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Inositol Metabolite Depletion Induces Hallmarks of ER Stress Without Activating the UPR

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ABSTRACT

The unfolded protein response (UPR) is a transcriptional pathway that responds to unfolded proteins in the endoplasmic reticulum (ER) through three well-defined pathways. However, the production of reactive oxygen species (ROS), membrane aberrancy, and the depletion of the sugar, lipid component, and signaling molecule inositol are also linked to UPR activation. While the mechanism for ROS-mediated UPR activation is understood, the mechanism for membrane aberrancy and inositol depletion—believed to be the same—is much less clear. Given inositol depletion's reduction of several inositol metabolites with divergent roles in the UPR, our study of the transcriptomic changes due to the metabolic depletion of certain inositols attempts to illuminate the poorly understood connections between inositol depletion, membrane aberrancy, and the UPR. We amalgamated and filtered RNA sequencing data from 19 studies knocking out inositol metabolic enzymes and conducted functional analyses. Our results indicated that while protein degradation, membrane stress, and redox stress—all hallmarks of ER stress and heavily associated with the UPR—were transcriptionally supported by inositol metabolite depletion, the UPR was not activated. As a result, inositol depletion studies could carry a confounding noise due to the depletion of inositol products that obfuscates attempts to define inositol's connections with membrane aberrancy and the UPR. The results of our study urge further research into the consequences of inositol metabolite depletion both to vitiate the resulting concerns about inositol depletion studies and to explain the lack of UPR activation even with the induction of ER stress hallmarks associated with UPR activation.

Introduction

The unfolded protein response (UPR) is a complex proteomic transcriptional pathway involving hundreds of genes that responds to stresses in the endoplasmic reticulum (ER) (Radanović and Ernst, 2021). Specifically, the UPR is activated by accumulations of unfolded proteins in the ER and ER membrane aberrations (Radanović & Ernst, 2021). UPR activation with unfolded proteins occurs through three canonical pathways shown in Figure 1: (1) unfolded proteins directly bind to and oligomerize inositol-requiring enzyme 1 (IRE1), leading to XBP1 mRNA splicing and activating the UPR, (2) unfolded proteins bind to protein kinase RNA-like endoplasmic reticulum kinase (PERK) and oligomerize PERK, phosphorylating translation initiation factor 2 (eIF2), promoting activating transcription factor 4 (ATF4) and activating the UPR, and (3) unfolded proteins interact with the lumenal domain of activating transcription factor 6 (ATF6), activating the UPR (Karagöz et al., 2019). IRE1 oligomerization is the most evolutionarily conserved mechanism for UPR activation, making it essential for a functional understanding of the UPR's role in the body (Karagöz et al., 2019). UPR dysregulation has been connected to cancers, Type II diabetes mellitus, and several heart diseases (Snapp, 2012).

