Available online at www.sciencedirect.com

SciVerse ScienceDirect

Current Opinion in Biotechnology

Integrative avenues for exploring the dynamics and evolution of protein interaction networks

Guillaume Diss[1], Marie Filteau[1], Luca Freschi[1], Jean-Baptiste Leducq[1], Samuel Rochette[1], Francisco Torres-Quiroz[1] and Christian R Landry

Over the past decade, the study of protein interaction networks (PINs) has shed light on the organizing principles of living cells. However, PINs have been mostly mapped in one single condition. We outline three of the most promising avenues of investigation in this field, namely the study of first, how PINs are rewired by mutations and environmental perturbations; secondly, how inter-species interactions affect PIN achitectures; thirdly, what mechanisms and forces drive PIN evolution. These investigations will unravel the dynamics and condition dependence of PINs and will thus lead to a better functional annotation of network architecture. One major challenge to reach these goals is the integration of PINs with other cellular regulatory networks in the context of complex cellular phenotypes.

Address
Département de Biologie, Institut de Biologie Intégrative et des Systèmes (IBIS), PROTEO, Université Laval, Québec, Canada G1V 0A6

Corresponding author: Landry, Christian R (Christian.Landry@bio.ulaval.ca)

[1] Contributed equally to this work.

Current Opinion in Biotechnology 2013, **24**:775–783

This review comes from a themed issue on **Systems biology**

Edited by **Orkun S Soyer** and **Peter S Swain**

For a complete overview see the Issue and the Editorial

Available online 7th April 2013

0958-1669/$ – see front matter,

http://dx/doi:10.1016/j.copbio.2013.02.023

Introduction

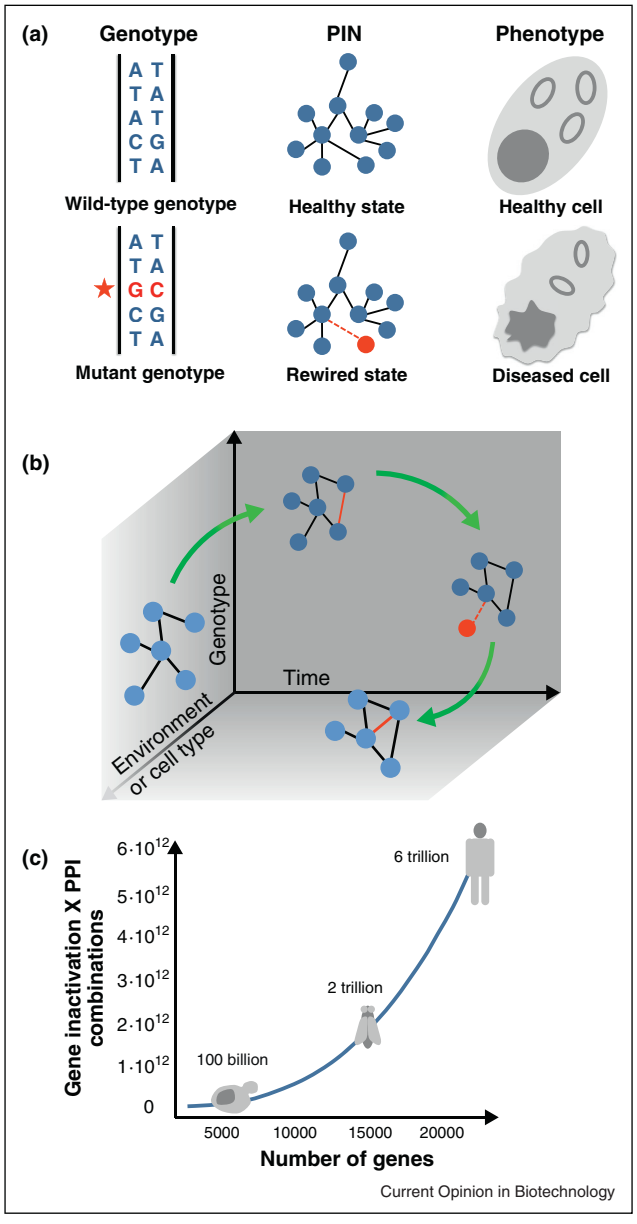
Proteins associate with each other to perform most functions encoded in the genome and to relay information between the environment, the genome and the cell [1]. Protein interaction networks (PINs) therefore play a key role in connecting genotypes to phenotypes [2]. For this reason, many human diseases result from, or lead to the dysfunction of protein–protein interactions (PPIs) [3••,4,5] (Figure 1a). The last decade has witnessed substantial progress in the implementation of large-scale approaches to study eukaryotic PINs in several organisms, including fruit flies [6], nematodes [7], yeasts [8–11], the model plant *Arabidopsis* [12] and humans [13–15]. These maps describe how cellular processes are connected with one another and are thus of fundamental and applied interests (*e.g.* study of the evolution of cellular functions and development of disease treatments). For instance, it is now clear that targeting PPIs rather than inhibiting all functions of a protein has a great potential for disease diagnosis [16], drug discovery [17] and treatment. This approach increases the target space and allows a more precise control of the disease causes by targeting specific functions, hence potentially decreasing side effects [17–20].

The majority of diseases and phenotypic traits are complex and involve dynamic PPIs, while most of the current interactome maps are static representations of the cellular architecture. Therefore, there is a requirement for improved models of PINs, which means that we need to annotate connections (edges) of the network in terms of the genetic, environmental or developmental context in which they take place. This implies that PINs should be mapped in several dimensions, that is, in different conditions, cell types, developmental stages and sets of genetic backgrounds as well as at different time scales, both short and evolutionary (Figure 1b). Here we discuss current and forthcoming empirical avenues this research is leading to and how these investigations will enable a better understanding of biological functions that emerge from the regulated association of proteins in the cell and the evolution of these networks.

