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DECIPHERING THE CROSS-TALK BETWEEN SPHINGOLIPID MECHANISM AND SIGNALING PATHWAYS IN BAKER'S YEAST

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ABSTRACT

As most of the known diseases exhibit dysfunctional aspects in the signal transduction networks, there has been a great deal of enthusiasm to identify novel drug targets based on the knowledge of key signal transduction components and their links to diseases. In the present study, a computational framework was recruited for the reconstruction of protein-protein interaction networks of specific signaling mechanisms in Baker's yeast. The objective was to analyze the interconnection between these signaling networks in order to identify the possible crosstalks between sphingolipid signaling and other signaling mechanisms. These networks are composed of the candidate proteins belonging to sphingolipid signaling, target of rapamycin (TOR) signaling, high osmolarity glycerol (HOG) signaling, pheromone response and calcium (Ca) mediated signaling in Saccharomyces cerevisiae. A detailed map including physical and functional connections that link the relevant signal transduction components to each other and to adjacent networks is developed. The proposed framework can effectively be used as a tool to give insight into the important and complicated network of signaling in higher eukaryotes.
Keywords: Signal transduction networks, sphingolipid, TOR, calcium, HOG.

MAYADA SİFİNGOLİPİD İLE DİĞER SİNYAL İLETİMİ MEKANİZMALARI ARASINDAKİ ETKİLEŞİMLERİN BELİRLENMESİ

ÖZET

Bilinen birçok hastalığın, sinyal ileti ağyapılarında işlev bozukluğundan kaynaklandığı bulgusuna dayanarak, anahtar sinyal ileti unsurları ve bunların hastalıklarla bağlantıları üstüne bilinenlerin baz alınması yoluyla yeni ilaç hedefleri saptanması son yıllarda çok ilgi çekmektedir. Bu çalışmada da, mayaya özgü sinyal ileti mekanizmalarının protein-protein etkileşim ağyapıları oluşturulması hesapsal bir çerçevede incelenmiştir. Amaç, mayadaki değişik sinyal ileti ağyapılarının birbirleriyle bağıntılarını inceleyerek, özellikle sifingolipid sinyal ileti mekanizması ile diğer sinyal iletimi mekanizmaları arasındaki olası etkileşimleri belirlemektir. Bu ağyapıları oluşturan aday proteinler mayadaki sifingolipid, Rapamisin hedefi (TOR), yüksek ozmolarite gliserol (HOG), feromon tepki ve kalsiyum ilintili sinyal ileti ağyapılarında görev almaktadırlar. İlgili sinyal ileti unsurlarını birbirlerine ve komşuağyapılarına birleştiren fiziksel ve işlevsel bağlantıları detaylı gösteren bir yolizi haritası hazırlanmıştır. Burada önerilen hesapsal yöntem, yüksek ökaryotlardaki önemli ve karmaşık sinyal ileti ağyapılarını incelemede de etkili olarak kullanılabilir.
Anahtar Sözcükler: Sinyal ileti ağyapıları, sifingolipid, TOR, kalsiyum, HOG.

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1. INTRODUCTION

Signaling is the study of how cells communicate, and it impinges on all aspects of biology, from development to disease. Like all living organisms, yeast cells must continually sense their surrounding environment and make decisions on the basis of that information. As almost all known diseases exhibit dysfunctional aspects in these signaling networks, there has been a great deal of enthusiasm to identify novel drug targets based on the knowledge of key signal transduction components and their links to diseases. Understanding cellular signaling is therefore essential for gaining insight into the molecular mechanisms underlying the diseases as well as for the adaptation of living cells to changes in the environment.

Various signaling pathways interact closely with one another and the final biological response due to a given perturbation is therefore shaped by the interactions between or among many different pathways. A growing body of experimental evidences (reviewed in [1]) confirms ubiquitous interconnections and interdependencies between the different components of these pathways. Signaling pathways are often branched in an intertwined fashion and are therefore integrated into complex signaling networks with many levels of interconnectivity.

