Systems Biology Approaches for Host–Fungal Interactions: An Expanding Multi-Omics Frontier

Luka Culibrk, Carys A. Croft, and Scott J. Tebbutt

Abstract

Opportunistic fungal infections are an increasing threat for global health, and for immunocompromised patients in particular. These infections are characterized by interaction between fungal pathogen and host cells. The exact mechanisms and the attendant variability in host and fungal pathogen interaction remain to be fully elucidated. The field of systems biology aims to characterize a biological system, and utilize this knowledge to predict the system’s response to stimuli such as fungal exposures. A multi-omics approach, for example, combining data from genomics, proteomics, metabolomics, would allow a more comprehensive and pan-optic “two systems” biology of both the host and the fungal pathogen. In this review and literature analysis, we present highly specialized and nascent methods for analysis of multiple -omes of biological systems, in addition to emerging single-molecule visualization techniques that may assist in determining biological relevance of multi-omics data. We provide an overview of computational methods for modeling of gene regulatory networks, including some that have been applied towards the study of an interacting host and pathogen. In sum, comprehensive characterizations of host–fungal pathogen systems are now possible, and utilization of these cutting-edge multi-omics strategies may yield advances in better understanding of both host biology and fungal pathogens at a systems scale.

Introduction

Invasive fungal infections (IFIs) are caused by opportunistic fungi such as the filamentous Aspergillus fumigatus or the yeasts Candida albicans and Cryptococcus neoformans (Enoch et al., 2006). Though not typically a concern in healthy individuals, IFIs are able to afflict ill or immunocompromised patients severely, including individuals with leukemia, transplant recipients, and those with HIV/AIDS (Comely et al., 2015; de Oliveira et al., 2014; Klingspor et al., 2015; Neofytos et al., 2013).

The incidence of IFIs is increasing, and a large proportion of these IFIs are nosocomial (Beck-Sagué and Jarvis, 1993; Lehrnbecher et al., 2010). This is believed to be due to an increase in the population of immunocompromised individuals (Lehrnbecher et al., 2010; Warnock, 2007). IFIs tend to have high mortality rates (Comely et al., 2015; Lehrnbecher et al., 2010), and as a result the improvement of current prophylactic and curative treatments is of increasing interest. It is essential that we understand the fundamental and dynamic biological interactions between host and fungal cells in order to advance the care and treatment of patients with IFIs.

Pathogenesis requires an interaction between a pathogen and its host. There are numerous examples of host–fungal interactions in the context of organisms causing IFIs. Aspergillus fumigatus has been shown to adhere to extracellular matrix of the lung as well as the surface of human lung epithelial cells (Gil et al., 1996, Sheppard, 2011). Additionally, the internalization of A. fumigatus spores by epithelial cells in vitro has been observed numerous times (Gomez et al., 2010; Oosthuizen et al., 2011; Wasylnka and Moore, 2003).

Candida albicans has been observed to invade host cells by inducing endocytosis (Dalle et al., 2010) or through active invasion, a process by which hyphae breach epithelial cell membranes (Dalle et al., 2010, Wächtler et al., 2011). It has been demonstrated that C. neoformans infects its host through an actin-dependent internalization mechanism (Guerra et al., 2014). These initial interactions often lead to other interactions between the host and fungus on numerous levels. Host–fungal interaction networks are extremely complex, as there are many inherent differences between mammalian cells and fungal cells. A comprehensive analysis of these networks would entail the use of “-omics”-wide techniques in order to capture both the drastic...
and the subtle dynamic biological perturbations within both host and pathogen.

The study of various biological "-omics" is generally segregated into several major fields of high-throughput biology, notably genomics, transcriptomics, proteomics, and metabolomics. An ideal -omic analysis of an organism involves collection of complete and unbiased datasets representative of the entire set of biomolecules of interest. Techniques that do not select specific, or candidate, targets are of particular value as they permit identification of novel biological networks without prior knowledge. The use of high-throughput techniques such as these has recently become far more commonplace as they can provide a more complete picture of the complexities of an organism’s or cell’s responses to experimental or environmental conditions. More prevalent quantitative techniques such as western blots and reverse transcription quantitative PCR are only able to analyze specific targets and are thus unable to detect unexpected changes.

Historically, high-throughput biology has been associated with a prohibitive monetary cost, rendering many of these techniques inaccessible to most researchers. Despite this, high-throughput biology has undeniable potential for the systematic analysis of a complex biological system, such as a host–pathogen interaction (Fig. 1). Researcher uptake has been aided by an increase in affordability of several high-throughput biology techniques in recent years. A notable example is the price of commercial genome sequencing, with the full sequencing of a human genome now as low as US $1000 (Veritas Genomics, 2015).

There are previous reviews on topics related to the host–fungal interaction (Durmuş et al., 2015; Horn et al., 2012; Santamaría et al., 2011), and our review builds upon these by outlining and integrating both experimental and computational methods in high-throughput biology. We place particular emphasis on recent innovations in technology that promise to yield valuable insights into the relatively limited field of host–fungal interactions.

The “Omics”

Genomics

Early genomic studies of host–pathogen interactions involved molecular genetics and classical mutagenesis-screening-cloning experiments in order to link a gene to a phenotype (Fromtling et al., 1982; Kwon-Chung et al., 1982; Sandhu et al., 1976). These studies allowed the identification of essential genes and virulence factors (Chang and Kwon-Chung, 1994; Kwon-Chung et al., 1982). However, as these scientists did not have access to the same types of omics-platforms and data as we have today, their studies were necessarily more limited in their scope.

Today there are many fully sequenced genomes with at least some annotations, allowing for a “forward genetics” approach, looking for protein-coding genes based on predicted...
RNAi is a knock-introduced silencing RNA (Larsson et al., 2010). Furthermore, weakness in some respects is its transience; high-turnover cost, but comes with some significant challenges. Its primary out by gene editing, such as simplicity and a relatively low et al., 2011; Stroschein-Stevenson et al., 2009).

interaction with a host (Chen et al., 2015b; Hu et al., 2008; Qin et al., 2014; Kalleda et al., 2013) and in the context of in- been used to great effect in the study of fungi alone (Eslami et al., 2015). These targeted experiments have also been able to make use of newly developed gene knockdown techniques. One such method is RNA interference (RNAi); the use of double-stranded RNA that binds to a specific mRNA transcript of interest, inhibiting translation (Hannon, 2002). RNAi has been used to great effect in the study of fungi alone (Eslami et al., 2014; Kalleda et al., 2013) and in the context of interaction with a host (Chen et al., 2015b; Hu et al., 2008; Qin et al., 2011; Stroschein-Stevenson et al., 2009).