Perturbing PINs: mapping in 3D

Several studies have shown how standard tools for analysing PPIs can be used to study PINs along diverse gradients of regulation and perturbations, either at the level of binary interactions or at the level of protein complex assembly. One of the first systematic studies of this type was that of Matsumoto *et al.* [21], which investigated the capacity of nitric oxide (NO), an important ubiquitous signalling molecule, to regulate interactions between proteins. This was done using a yeast two-hybrid (Y2H) screen where procaspase-3 interactions were tested against thousands of different proteins. The authors found that the inducible NO synthase interacts with procaspase-3 and the apoptosis-related enzyme acid sphingomyelinase, and that these PPIs were NO-dependent, suggesting a mechanism by which PPIs could be regulated by this signaling molecule. The Y2H approach has also been applied to genetic perturbation of PINs in order to examine how

Figure 1



PINs lay at the interface between the genotype and the phenotype of a cell. **(a)** In genetic diseases, a phenotype unfolds through the perturbation of PINs directly or the perturbation of other regulatory mechanisms that will influence PINs. **(b)** PINs are rewired because of mutations (genotype), biochemical and biophysical variation of the environment (environment or cell type) and/or developmental stages, and cell cycle phases (time). These parameters represent a multi-dimensional space where a PIN can be in a different state according to its position in this space, and can dynamically go from one state to another in response to a variation in these parameters. **(c)** Even when considering only gene deletion or inactivation, the number of genotype–protein–protein combinations is immense and increases rapidly with the complexity of the proteome. The complexity is even larger when considering the different protein isoforms of each gene.

mutations affect network connections. When a gene is mutated, amino acid changes can modify PPIs with only some or many of a protein's partners ('edgetic perturbation') [22], thereby changing PIN architecture. Zhong *et al.* [23] investigated mutations associated with human diseases and identified interaction profiles of mutant proteins. They showed that distinct mutations in one gene could generate different phenotypes according to the PPIs that were perturbed. In the case of the transcription factor TP63, a key developmental regulator, they showed that mutations could cause two clinically distinct developmental disorders [23]. These results illustrate the fact that discrete mutations on the same protein can have distinct effects on PINs, which opens the possibility for developing edge-specific disease treatments.

Other approaches that aimed at understanding how PPIs respond to perturbations using binary interactions are on the basis of protein-fragment complementation assays (PCAs). In one of the first investigations of this type, MacDonald *et al.* [24] measured 49 PPIs across the human PIN using a fluorescent PCA to show that related drugs have similar profiles of effects on PPIs, suggesting that with sufficient data, drug effects on PINs could be predicted from the properties of the compounds. PCAs on the basis of survival assays in model systems such as yeast [10] also provide a quantifiable PPI signal [25], which allows to estimate and compare the impact of PIN perturbations on a larger scale. Recently, Schlecht *et al.* [26•] developed a multiplex survival assay to show how PPIs are modulated by chemicals and screened 238 PPIs in the presence of 80 compounds. The results led to the identification of a chemotherapeutic drug that can disrupt a PPI between Dst1, a transcription elongation factor for RNA Pol II, and Rpb9, a subunit of RNA Pol II required for transcription start site selection [26•].

Other approaches used mass-spectrometry to quantify specific PPIs in different genetic backgrounds [27] or through time. Bisson *et al.* [28••] provided a clear example of these approaches in a study of the interactome of the adaptor protein GRB2 in a temporal and receptor-specific fashion. The authors identified proteins constitutively associated with GRB2 and proteins whose interactions increased or decreased upon stimulation. Their work illustrates how the interaction dynamics of a key protein helps explaining its pleiotropic effects in cell regulation [28••]. These new techniques allow a deeper and more quantitative analysis of the perturbation dynamics of specific pathways, but they may miss how these perturbations affect the rest of the PIN as only a specific list of proteins can be followed through time. Therefore, the different approaches (specific and global) described above are complementary. The former is suitable for detailed studies while the latter provides the backbone

to integrate the results. Ideally, one would want to combine both approaches in order to be able to study how perturbations in one pathway percolate through PINs and affect their architecture and ultimately cell physiology. This would allow to identify global effects and indirect connections that may have been missed before by studies focusing on canonical members of these pathways [29].

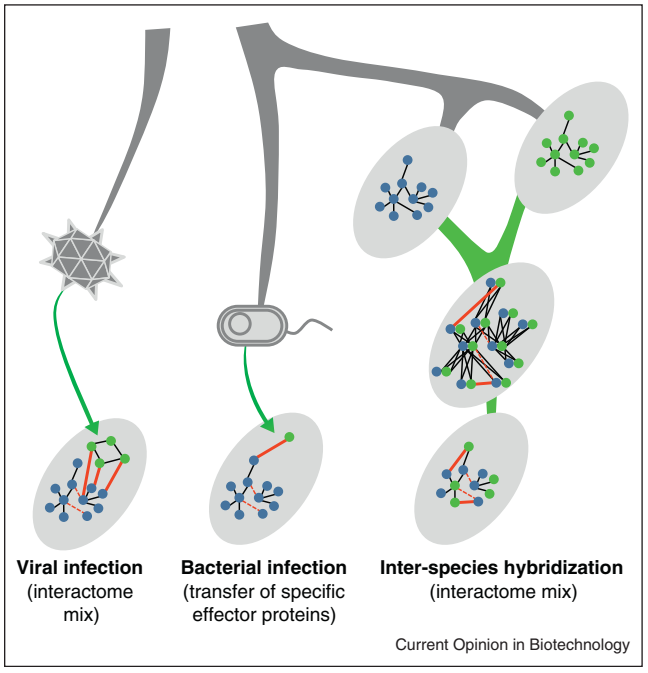
Altogether, these recent studies show how powerful the perturbation of PINs is for understanding the regulation of PPIs and dynamic cellular functions. The next challenge is to expand these approaches to the level of entire PINs. Large-scale perturbation of PINs could include for instance the measurement of all PPIs in a cell in the presence or absence of other proteins, which has only been done on a small scale so far [27,30]. This would enable the identification of PPI regulators, such as protein kinases and phosphatases [31•] and proteins acting as hubs in protein complexes [27] in a systematic manner. Because the size of this genetic perturbation space is immense (Figure 1c), further technological developments are required to explore these new PIN dimensions along with new computational approaches that will help prioritize relevant parts of this multidimensional space.