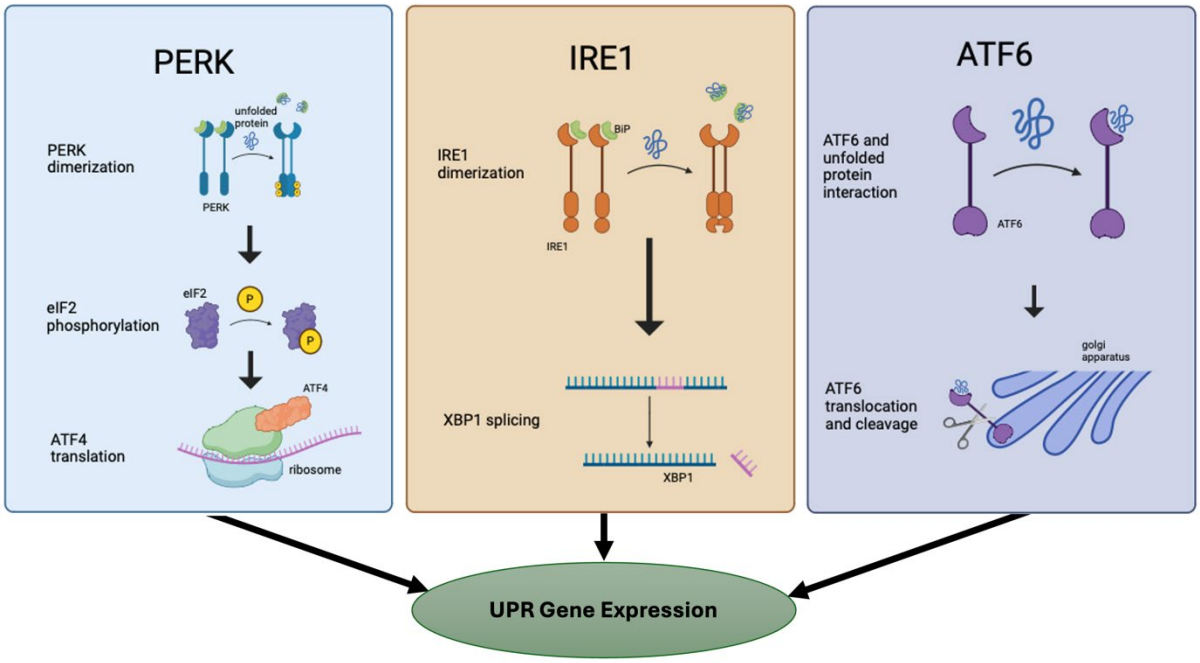

Figure 1. Canonical UPR Pathways. (1) BiP binds to an unfolded protein, allowing PERK dimerization, which triggers eIF2 phosphorylation, which upregulates ATF4 translation. (2) BiP binds to an unfolded protein, allowing IRE1 dimerization, which triggers XBP1 splicing. (3) ATF6 binds to an unfolded protein and translocates to the Golgi apparatus for cleavage. Made with BioRender.com.

Reactive oxygen species (ROS) are highly reactive molecules derived from oxygen and hydroxyl radicals and reactive nonradicals (Bhattarai et al., 2021). Cao and Kaufman find that oxidants and ROS stress activate the UPR by causing redox stress, disrupting disulfide bond formation, and membrane aberrations (2014). They further that redox stress also produces H_2O_2 and other ROS and that ER stress and protein misfolding can impair mitochondrial function, creating more mitochondrial ROS. The end result is a positive feedback loop between ROS accumulation and the UPR. Thus, in diseases where redox stress is dysregulated, this crosstalk can exacerbate symptoms.

Inositol and Membrane Aberrancy

Inositol is a carbocyclic sugar with five naturally occurring stereoisomers and three hydroxyl groups observed to be phosphorylated naturally (Balla, 2013). The most common stereoisomer is myo-inositol, with scyllo-inositol and d-chiro-inositol also naturally present in trace amounts (Balla, 2013). The metabolism of phosphatidylinositol (PtdIns), an inositol phosphatidylglyceride, is regulated by a set of kinases and phosphatases whose genetic expression is directly correlated with the presence of different PtdIns (Balla, 2013). The inositol pyrophosphate family also plays a dual role in regulating energy (Balla, 2013). Inositol regulation has been implicated as a player in several medical conditions, including cancers, atherosclerosis, neurodegenerative diseases, and sickle cell disease (Yang & Shamsuddin, 2015; Jadhav et al., 2016).

Inositols have been heavily associated with membrane aberrancy, especially in the ER (Promlek et al., 2011). For decades, inositol depletion has been used as a substitute for membrane aberrancy for UPR activation. Proteins' lipid-binding domains bind weakly to phosphoinositides, so multiple lipid-binding sites and additional lipids and proteins are necessary to stabilize these contacts (Posor et al., 2022). As a result, the cell can modulate

several factors to adjust phosphoinositide ligand binding (Posor et al., 2022). This powerful versatility is why phosphoinositides are heavily involved in regulating protein interactions with the membrane (Falkenburger et al., 2010). Several cytoplasmic proteins contain domains that interact with specific phosphoinositides, allowing a membrane to draw a different landscape of peripheral proteins depending on its phosphoinositide cocktail (Falkenburger et al., 2010). Phosphoinositides have also been given a primary role in regulating lipid transfer between specialized organelle membrane domains, and they regulate integral membrane proteins like K_{ATP} channels, TRP channels and Kir channels (Kim et al., 2013; Falkenburger et al., 2010). This power over membrane dynamics gives inositol a critical role in regulating membrane fluidity.