RNAi has numerous strengths when compared to a knock-out by gene editing, such as simplicity and a relatively low cost, but comes with some significant challenges. Its primary weakness in some respects is its transience; high-turnover transcripts cannot be silenced effectively as the rapid production of mRNA quickly overwhelms the experimentally introduced silencing RNA (Larsson et al., 2010). Furthermore, RNAi is a knock-down, rather than a true knock-out. Protein function may not be completely abolished within the organism, and can affect experimental results (Moore et al., 2010).

A relatively new technique, the CRISPR/Cas9 gene editing system, has been developed for true gene knockout in eukaryotic cells (Wang et al., 2013). It has been shown that A. fumigatus and C. albicans are both compatible with the use of the CRISPR/Cas9 system (Fuller et al., 2015; Vyas et al., 2015), illustrating proof-of-concept for use of the CRISPR/Cas9 system in the study of host–fungus interactions. Despite its many potential applications, CRISPR/Cas9 is still in its infancy and has been plagued with issues such as low rates of uptake of the CRISPR/Cas9 machinery and off-target reactions. Further optimization of the basic molecular machinery of the CRISPR/Cas9 system is still underway (Zetsche et al., 2015).

Epigenetic modifications are another important aspect to consider when characterizing an organism’s response, as they have an effect on the rate of gene expression. A major technique used to analyze distribution of DNA-binding proteins is ChIP-seq; chromatin immunoprecipitation coupled to high-throughput parallel sequencing (Robertson et al., 2007). ChIP-seq has been used extensively to examine the epigenomic profiles of fungi such as C. neoformans and A. fumigatus, under various experimental conditions (Chung et al., 2014; Toh-e et al., 2015).

Other forms of epigenetic modification represent interesting fields of research; some, such as DNA methylation, have been investigated in pathogenic fungi (Liu et al., 2012; Mishra et al., 2011). Of note is the recently reported ability of some pathogenic bacteria to modify a host’s gene expression through histone modifications and DNA methylation. A study by Sharma and colleagues (2015) determined that secreted bacterial methyltransferases were binding to host genes involved in the innate immune response. These observations corroborate a study that observed a downregulation of specific immune response genes due to a direct interaction between a pathogenic bacterial protein and host chromatin (Rennoll-Bankert et al., 2015).

In addition to its possible effects on pathogenesis, the ability to alter a host’s epigenome could have arisen from host–pathogen co-evolution. Observation of this behavior in a bacterial host–pathogen system provides biological precedent for the possibility of a similar interaction between a fungus and its host. Such mechanisms could present targets for future study in antifungal treatment.

Transcriptomics

The transcriptome of a cell can provide useful insights into differences in an organism’s gene expression under varying environmental conditions. The DNA microarray has allowed researchers to carry out analyses of the transcriptome of an organism for some time now (Schena et al., 1995), and has been applied extensively to the study of host–fungus interactions and changes in gene expression (Chow et al., 2007; Gomez et al., 2010; Kraus et al., 2004; Oosthuizen et al., 2011; Pukkila-Worley et al., 2005). Despite the many benefits, primarily ease of analysis and relatively low cost, microarrays have several limitations. Microarrays only detect selected gene transcripts that are based on the specific probes used on the array, preventing the discovery of novel transcripts and limiting the amount of data obtainable.

The more recently developed technique of RNA sequencing (RNA-seq) has provided a new way to analyze the transcriptome of an organism in more unbiased fashion (Mortazavi et al., 2008). Several studies have been conducted using RNA-seq to investigate dual-organism interactions (Chen et al., 2014; 2015a; Tierney et al., 2012), demonstrating its suitability as a tool for the analysis of host–fungus interactions from a transcriptomic perspective.

Validation of high-throughput transcriptomic data is essential for confidence in reported results. Quantitative PCR is a common method used to support the validity of RNA-sequencing and microarray data (Chen et al., 2015b; Gomez et al., 2010, Oosthuizen et al., 2011). While the need for validation has been questioned by some (Fang and Cui, 2011), many journals still require some form of validation of results obtained using these high-throughput methods.

Whilst quantitative PCR is limited in its multiplexity, there are other recently developed techniques capable of quantifying

**Table 1. Sequenced Genomes of Pathogenic Fungi**

| Species               | Reference                  |
|-----------------------|----------------------------|
| Aspergillus sp.       | Nierman et al., 2005       |
| Aspergillus fumigatus | Galagan et al., 2005       |
| Aspergillus nidulans  | Galagan et al., 2005       |
| Aspergillus flavus    | Nierman et al., 2015       |
| Candida sp.           | Jones et al., 2004         |
| Candida albicans      | Jones et al., 2004         |
| Cryptococcus sp.      | Loftus et al., 2005        |
| Cryptococcus neoformans | D'Souza et al., 2011     |

Opportunist pathogenic fungi are subdivided by genus, and individual species’ genome sequences are cited as shown.
Proteomics

While transcriptomic studies provide one view of gene expression, proteomics is another useful tool for the examination of a system’s gene expression patterns. The gold standard for proteomic analysis, particularly in the field of systems biology, remains mass spectrometry (Sabidó et al., 2012). Large-scale quantitative proteomics is of interest as it enables the determination of relative expression changes between different conditions.

Three label-based, quantitative proteomics techniques are of particular note: SILAC, iTRAQ, and PUNCH-P. Stable isotope labeling of amino acids in cell culture (SILAC) compares protein expression between samples due to experimental conditions through the incorporation of stable, heavy isotopes of amino acids into a cell’s proteins (Ong et al., 2002). Furthermore, SILAC can examine protein expression changes over specific time-points through “pulsing,” a process whereby cells are transferred to SILAC media at certain stages of experimental treatment (Schwanhausser et al., 2009). SILAC is only compatible with cells that can be cultured for extended periods of time; pulsed-SILAC requires 6 hours at the minimum to provide meaningful data (Schwanhausser et al., 2009).

Isobaric tags for absolute and relative quantitation (iTRAQ) is another tag-based proteomics technique that tags peptide fragments at their N-terminus after digestion (Ross et al., 2004; Wiese et al., 2007). The specific advantage of iTRAQ is its versatility; unlike SILAC, iTRAQ is capable of analyzing protein expression changes over short periods of time, as incorporation of metabolic tags into proteins is not required. SILAC and iTRAQ have been used to study fungi in detail alone (Cagas et al., 2011; Georgianna et al., 2008; Zhang et al., 2015), and in a dual-organism setting (Reales-Calderón et al., 2013).