Interaction of interactomes among species

Another avenue by which we can learn how PINs respond to perturbations and how we can manipulate them is by studying how ecological interactions and evolution shape PINs in the short and long terms (Figure 2). This opportunity occurs for instance when two organisms come into contact at the molecular level and have their PINs interacting. Good examples are host–pathogen interactions, such as those between viruses or bacteria and their host cells [32,33,34••]. The long-term co-evolution of pathogens and hosts is expected to favour pathogens that successfully manipulate their host by targeting key proteins in the host PIN. Accordingly, these PPIs become potential targets for the development of therapeutic solutions to treat infectious diseases, and dissecting this evolutionary tug of war can also indirectly reveal how a PIN can be manipulated for specific purposes.

Recent studies have indeed led to the identification of nodes with biological functions that are specifically targeted by parasites, such as those that occupy putative strategic regulatory positions in the host PIN [33,34••,35,36]. For instance, Mukhtar *et al.* [34••] analysed pairwise PPIs between effector proteins of two unrelated plant pathogenic bacteria and *Arabidopsis* proteins using a Y2H approach. They showed that effectors converge onto highly connected proteins more often than expected by chance alone. Similar results have been found with Epstein-Barr virus proteins [35] and for a large ensemble of viral proteins from different virus families with a similar approach [36]. In a recent study, a PPI screen between HIV proteins and the human proteome showed that EIF3D, a member of the eukaryotic translation initiation complex, interacts with the HIV protease (PR) protein [33]. A remaining challenge in this field is to study these inter-species PPIs in their biological context. This is particularly important in cases where pathogen effector proteins are modified in the cellular context of their hosts [37]. Another promising avenue will be to understand the role of inter-species interactomes in non-pathogenic situations to test whether similar node-targeting strategies are used by viruses and bacteria, in mutualistic or endosymbiotic interactions.

Figure 2



Species interactions at the interactome level represent important perturbations. (Left branch) Viral infections involve contacts between host and viral interactome components. The interactions between viral and host proteins (thick red lines) affect the host interactome (dashed red line) and consequently cellular processes. (Middle branch) In the case of a bacterial infection, the host interactome is affected by specific bacterial effector proteins rather than by the whole bacterial interactome. These effectors can also rewire the host interactome and manipulate the host cell. (Right branch) When a hybrid is formed between two species, the interactome is composed of within species (full lines) and between species PPIs (dashed lines). Perturbation of PPIs can result in the loss of interactions between proteins that normally interact within species or the formation of new interactions specific to the hybrids. These would result from cases where negative selection prevents proteins from interacting within species but this breakdowns in hybrids because proteins from the two species have not co-evolved to avoid each other.

Other cases of extreme PIN perturbations by inter-species interactions are found in inter-specific hybrids in which PINs of different species are completely mixed (Figure 2). Natural selection favours the maintenance of PPIs over time, promoting the co-evolution of interacting proteins and preventing unwanted interactions [38]. This co-evolution could lead to a breakdown of PPIs or to the formation of spurious interactions in cells where two proteomes co-exist. Theoretical work by Livingston and collaborators suggests that mutations arising independently in parental species could result in the formation of deleterious PPIs when combined in hybrids. This effect could be compensated by a high connectivity of the protein network [39]. Hybrid systems thus provide unique opportunities for the identification of critical protein residues responsible for PPIs and for understanding how PPI perturbations affect essential cellular processes. However, few studies have identified such incompatibilities at the interactome level. Zamir *et al.* [40••] recently showed how amino acid divergence between species can cause the breakdown of PPIs using very distantly related yeast species. More recently, Leducq *et al.* [41••] directly tested whether hybridization would perturb protein complexes in hybrids between two closely related yeast species. Their work indicates that PINs may be robust to hybridization, suggesting that orthologous proteins of divergent but similarly structured PINs could be permutated without affecting PIN structure [41••]. Taken together, these reports suggest that it will be soon feasible to interrogate entire inter-species interactomes and to use hybridization as a fundamental model to identify the mechanisms by which PINs could be disrupted or could withstand massive genetic perturbations.

