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REVIEW

Differential network biology

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Protein and genetic interaction maps can reveal the overall physical and functional landscape of a biological system. To date, these interaction maps have typically been generated under a single condition, even though biological systems undergo differential change that is dependent on environment, tissue type, disease state, development or speciation. Several recent interaction mapping studies have demonstrated the power of differential analysis for elucidating fundamental biological responses, revealing that the architecture of an interactome can be massively re-wired during a cellular or adaptive response. Here, we review the technological developments and experimental designs that have enabled differential network mapping at very large scales and highlight biological insight that has been derived from this type of analysis. We argue that differential network mapping, which allows for the interrogation of previously unexplored interaction spaces, will become a standard mode of network analysis in the future, just as differential gene expression and protein phosphorylation studies are already pervasive in genomic and proteomic analysis.

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Introduction

Physical and genetic interaction networks provide key insights into complex biological systems, from how different processes communicate to the function of individual residues on a single protein (Beltrao *et al*, 2010). For instance, the systematic identification of pairwise protein interactions (Stelzl *et al*, 2005; Tarassov *et al*, 2008; Yu *et al*, 2008; Consortium, 2011) or protein complexes (Butland *et al*, 2005; Gavin *et al*, 2006; Krogan *et al*, 2006; Sowa *et al*, 2009) has been a widely used strategy for understanding the physical architecture of the cell. Other types of physical interactions that are being mapped systematically include transcriptional protein–DNA interactions (Ren *et al*, 2000; Iyer *et al*, 2001) and kinase–substrate interactions (Ptacek *et al*, 2005; Linding *et al*, 2007). Genetic networks, in contrast, chart pairs of genetic mutations that in combination cause lethality or other phenotype—information that complements the structural information provided by the physical network (Tong *et al*, 2001; Roguev *et al*, 2007; Butland *et al*, 2008; Typas *et al*, 2008; Horn *et al*, 2011). Large network databases such as BioGRID, HPRD, IntAct, DIP and GeneMania (Xenarios *et al*, 2002; Keshava Prasad *et al*, 2009; Aranda *et al*, 2010; Warde-Farley *et al*, 2010; Stark *et al*, 2011) record hundreds of thousands of physical and genetic interactions from a wide variety of organisms.

Despite all of this exciting prior work in network mapping, at least one point stands out as remarkable: almost all physical and genetic networks, to date, have been examined under a single static (usually standard laboratory) condition. Biological systems, however, are highly dynamic entities that must continuously respond to a host of environmental and genetic changes or can be altered more slowly over an evolutionary period. It seems clear that if we are to develop a complete understanding of cellular dynamics, fast, slow or evolutionary, we must first understand how these dynamics effect, or are affected by, changes in the underlying physical and genetic networks.

Some understanding of the dynamics of large networks has been achieved by integrating static interaction measurements with dynamic changes in gene expression or metabolic fluxes (Ideker *et al*, 2002; Luscombe *et al*, 2004; Sauer, 2004; de Lichtenberg *et al*, 2005). These approaches seek to extract interactions from the static network that appear to be active under the new experimental conditions. However, these approaches are, by definition, unable to identify new interactions, complexes or pathways that are condition-specific, nor can they distinguish between changes in network state and changes in network wiring.

For this reason, an increasing number of studies have begun analyzing the dynamics of physical and genetic networks directly, through experimental mapping of networks across multiple conditions, species or times. As their main goal, these differential mapping approaches move away from characterizing absolute properties of the system to concentrate on a specific dynamic systems response. Rather than asking 'What parts of the system are the most abundant or dominant?' they ask 'What parts of the system are most affected by perturbation?'

Differential interaction maps thus chart a new type of interaction landscape that is fundamentally distinct from the

original static networks. The strongest differential interactions are not necessarily those that are strong in static conditions, they are those that are most clearly changing. Conversely, interactions present in both conditions are downplayed or removed from the differential network. For physical networks (protein–protein or protein–DNA), differential interactions imply mechanistic changes that are a result of an organism's response to environmental conditions. For genetic networks (synthetic-lethals or epistasis), interactions reflect functional consequences of mutations, not direct physical mechanisms. Thus, differential genetic interactions are a reflection of which cellular processes are differentially important under the studied condition.