Puromycin-assisted nascent chain proteomics (PUNCH-P) is another recently developed technique able to detect newly synthesized proteins over short time periods with high accuracy through incorporation of puromycin into polypeptide chains (Aviner et al., 2013). Puromycin, a protein synthesis inhibitor, can be integrated into polypeptide chains when present in low concentrations. A chimeric puromycin-biotin “tag” then allows for purification of the tagged polypeptides by use of solid-phase streptavidin beads. Digestion of puromycin containing proteins prior to elution followed by mass spectrometry then yields analysis of newly synthesised peptides. While this technique has not yet been applied to mycology, PUNCH-P is a viable alternative to iTRAQ for analysis of short-term protein expression changes.

The localization of proteins is essential to understand their function and the interactions they have with proteins of the cell or pathogen. In addition, the localization of newly synthesized proteins can provide useful information about the proteomic response of one organism to another. Green fluorescent protein (GFP) constructs have been the method of choice for in situ visualization of proteins for some time (Chalfie et al., 1994). While GFP is useful for long-term study of steady-state systems, limited multiplexity and the inability to monitor differential expression decreases its usefulness for proteomic studies.

Multiplexed protein localization using GFP constructs has been investigated through a number of methods, such as subcellular fractionation for organelle-level spatial resolution (Zimmermann et al., 2010), or through the lengthy process of producing many GFP–protein constructs (Dénervaud et al., 2013). While indirect visualization through fractionation or direct visualization through transfection with engineered plasmid constructs is useful for determining spatial localization of specific proteins upon a given treatment, the ability to directly visualize a variety of specific proteins and peptides in a high-throughput manner could be applied to numerous areas of research.

Surface sensing of translation (SU嫩SET) is a recently developed method that, like PUNCH-P, makes use of puromycin incorporation into a polypeptide chain, but follows this with anti-puromycin immunostaining for visualization of all newly synthesised proteins within a cell (Goodman et al., 2011; Schmidt et al., 2009). Recently, this has been refined to label specific proteins of interest in vivo by using an antibody pair: one anti-puromycin antibody, and one antibody against the target protein, and combining this approach with a proximity
ligation assay (Tom Dieck et al., 2015). The use of puromycin for visualization is particularly advantageous as it allows for simultaneous PUNCH-P analysis of protein expression.

In addition to this antibody-based method, mass spectrometry can also be utilized for detailed analysis of a cell’s proteome. A number of different methods have been developed for cellular mass spectrometry imaging, which have been described and summarized in a thorough review by Pól et al. (2010). The biologically relevant techniques described employ a precise laser that ionizes the surface of a biological sample, generating ions detectable through mass spectrometry.

Of particular interest is secondary ion mass spectrometry (SIMS), which provides a 2D resolution of below 1 μm, allowing for subcellular resolution (Pól et al., 2010). Stable isotope labeling has been combined with SIMS, resulting in multi-isotope imaging mass spectrometry (MIMS) (Steinhauser et al., 2012). MIMS has been used to analyze protein content in single cells, including within dendritic spines (Brismar et al., 2014) and hair cell stereocilia (Zhang et al., 2012). The ability to analyze and map individual proteins of interest is a potentially powerful tool for elucidating host–pathogen interactions in dual-organism networks.

Interactomics, a subset of proteomics, is particularly pertinent to host–pathogen interactions. The possibility that proteins of fungal and host cells not only interact but could be exchanged is intriguing and potentially quite valuable to our understanding of pathogenesis. The first challenge for dual-organism interactome analysis is to demonstrate the localization of host or pathogen proteins within the other organism. This can be accomplished using a SILAC-based technique, trans-SILAC (Rechavi et al., 2010). This method detects SILAC-tagged peptides within different cell samples, and has been applied towards the identification of human–human and bacterial–human protein delivery. This allows for the identification of inter-organism protein localization in a high-throughput, unbiased manner.

Direct observation of occurring protein–protein interactions is required subsequent to the identification of protein co-localization in an intercellular system. The yeast two-hybrid system has been a standard for examination of protein–protein interactions (PPIs) for some time (Fields, 1993; Fields and Song, 1989). While this technique provides critical tools for interactome analysis, it has a number of caveats to consider. A high percentage of the PPIs predicted by the system are false positives, necessitating stringent validation (von Mering et al., 2002).

Integration of high-throughput proteomics into the analysis of PPIs whilst eliminating the precision challenges observed with the two-hybrid system has been achieved by protein correlation profiling-SILAC (PCP-SILAC) (Kristensen et al., 2012). PCP-SILAC essentially utilizes a guilt-by-association approach by combining mass spectrometry with native chromatography; if two proteins migrate together in the chromatogram, they are assumed to be interacting (Fig. 2B). A combination of trans-SILAC and PCP-SILAC, a
Metabolomics

Metabolites are considered by some to be the final downstream effector molecules of the transcriptome and proteome. It therefore comes as no surprise that analysis of the metabolome would be valuable for the study of how fungi interact with their host, and vice-versa. Much like the genome, knowledge of an organism’s metabolome provides a notable advantage to metabolic research. The human metabolome is freely available through the Human Metabolome Database (Wishart et al., 2007). No such database exists for any pathogenic fungi, an undeniable challenge in the analysis of complex fungal metabolomes.

High-throughput techniques exist that are capable of analyzing metabolomes, namely mass spectrometry and nuclear magnetic resonance (NMR). Mass spectrometry is capable of analyzing extremely low quantities of metabolites within a sample, allowing for detection of materials at low concentrations within the target cell. Unfortunately, mass spectrometry is not as precise in quantification of metabolites within a sample unless stable isotope labeling methods are used (Creek et al., 2012; Veenstra, 2012).

By contrast, NMR technology is capable of determining relative quantitation of metabolites with ease (Griffiths, 1998), although it struggles with a sensitivity several orders of magnitude lower than that of mass spectrometry (Veenstra, 2012; Webb, 2012). Both mass spectrometry (Choi et al., 2012, Han et al., 2012, 2013; Smidsgaard and Nielsen, 2005) and NMR (de Souza et al., 2013; Forseth et al., 2011, Sanchez and Wang, 2012) have been applied to fungal metabolomics.

Resolution of the localization of fungal metabolites has recently been made possible using MIMS (see above). Quantitative imaging of specific metabolites has been reported within yeast to a high degree of precision (Siaardi et al., 2014; Steinhauser and Lechene, 2013), demonstrating a proof-of-concept for this method. To our knowledge, the metabolome has not been investigated in a dual-organism interaction setting. As the metabolome is capable of providing powerful insights into an organism’s response, the integration of metabolomics into a host–fungal interaction study would be a highly valuable addition to other “-omics” data.