Sphingolipids are highly bioactive compounds that serve not only as components of the biological structures such as membranes and lipoproteins, but also as regulators of cell proliferation, differentiation, cell–cell and cell–matrix interactions, cell migration, intracellular and extracellular signaling, membrane tracking, autophagy, and cell death [2,3]. Sphingolipids are abundant components of the *Saccharomyces cerevisiae* plasma membrane, with smaller amounts found in other cellular membranes. Despite their abundance, functional roles in cellular processes have only begun to be elucidated in the past decade. The interest in sphingolipids has been sparked by the indications that they regulate signal transduction pathways in mammals [4].

The functions of sphingolipids include facilitating transport of glycosyl phosphatidylinositol-anchored proteins from the endoplasmic reticulum to the Golgi apparatus, being the lipid moiety in many glycosylphosphatidylinositol-anchored proteins, playing some role in regulating calcium homeostasis or being components in calcium-mediated signaling pathways, and regulating the cell cycle [5-7]. Recent data suggest that sphingolipids should communicate with many other signaling pathway components in yeast, including those of pheromone, calcium, glucose, HOG and TOR signaling pathways [5-7]. Therefore, there is a great need for system-level analysis of interaction between sphingolipid metabolism and other cellular processes.

In the last decade, parallel to the completion of genome sequence projects, high-throughput functional genomics tools have been developed and protein-protein interaction databases [8-12] have expanded significantly. The Gene Ontology (GO) project was established to provide a common language to describe aspects of a protein represented by three categories, i.e. molecular function, biological process and cellular component, each one structured as a directed acyclic graph [13]. Now the challenge is to develop computational tools that allow system-level evaluation of the "omic" data with the objective of identifying the signal transduction pathways operating in the cell. Since the final biological response due to a given perturbation is shaped by the interactions between or among the relevant pathways, a reconstruction process related to signal transduction in living organisms would result in networks that are quite complex with many levels of interconnectivity indicating the interaction of several different signal transduction pathways.

Previously [14], we hypothesized that a protein has a high probability of having characteristics similar to the proteins classified in a functional category if its GO annotations are similar to those already present in that particular category. The Selective Permissibility Algorithm (SPA) was developed to integrate GO annotations with protein-protein interaction data to reconstruct a protein interaction network that has the potential for predicting signal transduction pathways in yeast. The success of this SPA methodology was shown by the reconstruction of glucose sensing and signaling pathways in *Saccharomyces cerevisiae* [14].

In the present study, in order to identify the possible interconnections of sphingolipid metabolism and signaling with other signaling pathways, the Selective Permissibility Algorithm was recruited for the reconstruction of protein-protein interaction networks of (i) sphingolipid signaling, (ii) Target of Rapamycin (TOR) signaling, (iii) High Osmolarity Glycerol (HOG) signaling, (iv) pheromone response and (v) calcium (Ca) mediated signaling in Saccharomyces cerevisiae. The objective was to analyze the interconnections between these signaling networks in order to identify the possible crosstalks between sphingolipid signaling and other signaling mechanisms. A detailed map including physical and functional connections that link the sphingolipid signaling network to other relevant signal transduction components is developed. Essentially, this reconstructed scheme is expected to serve as a starting point for future experimental work.

2. MATERIALS AND METHODS

A systems biology approach for the reconstruction of signal transduction networks requires the integration of several types of biological data and the use of the numerous analyses techniques.

2.1. Protein-Protein Interaction Data

Three public databases, BioGRID, DIP and STRING [8,11,12] use the results of high-throughput experiments and also collect experimentally determined protein-protein interactions from the literature. In the present study, the databases of interacting protein pairs for yeast were assembled from these three public databases as available on February 9, 2006. The assembly consists of 65,673 protein-protein interactions between 5,253 proteins.