Evolution of PINs

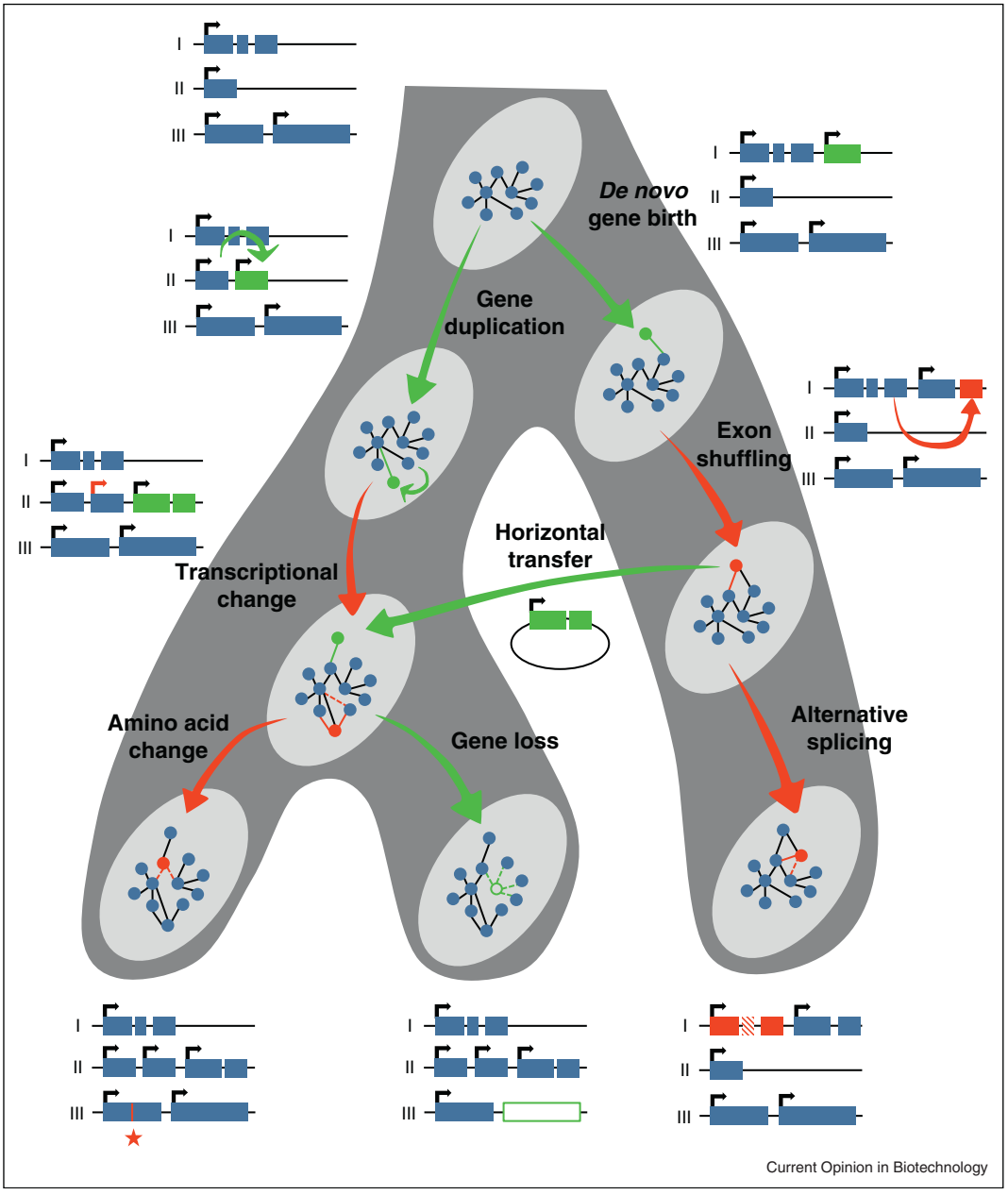
Another major challenge in the study of PINs is to understand what mechanisms and evolutionary forces are driving the evolution of PINs over time. PINs evolve either by changes in the edges that connect existing proteins (network rewiring) or by the addition or removal of nodes through diverse types of mutational changes (Figure 3). One key mechanism involved in network rewiring is thought to be amino acid substitution at the interaction interface [42,43]. A striking example of rewiring is given by the two duplicated yeast deacetylases Sir2 and Hst1 that subfunctionalized by acquiring complementary inactivating mutations in distinct interaction domains [44].

Other mechanisms of rewiring have recently been identified. For instance, changes involving non-coding sequences could play a role. Gagnon-Arsenault *et al.* [45•] experimentally showed that genome-wide PPI profiles of paralogous proteins can be rewired by placing the coding sequence of a paralog under the control of the regulatory elements of the other, showing the potential for transcriptional divergence in PIN rewiring. Other groups experimentally and computationally showed that splicing isoforms have different PPIs [46,47], highlighting again the potential for transcriptional programs to modify PINs among species.

A central mechanism that leads to the addition of nodes in PINs is gene duplication [48], such as in the case of Sir2 and Hst1. However, other mechanisms have been hypothesized to play a role, including horizontal gene transfer [49] and *de novo* gene birth [50] (Figure 3). A foremost challenge will be to determine the relative importance of each of these mechanisms and to examine whether they are dependent on the existing architecture of PINs and/or on the proteins themselves. For instance, some protein features, such as disordered regions, have been linked to accelerated rates of PPI evolution [51,52] and could ultimately have a significant impact in shaping PPI profiles. Further, Papp et *al.* [53] provide evidence that the architecture of protein networks may itself affect the fate of duplicated genes. They suggest that gene duplication of a single subunit of a protein complex could be harmful because it impairs the stoichiometric balance of complexes. They provide evidence for this by showing that dosage-sensitive genes are twice as likely to be part of protein complexes than genes with low dosage-sensitivity. Further, they show that ribosomal proteins, which are known to be harmful when imbalanced, are enriched in whole-genome duplicated genes [53]. This suggests that subunits of protein complexes that are dosage-sensitive can undergo duplication if all subunits are duplicated at the same time. More experimental studies and new theoretical and bioinformatics approaches are needed to estimate the relative contribution and the rate at which each one of these mechanisms shapes PINs.

The respective role of natural selection and random genetic drift on the evolution of PINs is another subject of critical importance in our understanding of the functional meaning of PPIs. A recent study suggested that the accumulation of mildly deleterious mutations in small populations of complex organisms might be a non-adaptive mechanism of PIN evolution, since it may induce secondary selection for compensatory protein interactions, thus leading to a complexification of PINs that does not necessarily involve novel functions [54••]. Furthermore, studies on phosphorylation sites have provided evidence favoring the hypothesis that a large fraction of phosphorylation sites might be non-functional [51], adding support to the idea that many PPIs might evolve neutrally. It will be difficult to empirically demonstrate that any given PPI has no cellular function, as this would require testing every possible condition a cell could encounter. However, much progress can be made by estimating parameters that drive the evolution of PPIs. Recent models suggest that the fraction of non-functional PPIs will depend on