Computational Modeling

Gene Network Inference: Dare to DREAM

High-throughput datasets require a large degree of computational assistance for their analysis. These analyses may involve the use of transcriptomic, proteomic, or metabolomic datasets to generate interaction networks at the organism–organism level. These networks are of interest for the identification of key characteristics of host–pathogen interactions.

RNA-seq and microarray-based transcriptomic technologies allow for generation of transcriptome-wide gene expression datasets. Gene network inference is an attempt to use high-throughput expression data to infer a regulatory network through a variety of statistical methods (Liu, 2015) (Fig. 2A). The DREAM (Dialogue on Reverse Engineering Assessment and Methods) challenges are a set of open challenges for researchers to utilize their own computational methods for the completion of a certain systems biology task.

One recent and notable example is the International Cancer Genome Consortium–The Cancer Genome Atlas DREAM Somatic Mutation Calling Challenge, in which participants were requested to simulate genomes of tumor cells, culminating in the development of an algorithm for prediction of cancer genomes (Ewing et al., 2015). DREAM challenge 5, which compared the precision of 35 different methods for computing inferred gene regulatory networks, is the most recent competition examining the problem of network inference (Marbach et al., 2012).

The challenge provided gene expression data from micro-organisms with known gene expression pathways; DREAM participants were instructed to attempt to reverse-engineer the gene regulatory network using their own software. The competitors of the challenge were subset into five categories based on methodology: regression, mutual information, correlation, Bayesian, and a group of assorted “others.” Interestingly, the best performers were “GEne Network Inference with Ensemble of trees” (GENIE3), an algorithm using a tree-based methodology (Huynh-Thu et al., 2010), and an “ANalysis Of Variance” (ANOVA)-based method for nonlinear modeling (Küffner et al., 2012), two methods categorized as “others.”

Other techniques have been developed outside the DREAM challenges, including the popular weighted gene correlation network analysis (WGCNA), which calculates a Pearson correlation coefficient between every gene in the dataset and weights each interaction based upon the strength of the correlation (Langfelder and Horvath, 2008). Further studies evaluated these network tools relative to one another and determined that GENIE3 provided a significant increase in precision when compared to all other evaluated tools (Madhamshettiwar et al., 2012). Other types of gene network modeling techniques have been used to survey potential gene regulatory networks in human pathogenic fungi (Altwasser et al., 2012, 2015).

It is worth noting that despite the progress in computational modeling, the top-scoring algorithms still returned a high rate of false positive interactions (Madhamshettiwar et al., 2012). GENIE3’s random forest basis has been improved upon with the release of the iRaftNet algorithm by allowing for the integration of data from outside sources, a capability that GENIE3 lacks (Petralia et al., 2015).

The DREAM challenges outline a key difficulty in the inference of genetic regulatory networks; computational modeling is inherently limited by the precision and replicability of high-throughput datasets. These datasets can be subset into two categories: analog, where the data is collected by observation of relative arbitrary units (such as fluorescent intensity), and digital data, which is a direct count of molecules within a sample. As these validations and comparisons were conducted utilizing microarray data, which is analog, they suffer both from the background noise and compressed linear scale. Indeed, most methods of high-throughput data collection, including the DNA microarray and spectrometry-based
technologies are reliant on analog measurements, allowing only relative quantification of data at best, with significant noise introduced as a result.

Digital-based data, such as RNA-seq, provide a promising alternative to noisy analog datasets, despite still being only a relative quantification method. Models based upon this type of data show demonstrable improvement (Iancu et al., 2012). Despite this, current methods are still unable to provide a definitive model of gene regulatory networks (Ballouz et al., 2015). Nonetheless, it is difficult to obtain “perfect” datasets for these purposes that contain highly sensitive, precise data collected with a large dynamic range.

Integration of the “-omics”: Towards multi-omics biology

Given the high rates of false network discovery, the development of methods to overcome these issues is essential. The integration of information and data outside of gene expression datasets lends a greater degree of confidence in an inferred network. Integration of prior network knowledge from the literature also provides increased accuracy to an inferred network (Ellwanger et al., 2014; Olsen et al., 2014). Based upon the central dogma of molecular biology, the proteome should provide a less biased view of gene expression compared to measurements of mRNA. Therefore integration of proteomics and transcriptomics into inference networks provides valuable correlation and correction of inferred models (Poultney et al., 2012).

Other types of valuable prior data exist, including protein–protein interaction networks, CHiP-seq, and metabolomic data. Integration of some of these types of data has been conducted and demonstrated to increase confidence in an inferred network (Garcia-Reyero et al., 2014; Greenfield et al., 2013; Schöpke and Zeng, 2012). Although a few studies have examined the integration of metabolomic data into an inferred model of gene network inference, it remains a potentially powerful method of improving confidence in an inferred regulatory network.

Algorithms have been developed for multi-omic data integration, such as sparse generalized canonical correlation analysis (SGCCA) (Tenenhaus et al., 2014), which was specifically designed for this purpose. SGCCA has been applied to the integration of various types of datasets, including gene expression data from a microarray and DNA methylation (Wang et al., 2014), and microarray data with iTRAQ proteome data (Günter et al., 2014). The results from these studies are promising, indicating that SGCCA may be capable of providing excellent correlative assistance in the interpretation of systems-wide observations.

Spatial Visualization

Several of the techniques discussed earlier in this review require the use of microscopy. Confocal microscopy has been the staple for visualization of fluorescent molecules within cells for some time. Development of super-resolution fluorescent microscopy has permitted the imaging of single molecule fluorescence, and a number of specific methods have been developed (Betzig et al., 2006; Hess et al., 2006; Rust et al., 2006). This development has allowed for the implementation of quantitative fluorescent techniques such as FISSEQ and MERFISH, and provides the basis for their usage in a biological study.

The use of visualization techniques allows for the validation and observation of novel interactions that occur within an organism or between a pair of interacting organisms. Spatio-temporal modeling is the integration of time-course and spatial distribution. Currently, this has been applied to spatial resolution of the transcriptome between multiple regions of an organism, such as the different regions of the human brain (Kang et al., 2011) or different regions of a germ layer (Hashimshony et al., 2015).