2.2. Gene Ontology Annotations

Gene Ontology (GO) is a controlled vocabulary used to describe the biology of a gene product in any organism [13]. There are three independent sets of vocabularies, or ontologies, which describe the molecular function of a gene product, the biological process in which the gene product participates, and the cellular component where the gene product can be found. The ontology is represented as a directed acyclic graph (DAG), in which terms may have multiple parents and multiple relationships to their parents. In addition, each term inherits all the relationships of its parents. GO is a dynamic, evolving project of the Gene Ontology (GO) Consortium (www.geneontology.org).

2.3. Yeast Databases

Saccharomyces Genome Database, SGD, [10] is a daily-updated scientific database of the molecular biology and genetics of the yeast *Saccharomyces cerevisiae*. It includes all types of biological information, (such as systematic names, product descriptions, GO annotations, mutant phenotype, physical and genetic interactions, sequence information, literature summary) on 6609 yeast ORFs and is achievable by internet (www.yeastgenome.org).

The MIPS Comprehensive Yeast Genome Database [9], CYGD, aims to present information on the molecular structure and functional network of the budding yeast, *Saccharomyces cerevisiae*. It includes a Functional Catalog (MIPS-FunCat), in which manually-annotated proteins are functionally classified into 17 specific categories.

2.4. Selective Permissibility Algorithm (SPA)

Selective Permissibility Algorithm, SPA, [14] integrates protein-protein interaction data with ene Ontology (GO) annotations to reconstruct signaling networks composed of candidate proteins for signal transduction mechanisms in *Saccharomyces cerevisiae*. The algorithm has two distinctive elements, the input protein(s) and the selection criterion (Figure 1). The input to the algorithm is a set of proteins known to have a certain function in the signaling pathway to be reconstructed. In the first step, all interactions of the proteins were extracted from interactome data sources. In the next step, the relevance of these interacting proteins was tested through a selection criterion which was designed by employing GO Annotations. Hereby interacting proteins were either accepted or rejected (the procedure is explained below), and in the next step of the algorithm the accepted proteins were used as input proteins and the cycle was repeated. This cycle was continued until the whole interaction data source was scanned, i.e. no new interacting partners were identified.

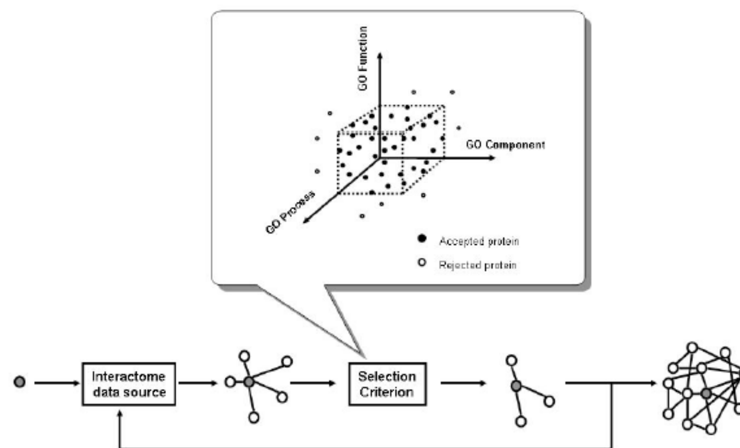

Figure 1. The geometrical representation of the selection step of SPA [14]

The selection criterion of SPA is based on an Annotation Collection of proteins, which was collected from GO annotations used to describe proteins belonging to the "cellular communication and signal transduction mechanism" category in the MIPS Comprehensive Yeast Genome Database [9] in terms of component, function and process.

A candidate protein was included into the network if all of the 3 GO annotations (component/function/process) of the protein are present in the Annotation Collection. It has a high probability of having characteristics similar to the proteins classified in "cellular communication and signal transduction mechanism" functional category in MIPS, and therefore, having a possible role in the signaling pathway to be reconstructed. In Figure 1, the Annotation Collection is represented as a 3-D rectangular subspace, where the coordinates are the GO annotations in terms of component, function and process, respectively. Any protein is described as a point in the 3-D space, p(x,y,z). If the point p is inside the rectangular subspace (S), the corresponding protein is selected.