In the remainder of this review, we summarize recent experimental and bioinformatic approaches for charting the dynamics of large physical and genetic interaction maps. We illustrate parallels between differential analysis of networks and previous differential analysis of a wide array of large-scale data sets. In addition, we discuss recently proposed quantitative methods for differential network analysis based on subtraction of interaction scores across conditions. These methods distinguish interactions that enable dynamic cellular processes from those that support the housekeeping functions of a cell.

Precedence for differential approaches in biology

Conceptually, differential network analysis is very similar to the way in which many other large-scale biological data types are now analyzed (Figure 1). For example, mRNA differential display and the two-color microarray revolutionized gene expression analysis because these techniques permitted direct comparison of two conditions and thus identification of differentially expressed genes (Liang and Pardee, 1992; Schena *et al*, 1995). A few years later, the microarray was adapted for a very different type of differential analysis: competitive growth phenotyping of barcoded mutations in budding yeast, allowing for identification of genes that are required for growth in certain conditions (Winzeler *et al*, 1999). In this same vein, use of mass spectrometry to uncover differentially expressed protein levels or protein post-translational modifications under different conditions or species has provided unique insight into the regulation of the cell (Gygi *et al*, 1999; Ong *et al*, 2002; Aebersold and Mann, 2003; Linding *et al*, 2007; Beltrao *et al*, 2009; Holt *et al*, 2009; Tan *et al*, 2009). Given this prior history, it is not surprising that differential network analysis is being recognized as a powerful approach to help understand a cellular response.

Differential mapping of physical interactions

An early case in which large network maps were analyzed differentially is the comparison of protein–protein interaction (PPI) data across species. Cross-species PPI analysis can be illuminating in terms of uncovering evolutionary conserved structures. For example, the PPI networks of *Saccharomyces cerevisiae* were compared against other microbial species such as *Helicobacter pylori* to predict previously uncharacterized PPIs (Matthews *et al*, 2001; Yu *et al*, 2004) or to identify evolutionarily conserved protein complexes (Kelley *et al*, 2003). Further work used multiple network alignment across three species simultaneously—yeast, fly and worm—to accurately infer conserved protein complexes and pathways in all three organisms (Sharan *et al*, 2005). However, all of these evolutionary network comparisons focused on interactions that are found in common across species, not those that differ. Differential interactions were difficult to detect because the networks of each species were measured in independent studies with relatively low network coverage in each study, resulting in a high false negative rate. Thus, failure to find