Groundbreaking examples of protein–RNA co-localization research include studies into the role of Drosophila patterning genes, resulting in the discovery that these proteins and their transcripts are co-localized in the Drosophila embryo (Bergsten and Gavis, 1999; Berleth et al., 1988; Spirov et al., 2009). These studies were conducted using methods that can be effectively outperformed by newer techniques. FISSEQ and MERFISH are both capable of localizing RNA within a single cell to a high degree of accuracy (Chen et al., 2015a, Lee et al., 2015), and protein visualization techniques have likewise been drastically improved upon (Tom Dieck et al., 2015), allowing for the possibility of visualization of protein–mRNA co-localizations with increased resolution and multiplexity. These techniques allow both mRNA and protein localization to be correlated in-situ.

The mass spectrometry-based methods for visualisation (MIMS) of cellular components have been developed, and are capable of analysis of nucleic acids (Steinhauser et al., 2012), proteins (Brismar et al., 2014; Zhang et al., 2012), and metabolites (Saiardi et al., 2014). However, these methods are mass spectrometry-based, and as a result are quite expensive. Nevertheless, in a dual organism setting, such methods can potentially identify a myriad of novel interactions, such as protein–protein interactions between host and pathogen or changes in mRNA localization as a result of interaction with host or pathogen. The ability to visualize a dual organism interaction in this fashion could greatly elucidate our understanding of the interaction in a spatio-temporal context. The combination of previously discussed analytical methods with these visualisation techniques may provide key context for drawing conclusions for biological relevance (Fig. 3).

Dual Organism Experiments

The utilization of the methodologies described above, in a dual organism setting, has been limited. A notable example of one such experiment involved the inference of a trans-species interaction network between Mus musculus macrophages and C. albicans (Tierney et al., 2012). The experiment relied on dual organism RNA-seq to profile the transcriptome of C. albicans and M. musculus macrophages simultaneously. The authors then computationally inferred an interaction network demonstrating cross-species network regulation. To our knowledge, this is the only published example of a host–fungus experiment that used a modeling approach.

Dual organism transcriptomics has been utilized to characterize the transcriptomes of human lung airway cells and A. fumigatus simultaneously, however these studies could only draw conclusions based on gene ontology enrichment and direct changes in expression as they did not perform computational network modeling (Chen et al., 2015b; Gomez et al., 2010; Oosthuizen et al., 2011). The paucity of A. fumigatus functional gene annotations has been a limitation for
these studies; many differentially expressed genes identified by Oosthuizen et al. (2011) lacked functional annotation. Nevertheless, such findings provide useful biological insight at a high level. Putative genes were identified with evidence for their expression, and in some cases differential expression, under specific experimental conditions. These observations allow for future studies to focus on these hitherto hypothetical genes to derive more functional insight. Hi-Jack, a tool designed for the analysis of simultaneous host–pathogen interactions using metabolomic data has been described (Kleftogiannis et al., 2015). This tool infers metabolic networks where a pathogen may be “hijacking” metabolites of its host, and has been used in a Mycobacterium tuberculosis model (Kleftogiannis et al., 2015). The application of such a framework to other “omics” areas could be highly informative. However, to our knowledge, Hi-Jack is the only tool developed specifically for use in a dual organism study.

**Challenges and the Road Ahead**

Significant challenges remain in the analysis of a dual organism host–fungal system. In terms of experimental design, fungal anatomy may lead to issues with the effectiveness of some experiments. The fungal cell wall represents an obstacle for many aspects of experimental protocols, such as the internalization of reagents, which may interfere with metabolic labeling necessary for techniques such as SILAC or SUnSET. Cell walls also interfere with lysis of fungal cells, which may be problematic when complete lysis is desired for maximum yield and low levels of technical bias.

While the DNA sequences, and thus amino acid sequences of genes and proteins vary enough to distinguish a fungal and mammalian molecule, many metabolites have no such restrictions. In this regard, network analysis via transcriptomic and proteomic analysis may be able to assist by identifying metabolic pathways of interest. While studies have managed to analyze the metabolome of various pathogenic fungi, it may prove to be exceedingly challenging to distinguish the fungal metabolome from a host metabolome, or vice-versa. Careful experimental design and precise technique is necessary to eliminate cross-contamination of processed samples, ensuring complete separation of host and fungal cells, or to verify the species that produced any given metabolite.

The introduction of a novel methodology into the laboratory also comes with significant challenges. Many techniques that we have discussed have only recently been reported. The potential power that these methods have is tempered by the fact that they have yet to be fully validated and assessed by the wider scientific community. Techniques allowing visualization of biomolecules, such as FISSEQ, have a large amount of inherent noise and significant false discovery rates, both of which present challenges that need to be solved for them to be used effectively.

These techniques also come with significant equipment and monetary requirements. Outfitting a microscope for FISSEQ analysis can cost up to US $20,000 alone (Lee et al., 2014), in addition to other costs such as sequence library preparation.
Many accepted and validated techniques in high-throughput biology, such as RNA-seq and mass spectrometry may still be prohibitively expensive for many research laboratories.

Despite the difficulties associated with them, modern tools have resulted in a number of new and exciting ways to analyze host–pathogen systems. Within the scope of any individual field of the “-omics,” a number of questions have yet to be investigated in any sort of detail. The investigation into the inter-species regulatory network of *C. albicans* demonstrated the potential for the application of network inference between a host and fungus; this study is the only one of its kind within this field (Tierney et al., 2012).

Similar analyses directed towards other pathogenic fungi, such as *A. fumigatus* or *Cryptococcus*, or other types of host cells, would yield novel insights into the host–pathogen system. High-throughput proteomics is now capable of demonstrating protein–protein interactions between a host and pathogen, while simultaneously generating data on expression to corroborate transcriptomic observations by using PCP-SILAC and trans-SILAC. Metabolomic integration would determine phenotypic consequences of fungal pathogenic infection and support biological pathway enrichment observed by proteomic and transcriptomic experiments.

Finally, direct observations of experimentally-hypothesised dual organism responses are now possible through multiple methods of fluorescent subcellular visualization. The “-ome-wide” nature of the techniques we have discussed provide valuable new ways to examine this field. These methods may be applied beyond model systems to clinical specimens so as to provide biomedical relevance that could assist in development of treatments for conditions such as invasive fungal infections.

Ultimately, clinical applications of -omics technologies at a patient level may require innovation beyond any research setting. For example, the Prosinga® prognostic assay, utilizing the nanoString nCounter® system has been FDA approved for use in a prognostic breast cancer perspective (US Food and Drug Administration, 2013). Instrument platforms such as the nCounter® system that allow simultaneous measurement across the “-omics” space are coming into production for research purposes and may drive forward ultimate clinical applicability. The continual development of “-omics” technology in a clinical setting will provide new avenues for clinical diagnostic and treatment options for invasive fungal infections.

