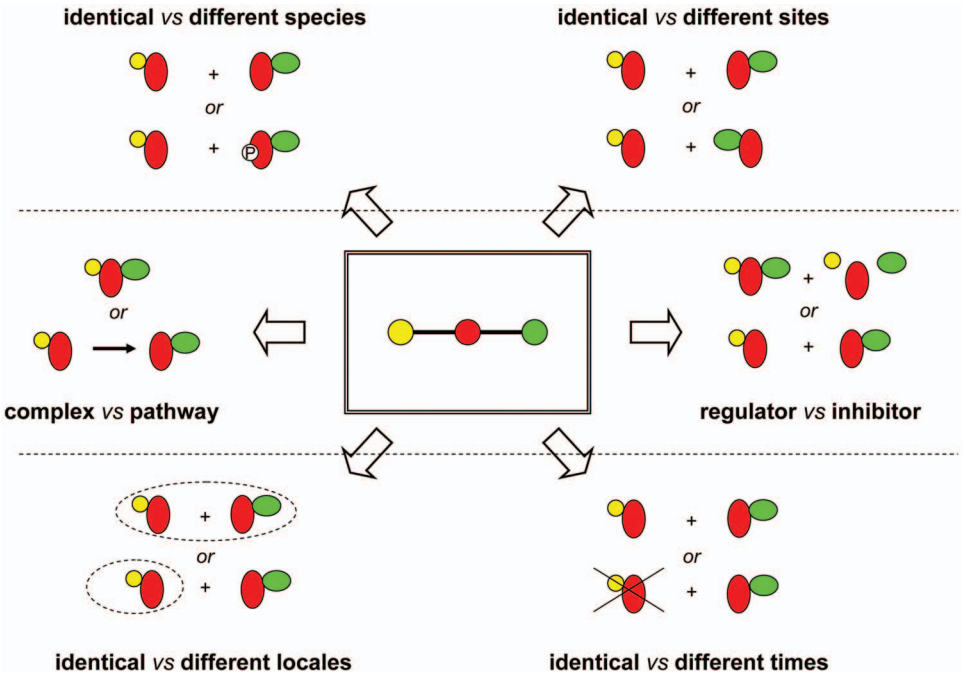

Figure 2. Examples for three-protein interactions resulting in a three-node-two-edge assembly in an interaction map. Top left: two proteins may bind to the same form of the bait protein or to two bait protein species that have different structures or sequences, e.g., caused by post-translational modifications such as phosphorylation. Top right: two proteins can associate via two different or the same binding site of the bait protein. Middle left: two proteins can bind the bait protein by forming a ternary complex or sequentially as part of pathway, e.g., in a kinase signaling cascade. Middle right: binding of a protein to the bait may be conditional upon binding of another protein such as a cofactor or regulator or could be prevented by the presence of another protein acting as inhibitor. Bottom left: two proteins could interact with the bait protein in the same cellular location or in two different compartments that are separated by a physical barrier such as a membrane. Bottom right: two proteins can bind to the bait protein independently of each other at the same time, or one of the interactions may be conditional upon the other not taking place at the same time, e.g., those that occur in two different phases of the cell cycle or in signaling cascades.

zyme's active site and prevent it from interacting with its usual substrates. Examples include traditional drugs, e.g., aspirin acetylating the active site of cyclo-oxygenase and more recent ones such as the anticancer drug Gleevec (imatinib), a tyrosine kinase inhibitor developed by rational drug design after initial screens against the *bcr-abl* fusion protein. Comprehensive protein–protein interaction screens may in fact represent excellent tools to assist in the identification of the mechanism of drug action but also in the definition of drug specificity. Many promising drugs ultimately fail because of interactions with secondary targets and the resulting unexpected side effects, imposing a financial burden to pharmaceutical companies. Large-scale interactome screens could provide a cost-efficient means to identify potential off-target effects early in the development process.