2.5. Gene Ontology (GO) Mapping Analysis

Gene Ontology mapping tool of Saccharomyces Genome Database [10], namely GO Term Finder, was used to analyze the GO annotations that are significantly common among products

encoded by a set of genes. GO Term Finder uses a hypergeometric distribution with multiple hypothesis correction (i.e., Bonferroni Correction) to calculate p-values:

$$p = \sum_{j=x}^{n} \frac{n!}{j!(n-j)!} \phi^{j} (1-\phi)^{n-j} \qquad (1)$$

where is Ø calculated as the number of genes annotated to a specific term divided by the total number of genes annotated to all terms, *n* is the number of genes in the investigated list, and *x* is the number of genes in the list annotated to the specific term.

2.6. Cross-Talk And Functional Linkage Analysis

To investigate possible cross-talks across the signaling pathways, we used the networks constructed specifically for each signaling pathway. The network proteins were analyzed for statistical enrichment of proteins from other signaling pathways by counting the proteins shared by two networks. A hyper-geometric test was used to estimate the significance of the counts:

$$P - value = 1 - \sum_{i=0}^{m-1} \frac{\binom{M}{i}\binom{N-M}{X-i}}{\binom{N}{X}} \qquad (2)$$

where X is the size (i.e. number of proteins) of the query network, M is the size of the cross-talk partner network, N is the total proteins in the global network (i.e. genome), and m is the number of shared proteins or GO terms in two networks. This calculation is repeated for all pair-wise combinations of networks, and p-value of 10^{-4} is considered as the threshold for significance [14].

2.7. Data Visualization Tool

Osprey [15] is a free software platform for visualization of complex interaction networks. Osprey builds data-rich graphical representations from Gene Ontology annotated interaction data maintained by BioGrid.

3. RESULTS AND DISCUSSION

The analysis of the protein-protein interactions is very important for unraveling signal transduction pathways. With the accumulation of genome sequence information of several organisms, large-scale genomic and proteomic experimental techniques have generated enormous amounts of interactome data, provided valuable resources, and offered insights into the components of the signaling pathways and the molecular and cellular responses to cell signaling [16].

The experimental evidences on functionality of sphingolipid molecules in regulating several molecular mechanisms in *Saccharomyces cerevisiae* indicated a need for the analysis of the interconnectivity of sphingolipid signaling with other signaling mechanisms. Therefore, in the present study, specific protein-protein interaction networks of Target of Rapamycin (TOR) signaling, High Osmolarity Glycerol (HOG) signaling, pheromone response signaling and calcium-mediated signaling are reconstructed together with the sphingolipid signaling in *Saccharomyces cerevisiae*.

3.1. Improvement of the Protein Interaction Data

In principle, protein-protein interaction data can be directly employed to reconstruct signaling networks. However, the full potential of these data cannot be utilized for discovering signal ransduction networks for several reasons: (i) the incompleteness of the interactome data, (ii) low reliability of the protein-protein interaction data due to high noise levels. Despite these potential problems, protein-protein interaction networks have been proved to be extremely useful for protein function prediction and characterization [17-20].

A potential difficulty with the available protein-protein interaction data obtained mostly by the high-throughput screens is the prevalence of false negatives –i.e. the interactions that are not detected but do occur in the cell [21-22]. 16-20% overlap of the interactions was reported by using different high-throughput datasets [23]. These results indicated that the individual datasets cover only a small percentage of the potential interactions. The low value calculated for the overlap in the datasets indicates the necessity for assembling the data. Therefore, in the present work, the interactome data were assembled from three publicly available large databases.

Another potential difficulty with the available protein-protein interaction data is the prevalence of false positives –i.e. the interactions that are seen in an experiment but never occur in the cell [21-23]. Several attempts have been made to assess and improve the quality of the data by integrating the different biological features, such as gene expression profiles and Gene Ontology annotations [24-27]. Lu and coworkers (2005) showed that any delicate dependencies between these features can confound the strength of the prediction in integrated frameworks, although there may not be any appreciable statistical dependence between the possible pairs of these features. The interactome assembly constructed in the present study was analyzed using the protein co-localization data [28] in yeast. Two interacting proteins are hypothesized to be in the same cellular location, at least at the time of interaction. Therefore, proteins that do not exist in the same cellular compartment are assumed to be non-interacting and labeled as false-positive. As a result of this analysis, a false-positive interaction data set consisting of a total of 2994 interactions was identified and removed from the protein-protein interaction assembly.