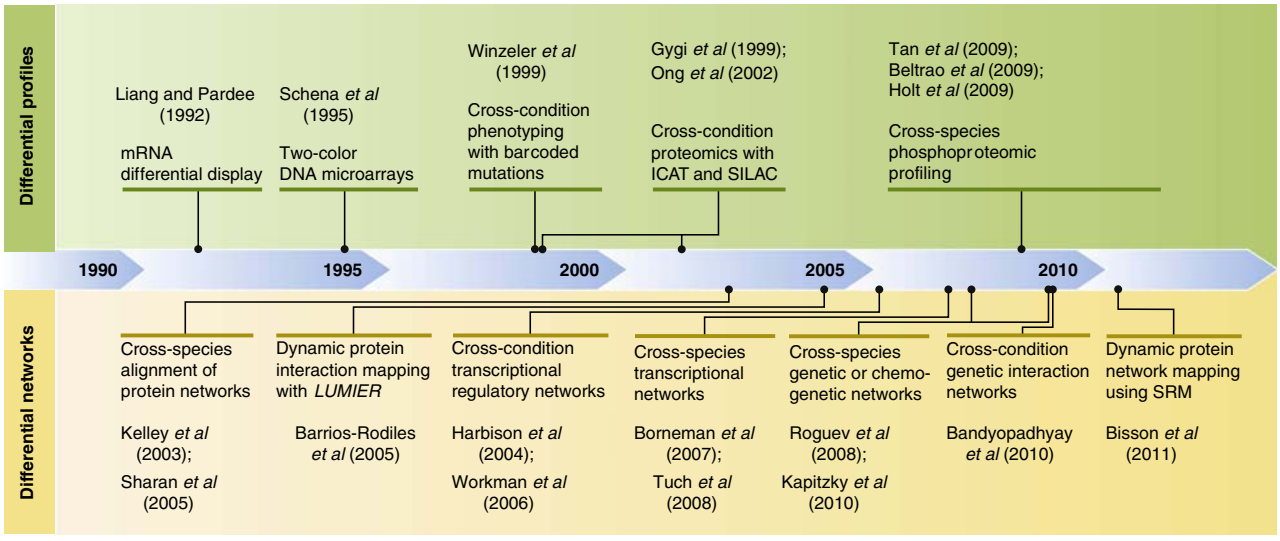

Figure 1 A historical timeline of differential approaches in biology. The top half of the timeline (green) tracks approaches used for differential analysis of molecular profiles over the past 20 years; the bottom half (yellow) tracks parallel approaches for differential analysis of molecular networks that have emerged more recently, within the past decade.

network conservation in a particular species was likely due to low network coverage, not evolutionary divergence.

Beyond comparison of PPIs across species, very little effort has been expended to characterize PPI networks under different conditions within a given species. In one early exception, Wrana and colleagues developed the LUMIER (luminescence-based mammalian interactome mapping) strategy to identify pairwise PPIs among a set of human factors with and without stimulation by transforming growth factor β (TGFβ) (Barrios-Rodiles *et al*, 2005). In the LUMIER approach, the luciferase enzyme is fused to protein 'baits' of interest and expressed in the same cell with Flag-tagged protein 'preys.' Using an anti-Flag antibody, prey proteins are immunoprecipitated in different conditions and the potential interactions measured quantitatively by the intensity of light in a luciferase assay. There is no formal score computed for the change in interaction strength between conditions; rather, baits with cross-condition interaction changes are qualitatively identified and validated biochemically. For instance, differential PPI mapping in the presence and absence of the TGFβ has allowed for the identification of functional links between the TGFβ pathway, the p21-activated kinase network and Occludin, a structural component regulating tight junctions during epithelial-to-mesenchymal transitions.

More recently, a quantitative approach has been presented for measuring differential interactions in PPI networks (Bisson *et al*, 2011). This approach, which the authors call affinity purification-selected reaction monitoring (AP-SRM), was used to map quantitative changes in interaction with the protein Grb2, an adapter protein that participates in diverse protein complexes involved in multiple aspects of cellular function. This network was generated in HEK293T cells at six time points after stimulation with epidermal growth factor (Figure 2) as well as in the presence of five other growth factors. SRM was used to measure integrated peak intensities for each peptide, which were combined into a weighted average intensity at each time point or condition. An intensity fold change was then calculated for each protein between two conditions, representing the change in interaction strength. The significance of this change was estimated using a statistic similar to the *t*-test, whose value increases with the difference in peak intensities but decreases with the variance observed over biological and technical replicates. Analysis of the resulting differential interactions showed that the composition of Grb2 complexes was remarkably dependent on the growth factor used for stimulation. By focusing on additional hub proteins beyond Grb2, this method is likely to be useful for obtaining a global overview of protein network remodeling in response to a stimulus.

Apart from PPI studies, a few studies have profiled transcriptional (protein–DNA) physical interactions under different conditions and across species. For example, Harbison *et al* (2004) carried out a genome-wide analysis of transcription factor binding in the yeast *S. cerevisiae* using the technique of chromatin immunoprecipitation followed by microchip hybridization (ChIP-CHIP), with some of the data being collected under different stimulating and stress conditions. Workman *et al* (2006) performed a ChIP-CHIP study focused specifically on the changes in transcriptional wiring that occur with respect to yeast transcription factor binding after exposure to the DNA damaging agent methyl methanesulfonate (MMS), for 30 different transcription factors. Cross-species analysis of a handful of transcription factors in both yeast (Borneman *et al*, 2007; Tuch *et al*, 2008) and mammalian cells (Schmidt *et al*, 2010) has revealed that protein–DNA interactions evolve quite rapidly over evolutionary time.