Clearly, the information needed for such applications goes beyond a simple yes/no answer. Instead, quantitative readouts would be required. The PCA approach would have to rely on a detection in cells by optical methods, and complementation of green fluorescent protein (GFP) or luciferase fragments is already available (Stefan *et al.*, 2007). Conversely, the TAP-MS approach would require quantitation of the copurifying proteins, ideally via their individual peptides as mass spectometry provides superior resolution. As an indirect measurement, it detects the signal of the ionized species of each peptide, which does not correlate well with the number of initial protein molecules. Semi-quantitative analyses have therefore been developed that rely on the comparison of the relative intensity of isotope-coded internal standards—synthetic peptides of known amounts (Gerber *et al.*, 2003) or similar protein mixtures subjected to the same sample processing steps (Ong *et al.*, 2002; Zhang *et al.*, 2005)—or of the same peptide species measured from samples of similar origin and complexity (Bondarenko *et al.*, 2002) to gauge the observed signal intensity. They have proved extremely powerful in revealing even minute changes in protein abundance and already find increasing use in the analysis of changed interaction patterns on the small scale (Foster *et al.*, 2006; Wang and Huang, 2008).

Another interesting question is whether organisms that are more complex than yeast can be subjected to these screens. Genetic manipulation becomes increasingly difficult in higher eukaryotes, and multicellular organisms increase the complexity even further because cell–cell interactions will have an additional influence on the status of a cell and its interactome. Cell cultures are commonly used surrogates for biological experimentation but often correlate poorly with the true *in vivo* model of the organism. While the

PCA approach will likely reach its limit, TAP-MS can still be employed in higher organisms if the affinity tags necessary for their purification can be introduced, e.g., by creating genetically modified organisms. Alternatively, the endogenous proteins could be targeted for affinity purification, which requires the availability of high-quality affinity reagents, usually antibodies. Still, one is bound by the fundamental limitations discussed earlier that lead to the loss of transient and low-stoichiometry interactions. Consequently, obtaining *in vivo* interaction maps from higher eukaryotes will likely be a daunting or even insurmountable task for these three large-scale approaches.

An alternative approach may be more adept at solving this problem. In analogy to PCA utilizing a process that occurs naturally in cells (protein folding driven by physical forces that involve establishing a large number of noncovalent bonds), protein cross-linking relies on the formation of covalent bonds between interacting proteins in cells (Sinz, 2006; Trakselis *et al.*, 2005). Not only is this strategy compatible with affinity purification methods and therefore extends the TAP-MS approach, but it can be quantitative when combined with isotope labeling. Finally, it can also provide detailed structural information on the interfaces used in protein–protein interactions. A simple example of the power such information would convey is also given by Tarassov *et al.* The size of the DHFR fragments and their position makes interactions that bring two proteins' C-termini within 82 Å more likely to be detected than those of longer distance. This allows inspection of the data for topographical information, as illustrated by the correlation with known complex geometry and membrane orientation, which may aid future modeling efforts.

In light of these obvious advantages, why has cross-linking not yet been applied to map the interactome of an entire cell or organism? There are two reasons: on one hand, the formation of covalent bonds results in a more complex peptide mixture that surpasses the technical capabilities of current mass spectrometers. As modern mass spectrometers become faster, more sensitive, and more accurate, this hurdle will eventually be overcome, allowing us to address the second challenge we face: delivering and distributing the cross-linking reagents in intact cells. Cross-linking reagents are typically too large to passively cross cell membranes (Petrotchenko *et al.*, 2005; Tang *et al.*, 2005). While hydrophobic reagents will be retained in the membrane, more hydrophilic ones require delivery systems that can significantly alter a cell's physiology. Conversely, cross-linking after cell lysis will result in the loss of cellular context and the disadvantages of the TAP-MS approach. So far, the only cross-linking reagent that has been found to enter and be distributed in cells by passive diffusion is formaldehyde. For this very reason, it is commonly used by several techniques for the formation of covalent bonds between proteins inside intact cells (Sutherland *et al.*, 2008). While formaldehyde has not been used to map protein interactions on a proteome-wide scale, a number of reports exist that demonstrate that there is no biological obstacle preventing us from doing so (Guerrero *et al.*, 2006; Schmitt-Ulms *et al.*, 2004; Vasilescu *et al.*, 2004). Indeed, it is our lack of understanding of the chemistry it involves and our capacity to comprehensively analyze the reaction products that hold us back. As these shortcomings are being addressed (Rinner *et al.*, 2008; Toews *et al.*, 2008), the use of protein cross-linking for the study of cellular interactomes will likely become feasible. It will then generate an even more detailed map by identifying the actual sites involved in the interactions. This will provide sufficient molecular detail to fuel already ongoing efforts to correlate protein structures and their domains with their interactions to generate interactomes *in silico*. It should be exciting to see more of these approaches come to fruition in the near future.