Acknowledgments

We would like to thank the reviewers of this manuscript for their comments and input that have improved this review. We also thank Drs. Margo M. Moore and Tillie L. Hackett for their continued support. Funding for this project was provided in part by NSERC Discovery Grant RGPIN-2015-05043.

Author Disclosure Statement

The authors declare that no competing financial interests exist.

References

Altwasser R, Baldin C, Weber J, et al. (2015). Network modeling reveals cross talk of MAP kinases during adaptation to caspofungin stress in *Aspergillus fumigatus*. PLoS One 10, e0136932.
Dalle F, Wächtler B, L’Ollivier C, et al. (2010). Cellular interactions of Candida albicans with human oral epithelial cells and enterocytes. Cell Microbiol 12, 248–271.

Dénervaud N, Becker J, Delgado-Gonzalo R, et al. (2013). A chemostat array enables the spatio-temporal analysis of the yeast proteome. Proc Natl Acad Sci USA 110, 15842–15847.

Durmuş S, Çakır T, Ozgür A, and Guthke R. (2015). A review on computational systems biology of pathogen-host interactions. Front Microbiol 6, 235.

Ellwanger DC, Leonhardt JF, and Mewes H-W. (2015). Large-scale modeling of condition-specific gene regulatory networks by information integration and inference. Nucleic Acids Res 42, 1–14.

Enoch DA, Ludlam HA, and Brown NM. (2006). Invasive fungal infections: A review of epidemiology and management options. J Med Microbiol 55, 809–818.

Esliami H, Khorramizadeh MR, Pourmand M, Moazeni M, and Rezaie S. (2014). Down-regulation of sidB gene by use of RNA interference in Aspergillus nidulans. Iran Biomed J 18, 55–59.

Ewing AD, Houlanan KE, Hu Y, et al. (2015). Combining tumor genome simulation with crowdsourcing to benchmark somatic single-nucleotide-variant detection. Nat Methods 12, 623–630.

Fang Z, and Cui X. (2011). Design and validation issues in RNA-seq experiments. Brief Bioinform 12, 280–287.

Feminio AM, Fay FS, Fogarty K, and Singer RH. (1998). Visualization of single RNA transcripts in situ. Science 280, 585–590.

Fields S. (1993). The two-hybrid system to detect protein–protein interactions. Methods 5, 116–124.

Fields S, and Song O. (1989). A novel genetic system to detect protein–protein interactions. Nature 340, 245–246.

Forseth RR, Fox EM, Chung D, Howlett BJ, Keller NP, and Schroeder FC. (2011). Identification of cryptic products of the gliotoxin gene cluster using NMR-based comparative metabonomics and a model for gliotoxin biosynthesis. J Am Chem Soc 133, 9678–9681.

Fromntling R, Shadomy HJ, and Jacobson ES. (1982). Decreased interferon responses in mice lacking mutants of Candida albicans and their virulence for mice. J. Bacteriol 150, 1414–1421.

Greenfield A, Hafemeister C, and Bonneau R. (2013). Robust data-driven incorporation of prior knowledge into the inference of dynamic regulatory networks. Bioinformatics 29, 1060–1067.

Griffiths L. (1998). Assay by nuclear magnetic resonance spectroscopy: Quantification limits. Analyst 123, 1061–1068.

Guerra CR, Seabra SH, de Souza W, and Rozental S. (2014). Cryptococcus neoformans is internalized by receptor-mediated or “triggered” phagocytosis, dependent on actin recruitment. PLoS One 9, e89250.

Güntner OP, Shin H, Ng RT, McMaster WR, McManus BM, Keown PA, Tebbutt SJ, and Lê Cao K-A. (2014). Novel multivariate methods for integration of genomics and proteomics data: Applications in a kidney transplant rejection study. OMICS 18, 682–695.

Han TL, Cannon RD, and Villas-Boás SG. (2012). The metabolic response of Candida albicans to farnesol under hyphae-inducing conditions. FEMS Yeast Res 12, 879–889.

Han T-L, Tumanov S, Cannon RD, and Villas-Boás SG. (2013). Metabolic response of Candida albicans to phenylethyl alcohol under hyphae-inducing conditions. PLoS One 8, e71364.

Hannon GJ. (2002). RNA interference. Nature 418, 244–251.

Hashimshony T, Feder M, Levin M, Hall BK, and Yanai I. (2015). Spatiotemporal transcriptomics reveals the evolutionary history of the endoderm germ layer. Nature 519, 219–222.

Hess ST, Girirajan TPK, and Mason MD. (2006). Ultra-high resolution imaging by fluorescence photoactivation localization microscopy. Biophys J 91, 4258–4272.

Horn F, Heinekamp T, Kniemeyer O, Pollma¨cher J, Valiante V, and Brakhage AA. (2012). Systems biology of fungal infection. Front Microbiol 3, 1–20.

Hu G, Hacham M, Waterman SR, et al. (2008). PI3K signaling of autophagy is required for starvation tolerance and virulence of Cryptococcus neoformans. J Clin Invest 118, 1186–1197.

Hyuhn-Thu VA, Irlthum A, Wehenkel L, and Geurts P. (2010). Inferring regulatory networks from expression data using tree-based methods. PLoS One 5, 1–10.

Iancu OD, Kawane S, Bottomly D, Searles R, Hitzemann R, and McWeeny S. (2012). Utilizing RNA-Seq data for de novo coexpression network inference. Bioinformatics 28, 1592–1597.

Kalleda N, Naorem A, and Manchikatla RV. (2013). Targeting fungal genes by diced siRNAs: A rapid tool to decipher gene function in Aspergillus nidulans. PLoS One 8, e75443.

Kang HJ, Kawasawa YI, Cheng F, et al. (2011). Spatio-temporal transcriptome of the human brain. Nature 478, 483–489.

Kleefogaarnis D, Wong L, Archer J, and Kalnis P. (2015). Hi-Jack: A novel computational framework for pathway-based inference of host-pathogen interactions. Bioinformatics 31, 2332–2339.

Klingspor L, Saedi B, Ljungman P, and Szakos A. (2015). Epidemiology and outcomes of patients with invasive mould infections: A retrospective observational study from a single centre (2005–2009). Mycoses 58, 470–477.

Kraus PR, Boily M-J, Giles SS, et al. (2004). Identification of Cryptococcus neoformans temperature-regulated genes with a genomic-DNA microarray. Eukaryot Cell 3, 1249–1260.

Kristensen AR, Gsponer J, and Foster LJ. (2012). A high-throughput approach for measuring temporal changes in the interactome. Nat Methods 9, 907–909.