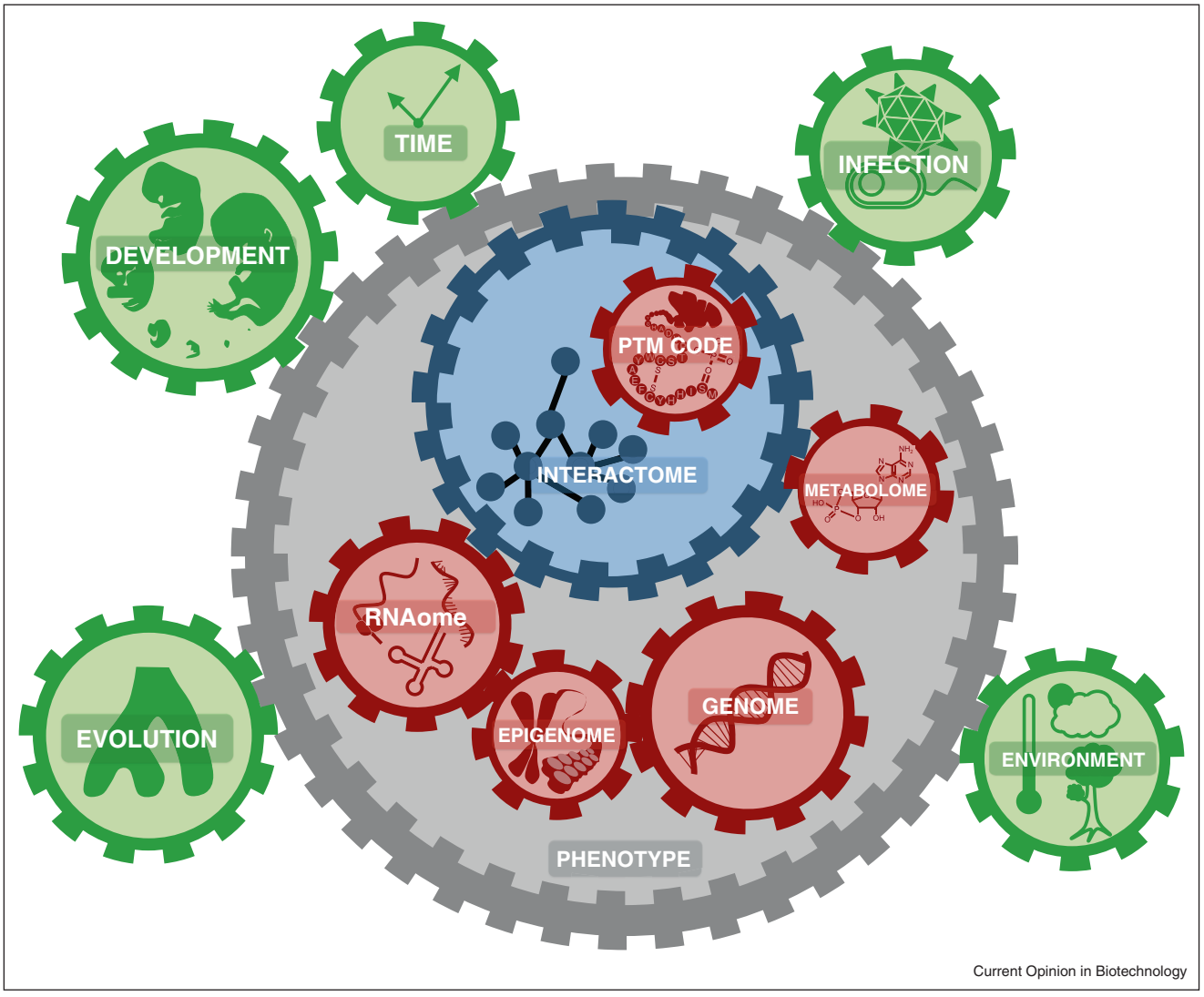
Figure 3



Mechanisms that shape PINs during evolution. Gene duplication, horizontal gene transfer and *de novo* gene birth (green arrows) contribute to add new nodes (green nodes) to PINs (edges in black, nodes in blue) while gene loss leads to the removal of nodes from PINs (empty green node). Alternative splicing, exon shuffling, transcriptional changes or amino acid replacements (red arrows) contribute to network rewiring, that is, changes in the connection between the nodes (red nodes and edges). For each state in the evolutionary trajectory, a graphical schematic description of the genomic configuration and the PIN configuration are shown. On each description the changes that occurred at genomic or network levels are highlighted (in green or red).

the equilibrium between mutations that create PPIs versus mutations that eliminate them and the strength of natural selection against the loss or introduction of new ones [29]. Physical parameters, such as protein disorder and abundance, will also need to be considered [55]. These parameters remain to be experimentally quantified and this is another forthcoming challenge in interactome studies.

Figure 4



PINs occupy a central position in the genotype–phenotype maps. Integrating together information from the genome, epigenome, transcriptome, metabolome, proteome, RNAome and post-translational modifications (PTM code) will reveal how the cellular machinery responds to extrinsic factors such as the environment, interaction with other organisms, developmental timing, timing of physiological processes such as the cell cycle and evolution. This integrative strategy will require appropriate analytical and experimental tools that can handle more than variation in a single dimension and that will incorporate the measurements of multiple networks at the same time in the same cell. This integration is necessary to be able to draw causal relationships between changes in the genotype or the environment and a direct versus indirect modification of PIN structure.

Conclusion

All the research avenues mentioned above have a common theme that is the study of PIN perturbation. Another common aspect is the need for new experimental approaches and better experimental integration of perturbations across the cellular regulatory layers to weigh their impact on PINs. For instance, approaches able to distinguish direct PPI perturbations from the ones acting indirectly through mechanisms that modify the abundance of the interaction partners would be most valuable. This is emphasized by the recent findings showing the effect of transcriptional regulation on the architecture of PINs [45]. So far, integration of PINs with other biological datasets has almost exclusively been limited to mapping one dataset onto another or to modeling the effects of one level of regulation onto PINs [5,47,56,57]. Yet, very few studies have examined different networks in the same cell simultaneously [32,45,58••] and this limits our ability to draw causal links.