Until such time, large-scale approaches such as the one described in the publication by Tarassov *et al.* that can be employed in high throughput to map the protein–protein interactions of an entire organism *in vivo* will be the benchmark for all subsequent studies. Moreover, their results are already available for perusal, helping researchers to form new hypotheses about local interaction networks, their arrangement into higher-order assemblies, and their integration into a framework that controls and coordinates the cellular processes. Being able to gain such detailed insight is incredibly rewarding for life scientists and will undoubtedly motivate them to go to even greater lengths to unravel the secrets of life.

REFERENCES

Aloy, P, and Russell, RB (2006). "Structural systems biology: modelling protein interactions." *Nat. Rev. Mol. Cell Biol.* **7**, 188–197.

Bondarenko, PV, Chelius, D, and Shaler, TA (2002). "Identification and relative quantitation of protein mixtures by enzymatic digestion followed by capillary reversed-phase liquid chromatography–tandem mass spectrometry." *Anal. Chem.* **74**, 4741–4749.

Collins, SR, Kemmeren, P, Zhao, XC, Greenblatt, JF, Spencer, F, Holstege, FC, Weissman, JS, and Krogan, NJ (2007). "Toward a comprehensive atlas of the physical interactome of *Saccharomyces cerevisiae*." *Mol. Cell Proteomics* **6**, 439–450.

Foster, LJ, Rudich, A, Talior, I, Patel, N, Huang, X, Furtado, LM, Bilan, PJ, Mann, M, and Klip, A (2006). "Insulin-dependent interactions of proteins with GLUT4 revealed through stable isotope labeling by amino acids in cell culture (SILAC)." *J. Proteome Res.* **5**, 64–75.

Gavin, AC, Aloy, P, Grandi, P, Krause, R, Boesche, M, Marzioch, M, Rau, C, Jensen, LJ, Bastuck, S, Dumpelfeld, B, Edelmann, A, Heurtier, M A, Hoffman, V, Hoefert, C, Klein, K, Hudak, M, Michon, AM, Schelder, M, Schirle, M, Remor, M, Rudi, T, Hooper, S, Bauer, A, Bouwmeester, T, Casari, G, Drewes, G, Neubauer, G, Rick, JM, Kuster, B, Bork, P, Russell, RB, and Superti-Furga, G (2006). "Proteome survey reveals modularity of the yeast cell machinery." *Nature (London)* **440**, 631–636.

Gavin, AC, Bosche, M, Krause, R, Grandi, P, Marzioch, M, Bauer, A, Schultz, J, Rick, JM, Michon, AM, Cruciat, CM, Remor, M, Hofert, C, Schelder, M, Brajenovic, M, Ruffner, H, Merino, A, Klein, K, Hudak, M, Dickson, D, Rudi, T, Gnau, V, Bauch, A, Bastuck, S, Huhse, B, Leutwein, C, Heurtier, MA, Copley, RR, Edelmann, A, Querfurth, E, Rybin, V, Drewes, G, Raida, M, Bouwmeester, T, Bork, P, Seraphin, B, Kuster, B, Neubauer, G, and Superti-Furga, G (2002). "Functional organization of the yeast proteome by

systematic analysis of protein complexes." *Nature (London)* **415**, 141–147.