Differential mapping of genetic interactions

Genetic interaction mapping has also been successfully used in a cross-species mode to compare budding and fission yeasts (Dixon *et al*, 2008; Roguev *et al*, 2008). When the data derived from these genetic networks are combined with PPI data, they provide a unique view of the conservation of the interaction architecture across eukaryotic organisms. For example, Roguev *et al* (2008) revealed that protein complexes were highly conserved between the different yeasts, but that the genetic interactions between protein complexes had diverged significantly. Cross-species drug profiling in these two same organisms revealed that the use of two very divergent eukaryotic species allows for a more accurate prediction of evolutionary conserved drug mode of action (Kapitzky *et al*, 2010). Related cross-species drug profiling studies have extended this type of analysis to other yeast species, including *Candida* and *Cryptococus* (Spitzer *et al*, 2011). Extension of these types of works to higher organisms will provide even greater insight into the genetic and physical architecture of the eukaryotic cell.

Quantitative analysis of genetic interactions, where both positive (alleviating) and negative (aggravating) interactions are observed along with their interaction strengths, was initially accomplished in budding yeast using the E-MAP (epistatic miniarray profile) approach (Schuldiner *et al*, 2005; Beltrao *et al*, 2010). Recently, we have developed a strategy for mapping genetic networks not only quantitatively but also differentially—an approach we call differential epistasis mapping or dE-MAP (Bandyopadhyay *et al*, 2010). To create a dE-MAP, large-scale quantitative genetic interaction screens are performed on solid agar in two different conditions, such as with and without treatment by a pharmacologic agent (Figure 3). Next, a differential interaction score is computed for each gene pair, by subtracting the static score in the first condition from the static score in the second, then indexing this value against the null distribution of values expected when the two conditions are equal replicates. A similar approach has been used to demonstrate changes in genetic interactions in a lower-throughput, liquid culture-based format (St Onge *et al*, 2007), and work has also been carried out in *Drosophila melanogaster* to map genetic interactions using RNAi in different genetic backgrounds (Bakal *et al*, 2008).

As a proof-of-principle, the first implementation of the dE-MAP approach was to analyze the differential network that arises when cells are challenged by DNA damage. Genetic interactions were interrogated among a set of 418 signaling and transcription genes, leading to the creation of ~80 000 double-mutant strains. Double mutants were grown with or without 0.02% MMS, a model DNA damaging agent. The