A full integration of the different levels of cell regulation and how they tie with the organization of PINs will

require integrative computational approaches, but most importantly, it will require the joint study of the activity of transcriptional, posttranscriptional, posttranslational, metabolic networks and PINs in the same cells and under the same perturbations (Figure 4). The power of data integration has recently been illustrated by a Bayesian network model applied to DNA variation, RNA levels, and metabolite profiles from a yeast cross and datasets from public databases including protein-metabolite and protein–protein interactions [59•]. The authors concluded that the incorporation of metabolite levels into the network reconstruction process significantly enhanced the utility of the network-based models. This type of integrative experiment will enable a better annotation of PINs, including the identification of condition-dependent links, and will ultimately contribute to close the gap between genotypes and phenotypes on physiological and evolutionary time-scales.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

We thank Nadia Aubin-Horth, Alexandre Dubé, Emmanuel Levy, Isabelle Gagnon-Arsenault and Nick Bisson for comments on the manuscript. CRL's research on protein interactomes is funded by the Canadian Institute of Health Research (CIHR) grant GMX-191597, a Human Frontier Science Program (HFSP) Grant and the Natural Sciences and Engineering Research Council of Canada (NSERC). CRL is a CIHR New Investigator. JBL was supported by a fellowship from the Fonds de Recherche du Québec – Santé (FRQS); GD, LF and FTQ were supported by fellowships from the Quebec Research Network on Protein Function, Structure and Engineering (PROTEO); LF was supported by a fellowship from the Fonds de Recherche du Québec – Nature et Technologies (FRQNT) and SR by a graduate scholarship from NSERC.

References and recommended reading

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

- • of special interest
- •• of outstanding interest

1. Scott JD, Pawson T: **Cell signaling in space and time: where proteins come together and when they're apart**. *Science* 2009, **326**:1220-1224.

2. Strohman R: **Maneuvering in the complex path from genotype to phenotype**. *Science* 2002, **296**:701-703.

3. •• Wang X, Wei X, Thijssen B, Das J, Lipkin SM, Yu H: **Three-dimensional reconstruction of protein networks provides insight into human genetic disease**. *Nat Biotechnol* 2012, **30**:159-164.
This work identifies mutations responsible for human disorders that are located at the interaction interface of proteins. This study is the most comprehensive analysis of the potential effects of amino acid substitutions on PINs and their relation with human diseases at the structural level.

4. Sóler-Lopez M, Zanzoni A, Lluis R, Stelzl U, Aloy P: **Interactome mapping suggests new mechanistic details underlying Alzheimer's disease**. *Genome Res* 2011, **21**:364-376.

5. Serra-Musach J, Aguilar H, Iorio F, Comellas F, Berenguer A, Brunet J, Saez-Rodriguez J, Pujana MA: **Cancer develops, progresses and responds to therapies through restricted perturbation of the protein–protein interaction network**. *Integr Biol (Camb)* 2012, **4**:1038-1048.

6. 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.

7. 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.

8. Gavin AC, Aloy P, Grandi P, Krause R, Boesche M, Marzioch M, Rau C, Jensen LJ, Bastuck S, Dumpelfeld B *et al.*: **Proteome survey reveals modularity of the yeast cell machinery**. *Nature* 2006, **440**:631-636.

9. Krogan NJ, Cagney G, Yu H, Zhong G, Guo X, Ignatchenko A, Li J, Pu S, Datta N, Tikuisis AP *et al.*: **Global landscape of protein complexes in the yeast *Saccharomyces cerevisiae***. *Nature* 2006, **440**:637-643.

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

11. Yu H, Braun P, Yildirim MA, Lemmens I, Venkatesan K, Sahalie J, Hirozane-Kishikawa T, Gebreab F, Li N, Simonis N *et al.*: **High-quality binary protein interaction map of the yeast interactome network**. *Science* 2008, **322**:104-110.

12. Consortium AIM: **Evidence for network evolution in an *Arabidopsis* interactome map**. *Science* 2011, **333**:601-607.

13. Rual JF, Venkatesan K, Hao T, Hirozane-Kishikawa T, Dricot A, Li N, Berriz GF, Gibbons FD, Dreze M, Ayivi-Guedehoussou N *et al.*: **Towards a proteome-scale map of the human protein–protein interaction network**. *Nature* 2005, **437**:1173-1178.

14. Havugimana PC, Hart GT, Nepusz T, Yang H, Turinsky AL, Li Z, Wang PI, Boutz DR, Fong V, Phanse S *et al.*: **A census of human soluble protein complexes**. *Cell* 2012, **150**:1068-1081.

15. Stelzl U, Worm U, Lalowski M, Haenig C, Brembeck FH, Goehler H, Stroedicke M, Zenkner M, Schoenherr A, Koeppen S *et al.*: **A human protein–protein interaction network: a resource for annotating the proteome**. *Cell* 2005, **122**:957-968.

16. Taylor IW, Linding R, Warde-Farley D, Liu Y, Pesquita C, Faria D, Bull S, Pawson T, Morris Q, Wrana JL: **Dynamic modularity in protein interaction networks predicts breast cancer outcome**. *Nat Biotechnol* 2009, **27**:199-204.

17. Mullard A: **Protein–protein interaction inhibitors get into the groove**. *Nat Rev Drug Discov* 2012, **11**:173-175.

18. Christ F, Voet A, Marchand A, Nicolet S, Desimmie BA, Marchand D, Bardiot D, Van der Veken NJ, Van Remoortel B, Strelkov SV *et al.*: **Rational design of small-molecule inhibitors of the LEDGF/p75-integrase interaction and HIV replication**. *Nat Chem Biol* 2010, **6**:442-448.

19. Filippakopoulos P, Qi J, Picaud S, Shen Y, Smith WB, Fedorov O, Morse EM, Keates T, Hickman TT, Felletar I *et al.*: **Selective inhibition of BET bromodomains**. *Nature* 2010, **468**:1067-1073.

20. Brehme M, Hantschel O, Colinge J, Kaupe I, Planyavsky M, Köcher T, Mechtler K, Bennett KL, Superti-Furga G: **Charting the molecular network of the drug target Bcr-Abl**. *Proc Natl Acad Sci U S A* 2009, **106**:7414-7419.

21. Matsumoto A, Comatas KE, Liu L, Stamler JS: **Screening for nitric oxide-dependent protein–protein interactions**. *Science* 2003, **301**:657-661.