3.2. Reconstruction of Yeast Signaling Networks

Literature data indicate that sphingolipid pathway communicates with many signaling pathways in yeast, including pheromone, calcium, glucose, HOG and TOR signaling pathways. It is stated that the inhibition of pheromone signaling due to the depletion of ergosterol is somehow linked to the sphingolipid metabolites [29]. It is also argued that sphingolipids take role in osmostress responses in yeast. Non-specific osmostress is exerted by moderate concentrations of various solutes such as NaCl, KCl or sorbitol and induces the high-osmolarity glycerol (HOG) pathway which rapidly raises the intracellular glycerol concentration up to molar levels [30]. It is reported that the homologue of the neutral sphingomyelinase in the mammalian sphingolipid metabolism, Isc1p, serves as a stress signalling mediator and ISC1 is required for the development of yeast halotolerance against Na+ and Li+ ions, again indicating a possible crosstalk between sphingolipid metabolism and HOG signaling pathway in yeast [30]. Furthermore, it is demonstrated that yeast cells deficient in TORC2 activity are impaired for de novo ceramide biosynthesis both in vivo and in vitro [31], and TORC2 regulates this step in part by activating the AGC kinase Ypk2 [32], this step is antagonized by the Ca^{2+}/calmodulindependent phosphatase calcineurin. Hence, sphingolipid metabolism interacts additionally with TOR and calcium signaling pathways. Another finding supporting that is the linkage of calcium signaling pathway to the sphingolipid metabolism in mammalian cells [33]. The possible crosstalks between these mentioned signaling pathways and sphingolipid metabolism are also summarized in recent reviews [2,3]. These experimental evidences on functionality of sphingolipid molecules in regulating several molecular mechanisms in *Saccharomyces cerevisiae* have indicated possible

interconnections of sphingolipid signaling with Target of Rapamycin (TOR) signaling, High Osmolarity Glycerol (HOG) signaling, pheromone response signaling and calcium (Ca) signaling in *Saccharomyces cerevisiae*.

Table 1. Signaling-annotated proteins used to built the Annotation Collection tables of signaling pathways

Sphingolipid	TOR	HOG	Pheromone	Calcium
Pkh1	Tor1	Sln1	Ste4	Cch1
Pkh2	Tor2	Ypd1	Ste2	Cdc31
Pil1	Kog2	Ssk1	Cdc24	Cmd1
Lsp1	Lst8	Ssk2	Far1	Cmk1
Pkc1	Avo1	Ssk22	Fus3	Cmk2
Ypk1	Avo2	Cdc42	Bem1	Cmp2
Ypk2	Sap190	Ste11	Cdc42	Cna1
Sch9	Bit61	Ste20	Ste5	Cnb1
	Slm1	Pbs2	Gpa1	Crz1
	Slm2	Hog1	Ste7	Csg2
	Sit4	Ptc1	Dig1	Frq1
	Tsc11	Ptp2	Dig2	Frt1
	Tco89	Ptp3	Ste11	Frt2
		Rck1	Ste18	Kex2
			Ste20	Lcb4
			Cdc28	Lcb5
			Ste12	Mid1
			Ste50	Plc1
			Kar4	Pmc1
			Prm7	Pmr1
			Prr2	Rcn1
			Plp1	Rsf1
			Far7	Tcb1

In the present work, for each signaling mechanism, the literature as well as the MIPS Functional Catalogue was scanned for signaling annotated proteins to reconstruct the Annotation Collection table. These proteins (Table 1) also form the initial protein set used as the starting point of the SPA algorithm. The GO terms of these proteins were extracted from the gene ontology database and the Annotation Collection tables were built.