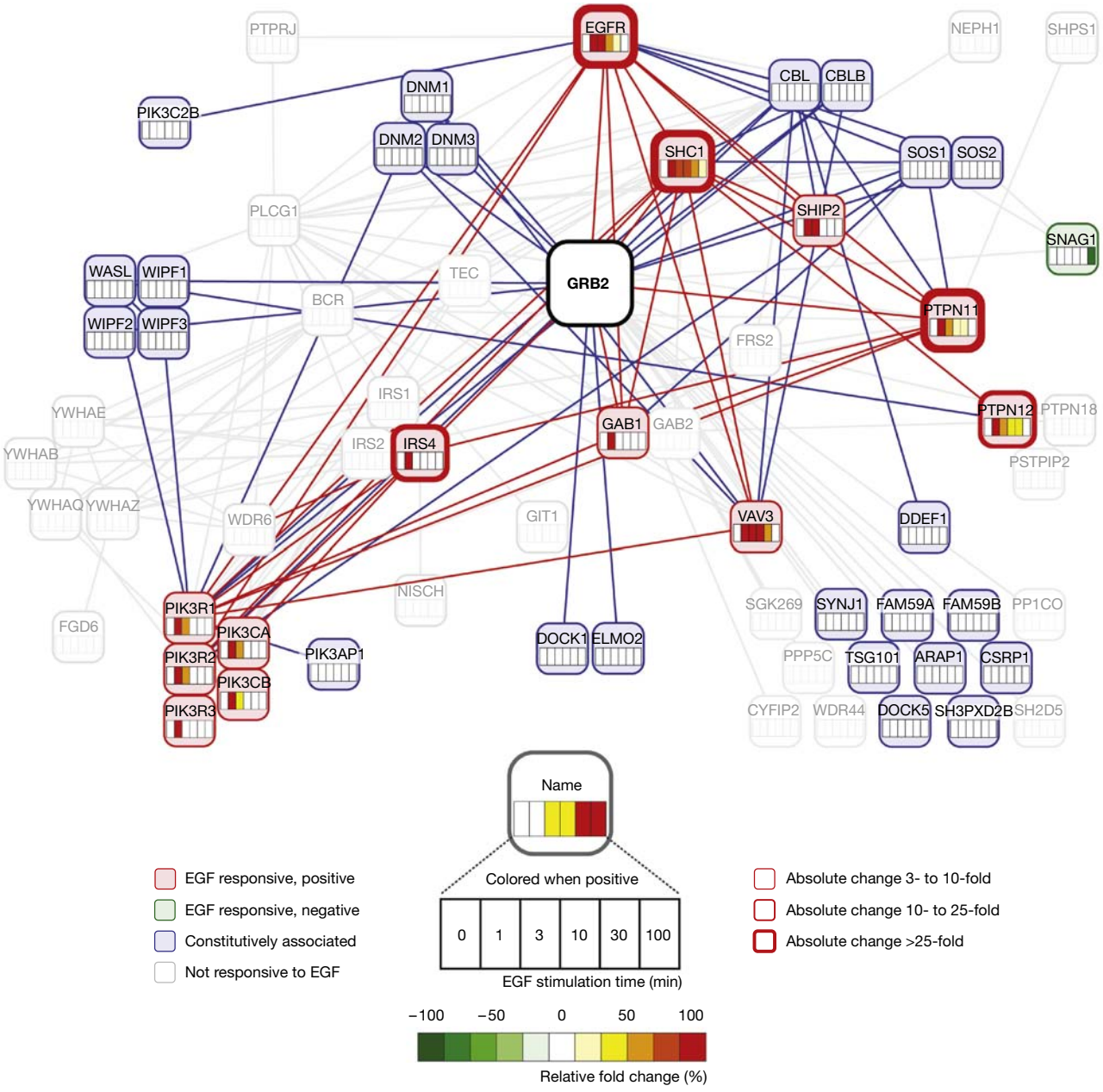

Figure 2 Differential physical interaction mapping with AP-SRM. Dynamic protein interaction network involving GRB2. Red-shaded nodes represent proteins that are recruited to GRB2 complexes after EGF stimulation irrespective of time, green-shaded nodes those that are decreased and blue-shaded nodes those present in GRB2 complexes in nonstimulated (control) cells. The thickness of the node border is proportional to the intensity of the change compared with control levels. Rectangles inside the nodes show the relative fold change for each time point. Reproduced from Bisson *et al* (2011).

resulting colony sizes were analyzed to compute static interaction scores in each condition and, from these static scores, a set of significant differential interactions was derived. The majority of static interactions detected in MMS-treated conditions (53%) were not observed in untreated conditions, indicating that a DNA damaging agent dramatically alters the genetic interaction landscape. The data also revealed that protein complexes are generally stable in response to perturbation, but the functional relations between these complexes are substantially reorganized. Interestingly, a similar trend was observed when analyzing genetic and physical data across different eukaryotic species (Roguev *et al*, 2008).

Parallels between housekeeping genes and housekeeping interactions

Housekeeping genes are genes such as actin, myosin, albumin and GAPDH that encode the most fundamental components of cell function and, as such, are expressed constitutively across cells and tissues (Lewin, 2007). Although dividing genes into

just two categories—housekeeping and other—is an oversimplification (certain housekeeping genes have significant variance in expression; Andersen *et al*, 2004), housekeeping genes have nonetheless been a useful concept for several reasons. First, due to their abundant and constant expression across conditions they are used frequently as controls for gene and protein expression analysis techniques. Second, they have motivated differential analysis methods such as cDNA library normalization, mRNA differential display, two-color gene expression microarrays, and ICAT and SILAC proteomics (Figure 1), in which the constant expression levels of housekeeping genes and proteins are normalized away in an attempt to highlight expression levels that are markedly changing.