22. Dreze M, Charloteaux B, Milstein S, Vidalain PO, Yildirim MA, Zhong Q, Svrzikapa N, Romero V, Laloux G, Brasseur R *et al.*: **'Edgetic' perturbation of a *C. elegans* BCL2 ortholog**. *Nat Methods* 2009, **6**:843-849.

23. Zhong Q, Simonis N, Li QR, Charloteaux B, Heuze F, Klitgord N, Tam S, Yu H, Venkatesan K, Mou D *et al.*: **Edgetic perturbation models of human inherited disorders**. *Mol Syst Biol* 2009, **5**:321.

24. MacDonald ML, Lamerdin J, Owens S, Keon BH, Bilter GK, Shang Z, Huang Z, Yu H, Dias J, Minami T *et al.*: **Identifying off-target effects and hidden phenotypes of drugs in human cells**. *Nat Chem Biol* 2006, **2**:329-337.

25. Freschi L, Torres-Quiroz F, Dubé AK, Landry CR: **qPCA: a scalable assay to measure the perturbation of protein–protein interactions in living cells**. *Mol Biosyst* 2013, **9**:36-43.

26. Schlecht U, Miranda M, Suresh S, Davis RW, St Onge RP: **Multiplex**
• **assay for condition-dependent changes in protein–protein interactions**. *Proc Natl Acad Sci U S A* 2012, **109**:9213-9218.
The authors develop a multiplex assay to measure *in vivo* binary protein–protein interactions that allows to identify chemical and other modulators of PPI in parallel and at high-throughput. This is a first step towards studying the condition dependence of entire interactomes.

27. Lee KK, Sardiu ME, Swanson SK, Gilmore JM, Torok M, Grant PA, Florens L, Workman JL, Washburn MP: **Combinatorial depletion analysis to assemble the network architecture of the SAGA and ADA chromatin remodeling complexes**. *Mol Syst Biol* 2011, **7**:503.

28. Bisson N, James DA, Ivosev G, Tate SA, Bonner R, Taylor L,
•• Pawson T: **Selected reaction monitoring mass spectrometry reveals the dynamics of signaling through the GRB2 adaptor**. *Nat Biotechnol* 2011, **29**:653-658.
The authors use tandem affinity purification coupled with mass spectrometry and selected reaction monitoring to monitor the dynamics of interaction of an adaptor protein following growth stimulation through time. This approach makes possible the tracking of protein complex assembly and dissolution in a time-dependent fashion.

29. Levy ED, Landry CR, Michnick SW: **Signaling through cooperation**. *Science* 2010, **328**:983-984.

30. Manderson EN, Malleshaiah M, Michnick SW: **A novel genetic screen implicates Elm1 in the inactivation of the yeast transcription factor SBF**. *PLoS ONE* 2008, **3**:e1500.

31. Friedman AA, Tucker G, Singh R, Yan D, Vinayagam A, Hu Y,
• Binari R, Hong P, Sun X, Porto M *et al.*: **Proteomic and functional genomic landscape of receptor tyrosine kinase and ras to extracellular signal-regulated kinase signaling**. *Sci Signal* 2011, **4**:rs10.
The authors identify new regulators of the RTK-Ras-ERK pathway by combining RNAi screens with PINs resulting from TAP-MS screens performed under different conditions. It is one of the few works that study tri-partite interactions between one gene product and interacting proteins.

32. Rozenblatt-Rosen O, Deo RC, Padi M, Adelmant G, Calderwood MA, Rolland T, Grace M, Dricot A, Askenazi M, Tavares M *et al.*: **Interpreting cancer genomes using systematic host network perturbations by tumour virus proteins**. *Nature* 2012, **487**:491-495.

33. Jäger S, Cimermancic P, Gulbahce N, Johnson JR, McGovern KE, Clarke SC, Shales M, Mercenne G, Pache L, Li K *et al.*: **Global landscape of HIV-human protein complexes**. *Nature* 2012, **481**:365-370.

34. Mukhtar MS, Carvunis AR, Dreze M, Epple P, Steinbrenner J,
•• Moore J, Tasan M, Galli M, Hao T, Nishimura MT *et al.*: **Independently evolved virulence effectors converge onto hubs in a plant immune system network**. *Science* 2011, **333**:596-601.
The authors show that two very divergent plant pathogens target common proteins of the *Arabidopsis* PIN and that these nodes tend to have a high degree in the host network. In addition, many host targets shared by both pathogens are essential for pathogen growth-suppression mechanisms.

35. Calderwood MA, Venkatesan K, Xing L, Chase MR, Vazquez A, Holthaus AM, Ewence AE, Li N, Hirozane-Kishikawa T, Hill DE *et al.*: **Epstein-Barr virus and virus human protein interaction maps**. *Proc Natl Acad Sci U S A* 2007, **104**:7606-7611.

36. Pichlmair A, Kandasamy K, Alvisi G, Mulhern O, Sacco R, Habjan M, Binder M, Stefanovic A, Eberle CA, Goncalves A *et al.*: **Viral immune modulators perturb the human molecular network by common and unique strategies**. *Nature* 2012, **487**:486-490.

37. Selbach M, Paul FE, Brandt S, Guye P, Daumke O, Backert S, Dehio C, Mann M: **Host cell interactome of tyrosine-phosphorylated bacterial proteins**. *Cell Host Microbe* 2009, **5**:397-403.

38. Lovell SC, Robertson DL: **An integrated view of molecular coevolution in protein–protein interactions**. *Mol Biol Evol* 2010, **27**:2567-2575.

39. Livingstone K, Olofsson P, Cochran G, Dagilis A, Macpherson K, Seitz KA Jr: **A stochastic model for the development of Bateson-Dobzhansky-Muller incompatibilities that incorporates protein interaction networks**. *Math Biosci* 2012, **238**:49-53.