Inositol's Role in the UPR

While inositol depletion activates the UPR, it does not do so through any of the canonical mechanisms (Promlek et al., 2011) (Halbleib et al., 2017) (Snapp, 2012). Because of inositol's involvement in regulating membrane dynamics, inositol depletion and membrane aberrancy appear to activate the UPR in the same manner, which is why inositol depletion is generally used as a substitute for membrane aberrancy (Promlek et al., 2011). Membrane aberrancy is not activated in the same way as unfolded protein accumulation. A causal relationship between membrane aberrancy and the UPR has been shown, but the exact connection is unknown exactly.

There are two plausible options for the interaction between inositol depletion and IRE1's amphipathic helix: (1) inositol depletion's introduction of an oligomerizing cofactor which causes IRE1 activation or (2) inositol depletion's direct modification of IRE1 presence to inhibit ER protein chaperone BiP's binding to IRE1 (dependent on BiP cycling off and on IRE1, rather than binding until the UPR is detected) (Snapp, 2012).

Ample evidence exists to support the oligomerizing cofactor theory. By replacing subregions of IRE1 with basic-region leucine zipper (bZIP) and measuring the reaction to inositol stress, Promlek et al. concluded that inositol depletion/membrane aberrancy activates the UPR through the transmembrane domain of IRE1 (2011). Halbleib et al. corroborated the results from Promlek et al. by testing the inositol auxotrophy of yeast mutants with changes to the amphipathicity of the amphipathic helix of IRE1, which overlaps the transmembrane domain of IRE1 (2017). The study found that it was the amphipathicity of IRE1's amphipathic helix that was essential to UPR activation through inositol depletion rather than the conservation of the transmembrane domain (Halbleib 2017). Volmer et al. expanded on this study by investigating how acyl chain saturation within membranes encourages membrane stiffening, allowing greater removal of lipid groups from transmembrane proteins and promoting protein-protein interactions (2013). Applied to membrane aberrancy in the ER, acyl chain saturation and membrane stiffening would promote PERK and IRE1 dimerization, activating the UPR (Volmer et al., 2013). However, the mechanism for acyl chain saturation from inositol depletion is unclear, and both Volmer et al. and Halbleib et al. acknowledge that the resolution of their assays leaves significant uncertainties.

More abstract evidence is present for the BiP binding theory. Lajoie et al. tagged the yeast homolog of BiP, Kar2, with GFP and observed that Kar2-sfGFP mobility decreased with unfolded protein accumulation independent of UPR activation but increased with inositol depletion, with which the UPR was always activated (2012). Although Lajoie et al. speculate that the increase in Kar2-sfGFP mobility could be linked to a simple increase in Kar2-sfGFP in general, these observations could also point to a decreased rate of Kar2 binding to IRE1 with inositol depletion and subsequent UPR activation, supporting the BiP binding theory. However, neither the BiP binding theory nor the oligomerizing cofactor theory has conclusive evidence supporting it.

Specific Inositols and the Unfolded Protein Response

Although we understand how inositol depletion affects the UPR, studying how different inositols specifically affect the UPR could help clarify this pathway. Given the highly specific function of phosphoinositides in cell

membranes, it makes sense that differently phosphorylated phosphoinositides would affect different cellular functions. In fact, a system of phosphatases and kinases maintains a specialized cocktail of phosphoinositides at the membranes of different organelles (Falkenburger et al., 2010).