Gerber, SA, Rush, J, Stemman, O, Kirschner, MW, and Gygi, SP (2003). "Absolute quantification of proteins and phosphoproteins from cell lysates by tandem MS." *Proc. Natl. Acad. Sci. U.S.A.* **100**, 6940–6945.

Guerrero, C, Tagwerker, C, Kaiser, P, and Huang, L (2006). "An integrated mass spectrometry-based proteomic approach: quantitative analysis of tandem affinity-purified *in vivo* cross-linked protein complexes (QTAX) to decipher the 26 S proteasome-interacting network." *Mol. Cell Proteomics* **5**, 366–378.

Ho, Y, Gruhler, A, Heilbut, A, Bader, GD, Moore, L, Adams, SL, Millar, A, Taylor, P, Bennett, K, Boutilier, K, Yang, L, Wolting, C, Donaldson, I, Schandorff, S, Shewnarane, J, Vo, M, Taggart, J, Goudreault, M, Muskat, B, Alfarano, C, Dewar, D, Lin, Z, Michalickova, K, Willems, AR, Sassi, H, Nielsen, PA, Rasmussen, KJ, Andersen, JR, Johansen, L E, Hansen, LH, Jespersen, H, Podtelejnikov, A, Nielsen, E, Crawford, J, Poulsen, V, Sorensen, BD, Matthiesen, J, Hendrickson, RC, Gleeson, F, Pawson, T, Moran, MF, Durocher, D, Mann, M, Hogue, CW, Figeys, D, and Tyers, M (2002). "Systematic identification of protein complexes in *Saccharomyces cerevisiae* by mass spectrometry." *Nature (London)* **415**, 180–183.

Ito, T, Chiba, T, Ozawa, R, Yoshida, M, Hattori, M, and Sakaki, Y (2001). "A comprehensive two-hybrid analysis to explore the yeast protein interactome." *Proc. Natl. Acad. Sci. U.S.A.* **98**, 4569–4574.

Ito, T, Tashiro, K, Muta, S, Ozawa, R, Chiba, T, Nishizawa, M, Yamamoto, K, Kuhara, S, and Sakaki, Y (2000). "Toward a protein-protein interaction map of the budding yeast: a comprehensive system to examine two-hybrid interactions in all possible combinations between the yeast proteins." *Proc. Natl. Acad. Sci. U.S.A.* **97**, 1143–1147.

Krogan, NJ, Cagney, G, Yu, H, Zhong, G, Guo, X, Ignatchenko, A, Li, J, Pu, S, Datta, N, Tikuisis, AP, Punna, T, Peregrin-Alvarez, J M, Shales, M, Zhang, X, Davey, M, Robinson, MD, Paccanaro, A, Bray, JE, Sheung, A, Beattie, B, Richards, DP, Canadien, V, Lalev, A, Mena, F, Wong, P, Starostine, A, Canete, MM, Vlasblom, J, Wu, S, Orsi, C, Collins, SR, Chandran, S, Haw, R, Rilstone, JJ, Gandi, K, Thompson, NJ, Musso, G, St Onge, P, Ghanny, S, Lam, MH, Butland, G, Altaf-Ul, AM, Kanaya, S, Shilatifard, A, O'Shea, E, Weissman, JS, Ingles, CJ, Hughes, T R, Parkinson, J, Gerstein, M, Wodak, SJ, Emili, A, and Greenblatt, JF (2006). "Global landscape of protein complexes in the yeast *Saccharomyces cerevisiae*." *Nature (London)* **440**, 637–643.

Ong, SE, Blagoev, B, Kratchmarova, I, Kristensen, DB, Steen, H, Pandey, A, and Mann, M (2002). "Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics." *Mol. Cell Proteomics* **1**, 376–386.

Petrotchenko, EV, Olkhovik, VK, and Borchers, CH (2005). "Isotopically coded cleavable cross-linker for studying protein–protein interaction and protein complexes." *Mol. Cell Proteomics* **4**, 1167–1179.