The same set of concepts and considerations applies not only to genes and proteins but also to gene and protein networks. While some interactions appear and disappear dynamically, many others remain strong irrespective of condition and some of these correspond to processes that might be considered 'housekeeping.' In mapping of static networks, it is perhaps not coincidental that many of the major discoveries in these networks to-date have related to essential housekeeping processes such as basic life support and the central dogma of transcription and translation. For example, static genetic networks have been very effective at identifying novel interactions underlying DNA replication (Collins *et al*, 2007; Nagai *et al*, 2008; Lambert *et al*, 2010), RNA splicing (Wilmes *et al*, 2008) and protein folding (Zhao *et al*, 2005).

On the other hand, of at least equal interest are those interactions that are activated only during a specific cellular response. For example, in our study of DNA damage-induced genetic networks using the dE-MAP technique (Bandyopadhyay *et al*, 2010), both the untreated and treated networks were strongly enriched for a common set of interactions with genes involved in transcription, translation, chromatin and other cellular housekeeping machinery (Figure 3B). In the differential analysis of these networks, however, new DNA damage response functions were identified for a number of genes (e.g., centromere binding factor 1 or *CBF1*) for which the new function becomes apparent only after subtraction of one network from the other. Interestingly, DNA repair factors also form a rich cluster of interactions in static genetic networks (Collins *et al*, 2007; Costanzo *et al*, 2010) although they are not interaction 'hubs' as they are in the differential DNA damage network (Bandyopadhyay *et al*, 2010). Thus, both static and differential maps provide useful information about a particular pathway or cellular response.

It is also important to realize that housekeeping genes (genes expressed uniformly across conditions) must not