Table 2. Size and average connectivity of reconstructed signaling networks

	Sphingolipid	TOR	HOG	Pheromone	Calcium	Overall
# of proteins (N)	181	398	332	102	319	1016
# of interactions (L)	918	1972	1685	333	1576	5298
Average connectivity $\left(\frac{2L}{N}\right)$	10.14	9.91	10.15	6.54	9.88	10.43

The execution of the SPA algorithm resulted in protein-protein interaction networks with different sizes for each signaling mechanism (Table 2). The sizes of the reconstructed networks differ from 102 to 398. Almost all of the reconstructed networks have average connectivity around 10, which indicates that a protein has 10 neighbors on the average.

A detailed map including physical and functional connections that link the sphingolipid signaling network to other relevant signal transduction components is then developed using Osprey (Figure 2). The overall network consists of 1016 proteins. Several proteins were shared between signaling networks. Two proteins, Cdc28 which is the catalytic subunit of the main cell cycle cyclin-dependent kinase, and Fus3, a mitogen-activated serine/threonine protein kinase involved in mating, were present in all five networks. Similarly, a total number of 60 proteins (6% of the network) were shared by at least three networks (shown in the middle of the figure 2 by yellow, red and blue colors). On the other hand, 779 proteins (77% of the network) were not shared; they are concluded to be mechanism-originated.

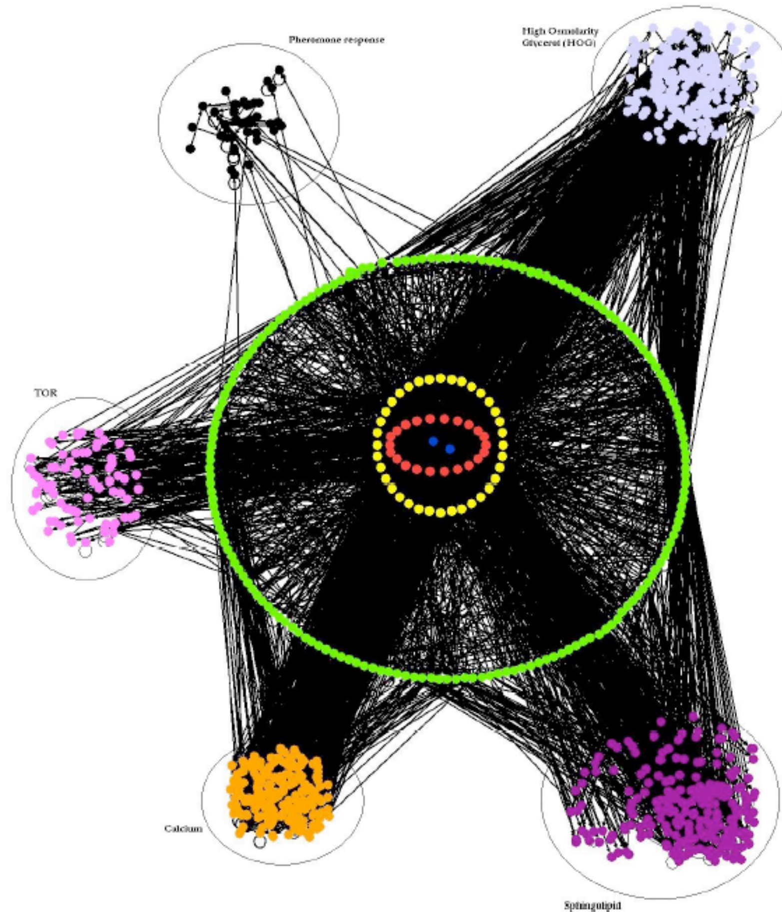

Figure 2. The reconstructed map including physical and functional connections that link the sphingolipid signaling to other relevant signal transduction pathways (Green: proteins shared by two pathways, yellow: protein shared by three pathways, red: proteins shared by four pathways, blue: proteins shared by all pathways)

3.3. Interconnectivity (Cross-talk) Between Yeast Signaling Networks

The presence of the common proteins between the reconstructed networks of signaling pathways was expected and that indicated the presence of a strong cross-talk. In order to elaborate the possible cross-talks among the signaling pathways, the networks reconstructed specifically for each signaling pathway were then quantitatively analyzed for statistical enrichment of proteins from other signaling pathways by counting the proteins shared by two networks. We have applied this approach to map possible cross-talk relations among the set of signaling pathways. The number of proteins shared between the network pairs and the corresponding p-values indicating their statistical significance were given in Table 3.