Küffner R, Petri T, Tavakkolkah P, Windhager L, and Zimmer R. (2012). Inferring gene regulatory networks by ANOVA. Bioinformatics 28, 1376–1382.

Kwon-Chung KJ, Polacheck I, and Popkin TJ. (1982). Melanin-lacking mutants of Cryptococcus neoformans and their virulence for mice. J. Bacteriol 150, 1414–1421.
Langfelder P, and Horvath S. (2008). WGCNA: An R package for weighted correlation network analysis. BMC Bioinformatics 9, 559.

Larsson E, Sander C, and Marks D. (2010). mRNA turnover rate limits siRNA and microRNA efficacy. Mol Syst Biol 6, 433.

Lee JH, Daugharthy ER, Scheiman J, et al. (2015). Fluorescent in situ sequencing (FISSEQ) of RNA for gene expression profiling in intact cells and tissues. Nat Protoc 10, 442–458.

Lee JH, Daugharthy ER, Scheiman J, et al. (2014). Highly multiplexed subcellular RNA sequencing in situ. Science 343, 1360–1363.

Lehrnbecher T, Frank C, Engels K, Kriener S, Groll H, and Schwabe D. (2010). Trends in the postmortem epidemiology of invasive fungal infections at a university hospital. J Infect 61, 259–265.

Liu S-Y, Lin J-Q, Wu H-L, et al. (2012). Bisulfite sequencing reveals that Aspergillus flavus holds a hollow in DNA methylation. PLoS One 7, e30349.

Liu Z-P. (2015). Reverse engineering of genome-wide gene regulatory networks from gene expression data. Curr Genomics 16, 3–22.

Mortazavi A, Williams B, McCue K, Schaeffer L, and Wold B. (2008). Mapping and quantifying mammalian transcriptomes using RNA-Seq. Nat Methods 5, 621–628.

Neofytos D, Treadway S, Ostrander D, et al. (2013). Epidemiology, outcomes, and mortality predictors of invasive mold infections among transplant recipients: A 10-year, single-center experience. Transpl Infect Dis 15, 233–242.

De Oliveira RB, Atobe JH, Souza SA, and de Castro Lima Santos DW. (2014). Epidemiology of invasive fungal infections in patients with acquired immunodeficiency syndrome at a reference hospital for infectious diseases in Brazil. Mycopathologia 178, 71–78.

Olsen C, Fleming K, Prendergast N, et al. (2014). Inference and validation of predictive gene networks from biomedical literature and gene expression data. Genomics 103, 329–336.

Ong S-E, Blagoev B, Kratchmarova I, Kristensen DB, Steen H, 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.

Oosthuizen JL, Gomez P, Ruan J, Hackett TL, Moore MM, Knight DA, and Tebbutt SJ. (2011). Dual organism transcriptomics of airway epithelial cells interacting with conidia of Aspergillus fumigatus. PLoS One 6, e20527.

Paddison PJ, Caudy AA, and Hannon GJ. (2002). Stable suppression of gene expression by RNAi in mammalian cells. Proc Natl Acad Sci USA 99, 1443–1448.

Petrailia F, Wang P, Yang J, and Tu Z. (2015). Integrative random forest for gene regulatory network inference. Bioinformatics 31, 197–205.

Pól J, Strohalm M, Havlíček V, and Volný M. (2010). Molecular spectrum imaging in biomedical and life science research. Histochem Cell Biol 134, 423–443.

Poultney CS, Greenfield A, and Bonneau R. (2012). Integrated inference and analysis of regulatory networks from multi-level measurements. Methods Cell Biol 110, 19–56.

Prokopek SD, Watson JD, Waggott DM, et al. (2013). Systematic evaluation of medium-throughput mRNA abundance platforms. RNA 19, 51–62.

Pukkila-Worley R, Gerrald QD, Kraus PR, et al. (2005). Transcriptional network of multiple capsule and melanin genes governed by the Cryptococcus neoformans cyclic AMP cascade. Eukaryot Cell 4, 190–201.

Qin Q-M, Luo J, Lin X, Pei J, Li L, Fichti TA, and de Figueiredo P. (2011). Functional analysis of host factors that mediate the intracellular lifestyle of Cryptococcus neoformans. PLoS Pathog 7, e1002078.

Reales-Calderón JA, Sylvester M, Strijbis K, Jensen ON, Nombela C, Molero G, and Gil C. (2013). Candida albicans induces pro-inflammatory and anti-apoptotic signals in macrophages as revealed by quantitative proteomics and phosphoproteomics. J Proteomics 91, 106–135.

Rechavi O, Kalman M, Fang Y, et al. (2010). Trans-SILAC: Sorting out the non-cell-autonomous proteome. Nat Methods 7, 923–927.

Rennoll-Bankert KE, Garcia-Garcia JC, Sinclair SH, and Dumler JS. (2015). Chromatin bound bacterial effector AnkA recruits HDAC1 and modifies host gene expression. Cell Microbiol. 17, 1640–1652.

Robertson G, Hirst M, Bainbridge M, et al. (2007). Genome-wide profiles of STAT1 DNA association using chromatin immunoprecipitation and massively parallel sequencing. Nat Methods 4, 651–657.

Ross PL, Huang YN, Marchese JN, et al. (2004). Multiplexed protein quantitation in Saccharomyces cerevisiae using amine-reactive isobaric tagging reagents. Mol Cell Proteomics 3, 1154–1169.

Rust MJ, Bates M, and Zhuang X. (2006). Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM). Nat Methods 3, 793–795.

Sabió E, Selevsek N, and Aebersold R. (2012). Mass spectrometry-based proteomics for systems biology. Curr Opin Biotechnol 23, 591–597.

Saiardi A, Guillermier C, Loss O, Poczatek JC, and Lechene C. (2014). Quantitative imaging of inositol distribution in yeast using multi-isotope imaging mass spectrometry (MIMS). Surf Interface Anal 46, 169–172.

Sanchez JF, and Wang CCC. (2012). The chemical identification and analysis of Aspergillus nidulans secondary metabolites. Methods Mol Biol 944, 97–109.

Sandhu DK, Sandhu RS, Khan ZU, and Damodaran VN. (1976). Conditional virulence of a p-aminobenzoic acid-requiring mutant of Aspergillus fumigatus. Infect Immun 13, 527–532.

Santamaría R, Rizzetto L, Bromley M, et al. (2011). Systems biology of infectious diseases: A focus on fungal infections. Immunobiology 216, 1212–1227.

Schena M, Shalon D, Davis RW, and Brown PO. (1995). Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science 270, 467–470.