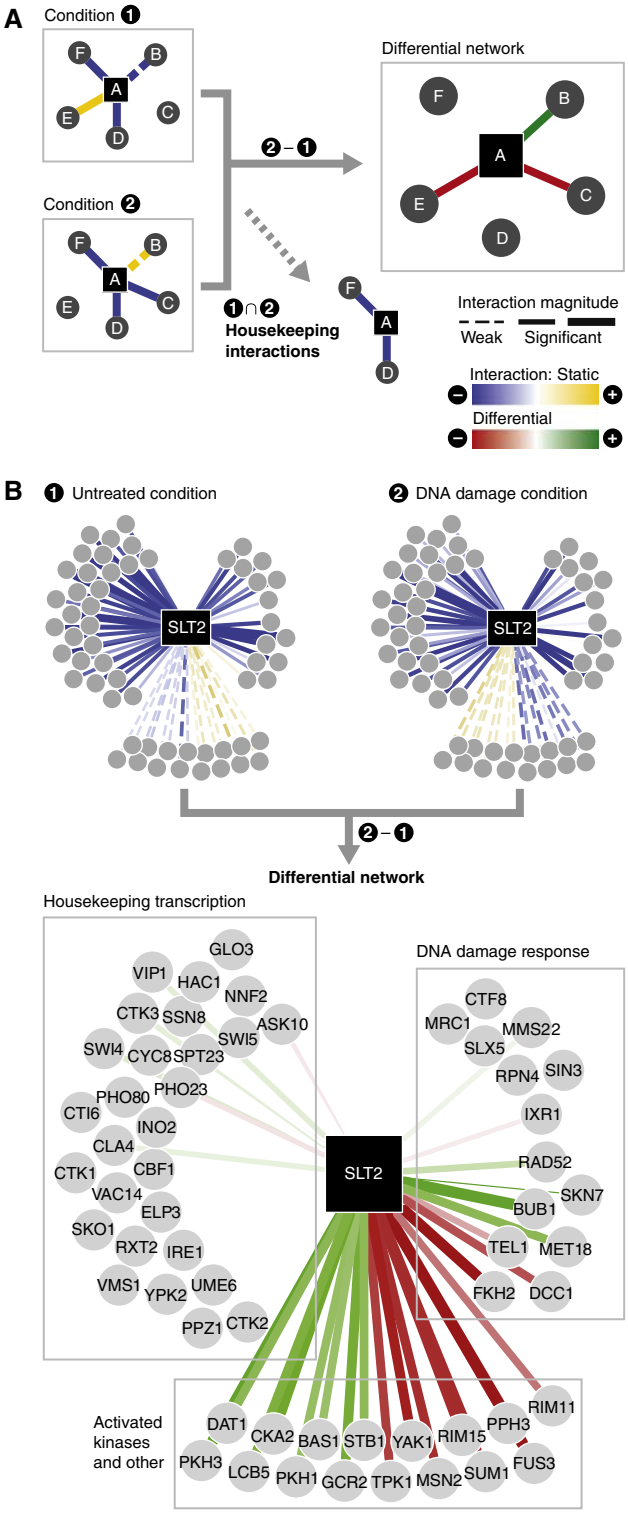

Figure 3 Differential genetic interaction mapping with dE-MAP. (**A**) Schematic showing principle of differential genetic interaction analysis. Static genetic interaction maps are measured in each of two conditions (left) resulting in both positive (yellow) and negative (blue) interactions. Condition 1 is subtracted from condition 2 to create a differential interaction map (right), in which the significant differential interactions are those that increase (green) or decrease (red) in score after the shift in conditions. In the differential map, weak but dynamic interactions (dotted edges) are magnified and persistent 'housekeeping' interactions are removed (bottom right). Note that (A, E) and (A, C) are decreasing differential interactions achieved by different circumstances: (A, E) is a positive interaction that disappears after the conditional shift, while (A, C) is a negative interaction that appears after the conditional shift. (**B**) Differential analysis for yeast gene *SLT2*. Genetic interaction data from Bandyopadhyay *et al* (2010) collected in either untreated or DNA-damage-treated conditions (top) are compared to create a differential interaction map (bottom). Interactions with transcriptional machinery are present in both conditions and thus downgraded in the differential map, while interactions with kinases and DNA damage response genes are highlighted. Functional annotations in the differential network summarize the predominant function within the demarcated set of genes.

necessarily give rise to housekeeping interactions (interactions present uniformly across conditions). Genes normally thought of as housekeeping may, at the network level, show a rich pattern of both static and differential interactions. Future differential analyses may allow us to better characterize the role that a variety of housekeeping genes play in a variety of dynamic cellular behaviors and responses.

Statistical treatment of differential networks: a 'Call to Arms'

Despite the increasing number of differential networks that have been generated to date, there has been very little work devoted to understanding the statistical issues associated with such networks. Differential network analysis introduces a number of statistical challenges, only some of which have been appreciated. For instance, differential analysis across conditions can mitigate experimental biases or errors that affect each condition in the same way, that is, systematic experimental artifacts that are reproducible. In this case, differences between a reference and a control may be meaningful even where absolute measurements are not (Kerr *et al*, 2000; Hatfield *et al*, 2003). However, in the general case, the difference of two static interaction measurements, with each influenced by independent errors, has variance equal to twice than that of either static measurement taken separately. Thus, it will be important to understand the relative contribution of the systematic and independent errors influencing an interaction mapping experiment. In the near future, we should attempt to tackle these and other statistical aspects of differential network maps, and doing so will undoubtedly lead to important insights and improvements in the published interaction maps. Certainly, there have been hundreds of manuscripts reporting on methods for differential analysis of gene expression data, which provides a pool of possible methods and suggests that there is at least some effort that should be pursued on the topic of differential analysis of networks.