The upregulation of different phosphoinositides, including Ins(3, 4, 5)P_3 and *myo*-inositol 3-phosphate, has a critical signaling role in bipolar disorder (Deranieh et al., 2009). Cells derived from patients with bipolar disorder have shown a hampered UPR and a modified ubiquitin-proteasome system suggesting that phosphoinositides specifically are involved in regulating the UPR in patients with bipolar disorder (Deranieh et al., 2009). Furthermore, valproate, a treatment for bipolar disorder that depletes inositol, specifically depletes *myo*-inositol 3-phosphate through activity of the protein MCK1, lowering phosphorylation of *myo*-inositol in general (Yu et al., 2017). Several inositol phosphates may thus be linked to bipolar disorder and the UPR.

Additionally, inositol stereoisomers have been shown to have different impacts on UPR regulation, as *scyllo*-inositol and *D-chiro*-inositol, two isomers of the more common *myo*-inositol, were unable to compensate for *myo*-inositol depletion, causing decreased cell viability, increased levels of phosphatidylinositol, and the upregulation of stress genes including ATF4, a key step in the PERK pathway to activate the UPR (Suliman et al., 2022). As a result, differing types of inositols do not play the same roles in intracellular communication and the unfolded protein response. Elucidating these differences is essential to understanding the UPR.

While the literature gives inositol monophosphates and *myo*-inositol a primary role in regulating the UPR, there is evidence to speculate that other phosphoinositides also play a role in this mechanism. Galactosemia-triggered UPR activation (i.e., another form of membrane stress), is not fully explainable by the downregulation of inositol monophosphatases, implying that inositol polyphosphates, pyrophosphates, or less-found isomers also have a role in membrane aberrancy-triggered UPR (De-Souza et al., 2014).

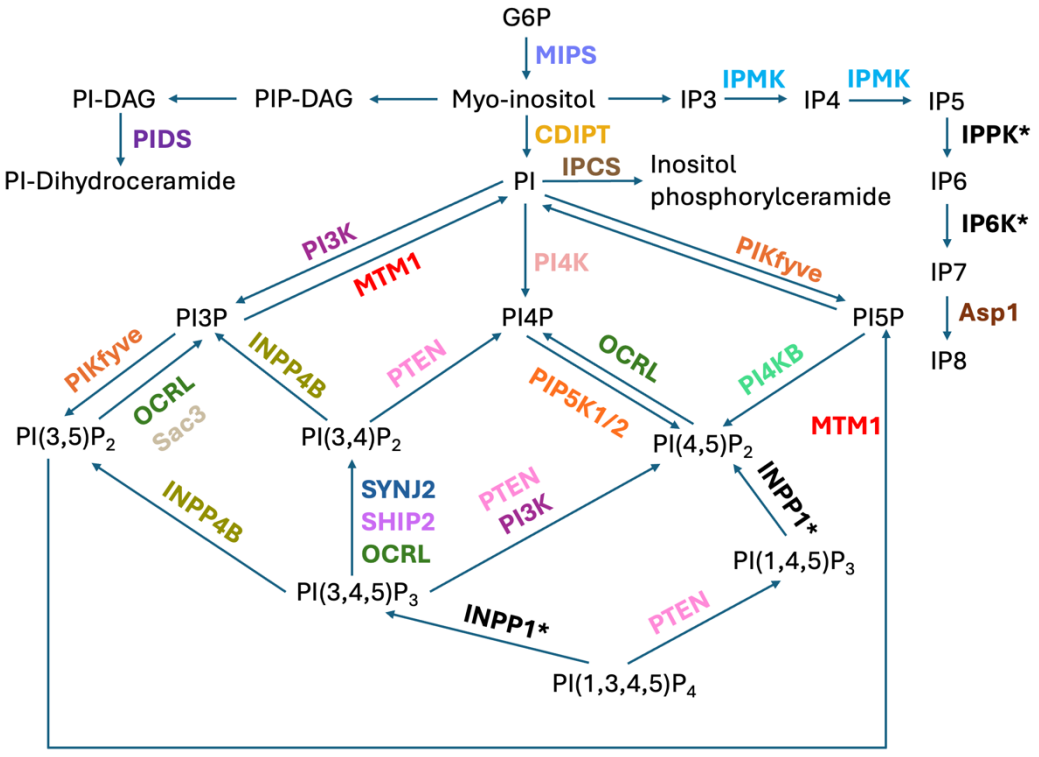

Figure 2. Select Inositol Metabolic Pathways Targeted in this Study. Metabolic enzymes targeted in this study are color-coded, while those excluded from this study and presented solely for clarity are in black and marked