40. Zamir L, Zaretsky M, Fridman Y, Ner-Gaon H, Rubin E, Aharoni A:
•• **Tight coevolution of proliferating cell nuclear antigen (PCNA)–partner interaction networks in fungi leads to interspecies network incompatibility**. *Proc Natl Acad Sci U S A* 2012, **109**:E406-E414.
The authors use chimeric proteins from different yeast species to demonstrate how coevolution between interacting proteins could generate strong incompatibilities among proteins, thus disrupting protein complexes.

41. Leducq J-B, Charron G, Diss G, Gagnon-Arsenault I, Dubé AK,
•• Landry CR: **Evidence for the robustness of protein complexes to inter-species hybridization**. *PLoS Genet* 2012, **8**:e1003161.
The authors show that PPIs in two major protein complexes are highly conserved between two yeast species and their hybrid, demonstrating robustness to hybridization even after substantial protein divergence. This is the first study to systematically assess the interactome in hybrids between species.

42. Sun MG, Sikora M, Costanzo M, Boone C, Kim PM: **Network evolution: rewiring and signatures of conservation in signaling**. *PLoS Comput Biol* 2012, **8**:e1002411.

43. Freschi L, Courcelles M, Thibault P, Michnick SW, Landry CR: **Phosphorylation network rewiring by gene duplication**. *Mol Syst Biol* 2011, **7**:504.

44. Froyd CA, Rusche LN: **The duplicated deacetylases Sir2 and Hst1 subfunctionalized by acquiring complementary inactivating mutations**. *Mol Cell Biol* 2011, **31**:3351-3365.

45. Gagnon-Arsenault I, Marois-Blanchet FC, Rochette S, Diss G,
• Dubé AK, Landry CR: **Transcriptional divergence plays a role in the rewiring of protein interaction networks after gene duplication**. *J Proteomics* 2012 http://dx.doi.org/10.1016/j.jprot.2012.09.038.
This work shows that the evolution of regulatory sequences may contribute to rewire PINs during evolution. The authors study the extent to which the PINs of yeast paralogous proteins are rewired by placing the coding sequence of each gene under the control of the other one. They present four examples in which the diverged PPI profiles were dependent on transcriptional regulation.

46. Ellis JD, Barrios-Rodiles M, Colak R, Irimia M, Kim T, Calarco JA, Wang X, Pan Q, O'Hanlon D, Kim PM *et al.*: **Tissue-specific alternative splicing remodels protein–protein interaction networks**. *Mol Cell* 2012, **46**:884-892.

47. Buljan M, Chalancon G, Eustermann S, Wagner GP, Fuxreiter M, Bateman A, Babu MM: **Tissue-specific splicing of disordered segments that embed binding motifs rewires protein interaction networks**. *Mol Cell* 2012, **46**:871-883.

48. Amoutzias G, Van de Peer Y: **Single-gene and whole-genome duplications and the evolution of protein–protein interaction networks**. *Evolutionary Genomics and Systems Biology*. John Wiley & Sons, Inc.; 2010:. 413–429.

49. Treangen TJ, Rocha EP: **Horizontal transfer, not duplication, drives the expansion of protein families in prokaryotes**. *PLoS Genet* 2011, **7**:e1001284.

50. Tautz D, Domazet-Loso T: **The evolutionary origin of orphan genes**. *Nat Rev Genet* 2011, **12**:692-702.

51. Landry CR, Levy ED, Michnick SW: **Weak functional constraints on phosphoproteomes**. *Trends Genet* 2009, **25**:193-197.

52. Mosca R, Pache RA, Aloy P: **The role of structural disorder in the rewiring of protein interactions through evolution**. *Mol Cell Proteomics* 2012, **11**:M111014969.

53. Papp B, Pal C, Hurst LD: **Dosage sensitivity and the evolution of gene families in yeast**. *Nature* 2003, **424**:194-197.

54. Fernández A, Lynch M: **Non-adaptive origins of interactome**
•• **complexity**. *Nature* 2011, **474**:502-505.
This work addresses the role that random genetic drift could play in PIN evolution. It shows that large organisms with small population sizes experience a significant increase in the power of drift that leads to the accumulation of mild structural deficiencies. These mutations provide opportunities for the recruitment of stabilizing protein-interactions that may contribute to increase molecular and organismal complexity.

55. Levy ED, Michnick SW, Landry CR: **Protein abundance is key to distinguish promiscuous from functional phosphorylation based on evolutionary information**. *Philos Trans R Soc Lond B: Biol Sci* 2012, **367**:2594-2606.

56. Mihalik Á, Csermely P: **Heat shock partially dissociates the overlapping modules of the yeast protein–protein interaction network: a systems level model of adaptation**. *PLoS Comput Biol* 2011, **7**:e1002187.

57. Davis MJ, Shin CJ, Jing N, Ragan MA: **Rewiring the dynamic interactome**. *Mol Biosyst* 2012, **8**:2054-2066.

58. •• van Noort V, Seebacher J, Bader S, Mohammed S, Vonkova I, Betts MJ, Kuhner S, Kumar R, Maier T, O'Flaherty M *et al.*: **Cross-talk between phosphorylation and lysine acetylation in a genome-reduced bacterium**. *Mol Syst Biol* 2012, **8**:571.

Using systematic perturbations by deletion in *Mycoplasma pneumoniae*, the authors demonstrate a multilevel impact of the perturbations, affecting proteins abundance and posttranslational modifications mostly without transcriptional modifications. They integrate these data with PINs to show that perturbed proteins are more likely to interact with each other.

59. • Zhu J, Sova P, Xu Q, Dombek KM, Xu EY, Vu H, Tu Z, Brem RB, Bumgarner RE, Schadt EE: **Stitching together multiple data dimensions reveals interacting metabolomic and transcriptomic networks that modulate cell regulation**. *PLoS Biol* 2012, **10**:e1001301.

This newly developed analytical approach successfully displays that combining multiple layers of biological information has a synergistic effect on biological insights discovery. The authors assess the contributions of different data types to the predictive power of the network including DNA variation, RNA levels, metabolite levels, protein–protein interaction data, protein–DNA binding data and protein small molecule interaction data.