Perspective and future directions

Where to now? In the future, more comprehensive and quantitative study of physical and genetic interaction maps across species and within a species under different conditions will be crucial for understanding both global evolutionary trends as well as how specific pathways are re-wired in the presence of an exogenous stress. We expect that many more interaction maps will be generated in the presence of drugs, as was done with the MMS dE-MAP (Bandyopadhyay *et al*, 2010), but using different concentrations and in a time-dependent manner after exposure to the compound. Such work would provide insight into drug response as well as how ultimately drug resistance is manifested within interaction networks. Another extension of this work would include genetic interaction mapping of analog-sensitive kinase mutants in the presence of the relevant compound, which specifically inhibits the kinase (Bishop *et al*, 2000) to more accurately ascertain which effects on signaling cascades are primary and which occur downstream (J Kliegman and K Shokat, personal communication). Also, innovative applications of microscopy may allow us to determine how networks change in cells as they come into contact with different cell types (Jorgensen *et al*, 2009), as well as how they spatially change within an organism in a condition and time-dependent manner (Maeder *et al*, 2007).

It is important to recognize that organisms normally do not exist in isolation, but participate in pathogenic or symbiotic relationships with other organisms which impinge on the biochemical and genetic make-up of all species involved (Fischbach and Krogan, 2010). Understanding the dynamics of inter-species, molecular level interactions will be key to help unravel these complicated relationships. Pathogenic organisms—in particular viruses—have relatively small genomes ($\sim$10–100) and thus rely heavily on key host machinery for infection and propagation, making them excellent probes for understanding the dynamics of mammalian biological systems. Viral proteins will be involved in hijacking key molecular machines within the host and reconfiguring their physical and genetic interactions during infection. In many ways, infection from another organism represents the ultimate 'stress' on a system and characterizing host–pathogen PPIs using strategies that target pairwise interactions (Mukhtar *et al*, 2011) or protein complexes (Jäger *et al*, 2011) will be key to understanding how host cellular pathways are re-wired during the course of infection. Analyzing these connections in a time-dependent and post-infection manner will also show how the host attempts to respond to the foreign host machinery.

Work is also ongoing to use networks to gain insight into different disease states (Braun *et al*, 2008; Pawson and Linding, 2008). For instance, Goh *et al* (2007) show that there is a higher degree of physical connectivity between proteins whose genes are mutated in the same disease state. More detailed studies of how these individual mutations would perturb specific interactions will provide greater insight into the molecular basis of these diseases (Zhong *et al*, 2009). In the future, network biology can also be used to predict the onset and severity of specific disease states. For example, several groups have shown that alterations in the physical interaction network can be a powerful indicator of breast cancer prognosis (Chuang *et al*, 2007; Taylor *et al*, 2009).

Finally, an important question moving forward is the extent to which network representations are able to faithfully capture cellular structure and behavior at all. Even allowing for dynamic interactions, it is not given that network models offer the best description of the processes ongoing in a cell. Network models typically represent a protein or other molecule as a single node 'wired' to others to represent known molecular interactions. These network representations have been very useful to recognize functional modules such as protein complexes (Bader and Hogue, 2003), transcriptional circuits (Tsong *et al*, 2003) and signaling pathways (Steffen *et al*, 2002). They have also been useful for the study of unifying network topologies and architectures, which link molecular biology with many other scientific disciplines that involve networks (Milo *et al*, 2002; Barabasi, 2009; Liu *et al*, 2011). On the other hand, although conceptually useful, it is clear that network 'wires' have little resemblance to the physical reality, in which a population of potentially many copies of a protein

diffuses or is transported throughout the cell and, in so doing, experiences a variety of opportunities for interaction that range in timing, affinity, specificity and stoichiometry. Nonetheless, there is likely still much mileage to be gained with the networks view before graduating to the next one, whatever shape that may take.

Conclusion

After years of static genetic and physical interaction mapping, differential network analysis is now becoming prevalent as a tool to more comprehensively interrogate biological systems in a variety of organisms. Conceptually, differential analysis is not new but has been a successful mode of genomic and proteomic analysis for decades. Rather, a major reason for this advancement can be attributed to the fact that experimental and computational methods for network mapping are becoming more robust, quantitative, and high-throughput. Even though many insights still remain to be extracted from static interaction maps, differential mapping will allow us to explore a previously unexplored interactome and biological space, ultimately providing a deeper understanding of complex biological phenomena.

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Conflict of interest

The authors declare that they have no conflict of interest.

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