Remy, I and Michnick, SW (1999). "Clonal selection and in vivo quantitation of protein interactions with protein-fragment complementation assays." *Proc. Natl. Acad. Sci. U.S.A.* **96**, 5394–5399.

Rinner, O, Seebacher, J, Walzthoeni, T, Mueller, L, Beck, M, Schmidt, A, Mueller, M, and Aebersold, R (2008). "Identification of cross-linked peptides from large sequence databases." *Nat. Methods* **5**, 315–318.

Schmitt-Ulms, G, Hansen, K, Liu, J, Cowdrey, C, Yang, J, DeArmond, SJ, Cohen, FE, Prusiner, SB, and Baldwin, MA (2004). "Time-controlled transcardiac perfusion cross-linking for the study of protein interactions in complex tissues." *Nat. Biotechnol.* **22**, 724–731.

Sinz, A (2006). "Chemical cross-linking and mass spectrometry to map three-dimensional protein structures and protein-protein interactions." *Mass Spectrom. Rev.* **25**, 663–682.

Stefan, E, Aquin, S, Berger, N, Landry, CR, Nyfeler, B, Bouvier, M, and Michnick, SW (2007). "Quantification of dynamic protein complexes using *Renilla luciferase* fragment complementation applied to protein kinase A activities *in vivo*." *Proc. Natl. Acad. Sci. U.S.A.* **104**, 16916–16921.

Sutherland, BW, Toews, J, and Kast, J (2008). "Utility of formaldehyde cross-linking and mass spectrometry in the study of protein-protein interactions." *J. Mass Spectrom.* **43**, 699–715.

Tang, X, Munske, GR, Siems, WF, and Bruce, JE (2005). "Mass spectrometry identifiable cross-linking strategy for studying protein-protein interactions." *Anal. Chem.* **77**, 311–318.

Tarassov, K, Messier, V, Landry, CR, Radinovic, S, Molina, MM, Shames, I, Malitskaya, Y, Vogel, J, Bussey, H, and Michnick, SW (2008). "An *in vivo* map of the yeast protein interactome." *Science* **320**, 1465–1470.

Toews, J, Rogalski, JC, Clark, TJ, and Kast, J (2008). "Mass spectrometric identification of formaldehyde-induced peptide modifications under *in vivo* protein cross-linking conditions." *Anal. Chim. Acta* **618**, 168–183.

Trakselis, MA, Alley, SC, and Ishmael, FT (2005). "Identification and mapping of protein–protein interactions by a combination of cross-linking, cleavage, and proteomics." *Bioconjugate Chem.* **16**, 741–750.

Uetz, P, Giot, L, Cagney, G, Mansfield, TA, Judson, RS, Knight, JR, Lockshon, D, Narayan, V, Srinivasan, M, Pochart, P, Qureshi-Emili, A, Li, Y, Godwin, B, Conover, D, Kalbfleisch, T, Vijayadamodar, G, Yang, M, Johnston, M, Fields, S, and Rothberg, JM (2000). "A comprehensive analysis of protein-protein interactions in *Saccharomyces cerevisiae*." *Nature (London)* **403**, 623–627.

Vasilescu, J, Guo, X, and Kast, J (2004). "Identification of protein-protein interactions using in vivo cross-linking and mass spectrometry." *Proteomics* **4**, 3845–3854.

Wang, X and Huang, L (2008). "Identifying dynamic interactors of protein complexes by quantitative mass spectrometry." *Mol. Cell Proteomics* **7**, 46–57.

Zhang, Y, Wolf-Yadlin, A, Ross, PL, Pappin, DJ, Rush, J, Lauffenburger, DA, and White, FM (2005). "Time-resolved mass spectrometry of tyrosine phosphorylation sites in the epidermal growth factor receptor signaling network reveals dynamic modules." *Mol. Cell Proteomics* **4**, 1240–1250.