with an asterisk. Includes several phosphoinositide and inositol-containing sphingolipid pathways. References for this figure are detailed in Table 1.

Importantly, almost all of these inositols with divergent roles in ER stress and the UPR would be affected by *myo*-inositol depletion. As shown in Figure 2, several phosphoinositides, inositol phosphates and pyrophosphates, and inositol-containing sphingolipids depend on the presence of *myo*-inositol for their anabolism. *Myo*-inositol depletion would thus deplete many inositol metabolites, with a range of uncertain consequences for membrane aberrancy and the UPR. Only one study—Deranieh & Greenberg— has attempted to systematically dissect the effects of inositol metabolite depletion, and even their study focused on reviewing the various macroscale health consequences of these inositol metabolites (2009). Our study will attempt to identify the transcriptomic effects of inositol metabolites' depletion and the implications for membrane aberrancy and the UPR. Such effects could explain or elucidate the connection between inositol depletion and the UPR in a way that studies focusing on *myo*-inositol could not, with ramifications for a number of prevalent, destructive diseases and our understanding of a key homeostatic regulatory pathway in the cell.

Methods

To study the effects of inositol depletion's disruption of inositol metabolism on the transcriptome, we sourced data from RNA sequencing studies that dysregulated specific segments of inositol metabolism, as in Table 1.

Table 1. Included Inositol Metabolic Enzyme Knockout Studies and Corresponding Metabolites.

Depleted Inositol Product	Metabolic Pathway	Mechanism	Data Source Study	Reaction Reference
IP8	IP7 -> IP8	Asp1 IPP kinase mutation	(Sanchez et al., 2019)	(Benjamin et al., 2022)
PI4P	PI -> PI4P	PI4K knockout via MMV390048	(Watt et al., 2018)	(Li et al., 2024)
Inositol phosphorylceramide	PtdIns + ceramide -> inositol phosphorylceramide + DAG	Inositol phosphorylceramide synthase knockout	(Figueiredo et al., 2005)	(Figueiredo et al., 2005)
PI(3,4)P2	PI(3,4,5)P3 -> PI(3,4)P2	SYNJ2 knockout	(Harbauer et al., 2022)	(Ramos et al., 2019)
Phosphoinositol dihydroceramide	PI-DAG + DHC -> PI-DHC + DAG	Phosphoinositol dihydroceramide synthase knockout	(Heaver et al., 2022)	(Heaver et al., 2022)
Myo-inositol	G6P -> myo-inositol-P	Myo-inositol phosphate synthase knockout	(Heaver et al., 2022)	(Deranieh et al., 2013)
PI(3,4)P2 and Inositol-1,3,4-triphosphate (IP3)	PI(3,4,5)P3 -> PI(3,4)P2 AND Ins(1,3,4,5)P4-> Ins(1,3,4)P3	SHIP1 knockout	(Iguchi et al., 2023)	(Ramos et al., 2019)
PI3P	PI(3,5)P2 -> PI3P	Sac3/Fig4 knockout	(Zylstra et al., 2023)	(Hasegawa et al., 2017)
PI3P and PI(3,5)P2	PI(3,4)P2 -> PI3P AND PI(3,4,5)P3 -> PI(3,5)P2	INPP4B knockout	(Zhang et al., 2018)	(Maekawa et al., 2014)