The comparative analysis of the reconstructed networks predicted several expected and ovel cross-talk relationships between the considered pathways that are supported by the experimental evidence in literature. It can seen from Table 3 that sphingolipid (SL) signaling is strongly interconnected with target of Rapamycin (TOR), high osmolarity glycerol (HOG) and calcium signaling pathways. The lowest p-value (1.51×10^{-18}) is obtained for the interactions between SL and TOR pathways indicating a strong crosstalk. Additionally, calcium signaling and high osmolarity glycerol (HOG) signaling pathways also show a good correlation with a low p-value (5.30×10^{-8}) compared to other interactions. Furthermore, high osmolarity signaling and mating pheromone response networks result in a noticeable crosstalk (p-value of 7.43×10^{-5}).

Table 3. Cross-talk analysis across signaling pathways. The rows of the table correspond to the query network and the columns correspond to the potential cross-talk partner network. The upper-triangular part indicates the number of proteins shared between the corresponding network pairs, whereas the lower triangular part gives the p-values indicating the statistical significance of these counts. Significant results are indicated as bold and italic.

	Size	Sphingolipid	TOR	HOG	Pheromone	Calcium
Sphingolipid	181	-	82	52	4	40
TOR	398	***1.51×10^{-18}***	-	70	9	68
HOG	332	***2.29×10^{-7}***	0.131	-	12	60
Pheromone	102	0.219	1.16×10^{-4}	***7.43×10^{-5}***	-	5
Calcium	319	***2.11×10^{-5}***	0.099	***5.30×10^{-8}***	0.013	-

Sphingolipids in yeast have been shown to mediate significant and diverse biological responses. The erectors of ceramide production include Fas ligands, ultraviolet light, heat shock, DNA damage, chemotherapeutic drugs and many other agents. The activated signal transduction pathways control a variety of cellular processes including the cell cycle, apoptosis and senescence, immune responses, and cell-cell interactions. Ceramide is one of many intermediates in sphingolipid metabolism and it seemed likely that other sphingolipid metabolites might also function as signaling molecules. Subsequently, it has been shown that sphingosine and sphingosine-1-phosphate, sphingosylphosphorylcholine, and possibly di- and tri-Nmethylsphingosine are signaling molecules that regulate numerous cellular processes in mammalian cells.

The target of Rapamycin (TOR) signaling pathway is an important mechanism by which cell growth is regulated by nutrient availability in eukaryotes. Similarly, sphingolipids are essential for yeast cell growth and survival based on the observation that the inactivation of the biosynthesis of sphingolipids leads to lethality of yeast cells [31]. Yeast cells with the deletion of IPC synthase fail to synthesize complex sphingolipids and die [3]. These studies suggest that complex sphingolipids are essential for yeast survival and growth. The significant interconnectivity between TOR signaling and sphingolipid signaling (p-value of 1.51×10^{-18}) should be due to their regulatory function on cell growth and needs further experimentation.