Schmidt EK, Clavarino G, Ceppi M, and Pierre P. (2009). Sorting out the non-cell-autonomous proteome. Nat Methods 7, 923–927.
consideration of the metabolic network structure. Integr Biol 4, 888–889.

Schwanhauser B, Gossen M, Dittmar G, et al. (2009). Global analysis of cellular protein translation by pulsed SILAC. Proteomics 9, 205–209.

Sharma G, Upadhyay S, Srilalitha M, Nandicoori VK, and Kholas A (2015). The interaction of mycobacterial protein Rxv2966c with host chromatin is mediated through non-CpG methylation and histone H3/H4 binding. Nucleic Acids Res 43, 3922–3937.

Sheppard DC (2011). Molecular mechanism of Aspergillus fumigatus adherence to host constituents. Curr Opin Microbiol 14, 375–379.

Smidsgaard J, and Nielsen J. (2005). Metabolite profiling of fungi and yeast: From phenotype to metabolome by MS and informatics. J Exp Bot 56, 273–286.

De Souza WR, Morais ER, Krohn NG, et al. (2013). Identification of metabolic pathways influenced by the G-protein coupled in Aspergillus nidulans. PLoS One 8, e62088.

Spirov A, Fahmy K, Schneider M, Frei E, Noll M, and Baumgartner S. (2009). Formation of the bicoid morphogen gradient: An mRNA gradient dictates the protein gradient. Development 136, 605–614.

Steinhauser ML, Bailey AP, Senyo SE, Guillermier C, Perlstein TS, Gould AP, Lee RT, and Lechene CP. (2012). Multi-isotope imaging mass spectrometry quantifies stem cell division and metabolism. Nature 481, 516–519.

Steinhauser ML, and Lechene CP. (2013). Quantitative imaging of subcellular metabolism with stable isotopes and multi-isotope imaging mass spectrometry. Semin Dev Cell Biol 24, 661–667.

Stroschein-Stevenson SL, Foley E, O’Farrell PH, and Johnson AD. (2009). Phagocytosis of Candida albicans by RNAtreated Drosophila S2 cells. Methods Mol Biol 470, 347–358.

Tenenhaus A, Philippe C, Guillemot V, Le Cao K-A, Grill J, and Frouin V. (2014). Variable selection for generalized canonical correlation analysis. Biostatistics 15, 569–583.

Tierney L, Linde J, Müller S, et al. (2012). An interspecies regulatory network inferred from simultaneous RNA-seq of Candida albicans invading innate immune cells. Front Microbiol 3, 85.

Toh-e a, Ohkus M, Li H-M, et al. (2015). Identification of genes involved in the phosphate metabolism in Cryptococcus neoformans. Fungal Genet Biol 80, 19–30.

Tom Dieck S, Kochen L, Hanus C, et al. (2015). Direct visualization of newly synthesized target proteins in situ. Nat Methods 12, 1–7.

US Food and Drug Administration. (2013). 510(k) Summary: K130010. 1–8.

Veenstra TD. (2012). Metabolomics: The final frontier. Genome Med 5, 2010–2012.

Vyas VK, Barrasa MI, and Fink GR. (2015). A Candida albicans CRISPR system permits genetic engineering of essential genes and gene families. Sci Adv 1, e1500248–e1500248.

Wächter B, Wilson D, Haedicke K, Dalle F, and Hube B. (2011). From attachment to damage: Defined genes of Candida albicans mediate adhesion, invasion and damage during interaction with oral epithelial cells. PLoS One 6, e17046.

Wang Z, Curry E, and Montana G. (2014). Network-guided regression for detecting associations between DNA methylation and gene expression. Bioinformatics 30, 2693–2701.

Wang H, Yang H, Shivaila CS, Dawlaty MM, Cheng AW, Zhang F, and Jaenisch R. (2013). One-step generation of mice carrying mutations in multiple genes by CRISPR/cas-mediated genome engineering. Cell 153, 910–918.

Warnock DW. (2007). Trends in the epidemiology of invasive fungal infections. Jap J Med Mycol 48, 1–12.

Wasylnka JA, and Moore MM. (2003). Aspergillus fumigatus conidia survive and germinate in acidic organelles of A549 epithelial cells. J Cell Sci 116, 1579–1587.

Webb A. (2012). Increasing the sensitivity of magnetic resonance spectroscopy and imaging. Anal Chem 84, 9–16.

Wiese S, Reidegeld K a, Meyer HE, and Warscheid B. (2007). Protein labeling by iTRAQ: A new tool for quantitative mass spectrometry in proteome research. Proteomics 7, 340–350.

Wistart DS, Tzur D, Knox C, et al. (2007). HMDB: The Human Metabolome Database. Nucleic Acids Res 35, D521–526.

Zetsche B, Gootenberg JS, Abudayeh OO, et al. (2015). Cpf1 is a single RNA-guided endonuclease of a Class 2 CRISPR-Cas system. Cell 163, 759–771.

Zhang D-S, Piazza V, Perrin BJ, et al. (2012). Multi-isotope imaging mass spectrometry reveals slow protein turnover in hair-cell stereocilia. Nature 481, 520–524.

Zhang J, Zhang L, Qiu J, and Nian H. (2015). Isobaric tags for relative and absolute quantitation (iTRAQ)-based proteomic analysis of Cryptococcus humicola response to aluminum stress. J Biosci Bioeng 120, 359–363.

Zimmermann AC, Zarei M, Eiselein S, and Dengjel J. (2010). Quantitative proteomics for the analysis of spatio-temporal protein dynamics during autophagy. Autophagy 6, 1009–1016.

Address correspondence to:
Associate Professor Scott J. Tebbutt
Centre for Heart Lung Innovation
University of British Columbia
St. Paul’s Hospital
1081 Burrard Street
Vancouver
British Columbia V6Z 1Y6
Canada
E-mail: scott.tebbutt@hci.ubc.ca

Abbreviations Used

CHIP = chromatin immunoprecipitation
CRISPR = clustered regularly interspaced short palindromic repeats
FISH = fluorescent in situ hybridization
FISSEQ = fluorescent in situ sequencing
IFI = invasive fungal infection
iTRAQ = isobaric tags for relative and absolute quantitation
MERFISH = multiplexed error-robust FISH
MIMS = multi-isotope imaging mass spectrometry
PCP-SILAC = protein correlation profiling-SILAC
PUNCH-P = puromycin-assisted nascent chain proteomics
RNAi = RNA interference
SILAC = stable isotope labeling by amino acids in cell culture
SIMS = secondary ion mass spectrometry
SUnSET = surface sensing of translation