Recent data suggest that sphingolipids acting as signaling molecules or secondary messengers are playing a role during the response to heat stress [5]. The protective role of sphingolipids in cell growth and survival under heat stress conditions was first speculated based on the observation that a yeast mutant SLC-1 lacking the ability to synthesize sphingolipids was unable to grow at an elevated temperature (>37 °C). Although ceramides, sphingoid bases, and their phosphates are increased in response to heat stress and are metabolically interchangeable in yeast cells, it is still unknown whether one or all of these lipids has a protective effect against heat stress and heat shock [6]. It may be possible to address this issue by constructing yeast mutants that generate one but not the other lipids. On the other hand, the exact pathways for the generation of sphingoid bases, ceramide, and sphingoid base phosphates upon heat stress have not been defined yet. It was initially thought that ceramides, sphingoid bases and their phosphates were generated only *de novo* in response to heat stress. However, the identification of the enzymes responsible for the breakdown of complex sphingolipids and ceramides raises the possibility that ceramides, sphingoid bases and their phosphates can also be generated from the breakdown of sphingolipids. However, the increased ceramide concentration in response to the heat stress appears to arise from *de novo* biosynthesis because australifungin, the specific inhibitor for ceramide synthase, is able to block most of the increase in ceramide induced by heat stress [5,6]. These evidences lead the researchers to hypothesise on the possible roles of sphingolipids during osmotic stress and low pH stress [29,30]. In the present work, the strong interconnectivity between the reconstructed networks of SL and HOG (p-value of 2.29×10^{-7}) also supports this hypothesis, but needs validation with further experimentation.

The crosstalk between sphingolipid signaling and calcium-mediated signaling (p-value $2.11\text{x}10^{-5}$) can be explained by the functional roles of sphingolipids in the regulation of calcium homeostasis. Moreover, sphingolipid metabolites have been recognized as capable of also mobilizing intracellular calcium. In the mammalian cells, the action of two sphingolipid metabolites, S1P and sphingosylphosphocholine (SPC), is of particular interest since they induce calcium ion release not only via inositol trisfosfate (IP3), generated by the activation of specific extracellular receptors, but also by a direct action on intracellular Ca ion stores [32,33].

Another strong crosstalk (p-value of 7.43×10^{-5}) is observed between the networks reconstructed for two MAPK pathways, namely high osmolarity signaling and mating pheromone response (Table 3), since a significant number of proteins were shared between these networks. The high osmolarity signaling pathway operates through Hog1, which plays a key role in global gene expression. In *Saccharomyces cerevisiae*, two trans-membrane proteins (Sln1 and Sho1) act as receptors, and five transcription factors (Hot1, Mcm1, Msn2, Msn4 and Sko1) are controlled by Hog1 [34]. These transcription factors regulate numerous genes that are involved mainly in carbohydrate metabolism, general stress protection, protein production and signal transduction. Mating pheromone response pathway is involved in mating of haploid yeast cells [35]. The mating response to generate diploids is initiated by the binding of .-and A-pheromones to G-protein-coupled receptors Ste2 and Ste3, respectively [35] and results in the activation of two transcription factors, Fus1 and Far1, which have regulatory roles in blocking cell cycle progression. A three-component cascade was reported to be conserved in these two signaling pathways from yeast to humans [34,35]. The cross-talk between MAPK pathways is attributed to the basic assembly of these cascades (MAPKKK→ MAPKK→ MAPK) in the organization of these pathways. The upstream elements of these cascades, Ste11 and Ste7, are common in all MAPK pathways. Moreover, the possible cross-talk between the high osmolarity signaling (HOG) pathway and the mating pheromone response pathway can be explained by the role of high osmolarity signaling in repression of the mating pheromone response pathway. It has been reported that the HOG pathway kinases are possibly controlling the activity of Fus3 protein kinase of the mating pheromone response pathway. Inactivating mutations in the HOG pathway kinases cause an increase in phosphotyrosine content of Fus3 protein and expression of pheromone-response genes, and increased sensitivity to growth arrest by pheromone [35].

4. CONCLUSIONS

A detailed map including physical and functional connections that link the sphingolipid metabolism and signaling to other relevant signal transduction networks is developed using Osprey software. These reconstructions form a first draft of these signaling networks operating in the yeast cell and can be used as a starting point for designing further experimental and computational studies to (i) identify the unknown elements of the signaling pathways, (ii) understand the functional linkages within the signaling network of yeast, and (iii) decipher the general organizational principles such as pathway crosstalk. The proposed framework can effectively be used as a tool to give insight into the important and complicated network of signaling in higher eukaryotes.

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