PI5P, some PI	PI(3,5)P2 -> Pi5P, also some PI3P -> PI	MTM1 knockout	(Sarikaya et al., 2022)	(Mansat et al., 2024)
PI(4,5)P2, some PI4P and PI(1,4,5)P3	PI(3,4,5)P3 -> PI(4,5)P2, also some PI(3,4)P2 -> PI4P and some PI(1,3,4,5)P4 -> PI(1,4,5)P3	PTEN knockout	(Cheung et al., 2023)	(Malek et al., 2017)
PI4P	PI(4,5)P2 -> PI4P, some 5-phosphatase activity on PI(3,4,5)P3, PI(3,5)P2, Ins(1,3,4,5)P4, and Ins(1,4,5)P3	OCRL knockout	(Naik et al., 2021)	(Ramos et al., 2019)
PI(3,5)P2 and PI5P	PI3P -> PI(3,5)P2 and PI -> PI5P	PIKfyve inhibition via APY0201	(Campos et al., 2019)	(Burke et al., 2022)
PI(4,5)P2	PI4P -> PI(4,5)P2	PIP5K1 and PIP5K2 knockout	(Qin et al., 2020)	(Qin et al., 2020)
PI(3,4,5)P3, PI3P	PI(4,5)P2 -> PI(3,4,5)P3, PI -> PI3P	PIK3CA (PI3K) knockout	(Ladewig et al., 2022)	(Malek et al., 2017)
PI4P, PI(4,5)P2	PI -> PI4P, PI5P -> PI(4,5)P2	PI4KB1/PI4KB2 knockout	(Starodubtseva et al., 2022)	(Balla, 2013)
PI	CDP-DAG + myo-inositol -> PI + CMP	CDIPT knockout	(Iyer et al., 2018)	(Lykidis et al., 1997)
Principally Ins(1,3,4,5,6)5P	Principally Ins(1,4,5)3P -> Ins(1,3,4,5)4P -> Ins(1,3,4,5,6)5P	IPMK knockout	(Yuk et al., 2024)	(Maag et al., 2011)
PI(3,4)P2	PI(3,4,5)P3 -> PI(3,4)P2	SHIP2 knockout	(Wei et al., 2023)	(Fradet & Fitzgerald, 2017)

Gene Data Processing

We filtered the data for significance with the DeSeq2 Package in R for a p-value less than 0.05 and an absolute $\log_2$ Fold Change greater than 2. To address nonstandard gene identifiers, Uniprot's batch retrieval service, g:Profiler's g:Convert, and the National Institutes of Health's DAVID Gene ID Conversion tool were used iteratively to retrieve the gene symbol, protein name, UniprotKB ID, and protein family associated with each gene identifier.

Functional Classification

To discern the main characteristics of the transcriptome following inositol metabolism disruption and determine whether the UPR is activated in response to other inositols' depletion, the dataset was further restricted to gene targets and protein families significantly transcriptionally regulated in multiple studies. After 371 genes previously identified by Travers et al., Reich et al., and the Harmonizome 3.0 as UPR targets underwent the same gene identifier conversion process as the RNA sequencing data, they were compared to both the restricted and original datasets (2000; 2020; Rouillard et al., 2016). Uniprot was used to determine the general localization and function of certain protein families, and g:Profiler's g:GOst feature was used to identify enriched Gene Ontology terms for the genes in the restricted dataset.

Results

Filtering left 1897 genes significantly up- or down-regulated by the knockout of an inositol metabolic enzyme. Only 66 genes, 118 proteins, and 146 protein families were significantly up- or down-regulated by multiple inositol metabolic enzymes. Most proteins were involved in ROS regulation, protein synthesis, and membrane structure.

Table 2. Common Protein Families.

Protein Family	Occurrences
Cytochrome P450 family	29
Oxidoreductases	27
Cytokines	16
Peroxidases	13
Lipolytic enzymes	11
Glycosyl hydrolases	10

As depicted in Table 2, the most frequent protein families in our dataset were involved in redox stress (cytochrome P450, oxidoreductases, and peroxidases) and membrane stress (lipolytic enzymes) (Veith & Moorthy, 2018; Bissaro et al., 2018; Schaffer & Bronnikova, 2012).

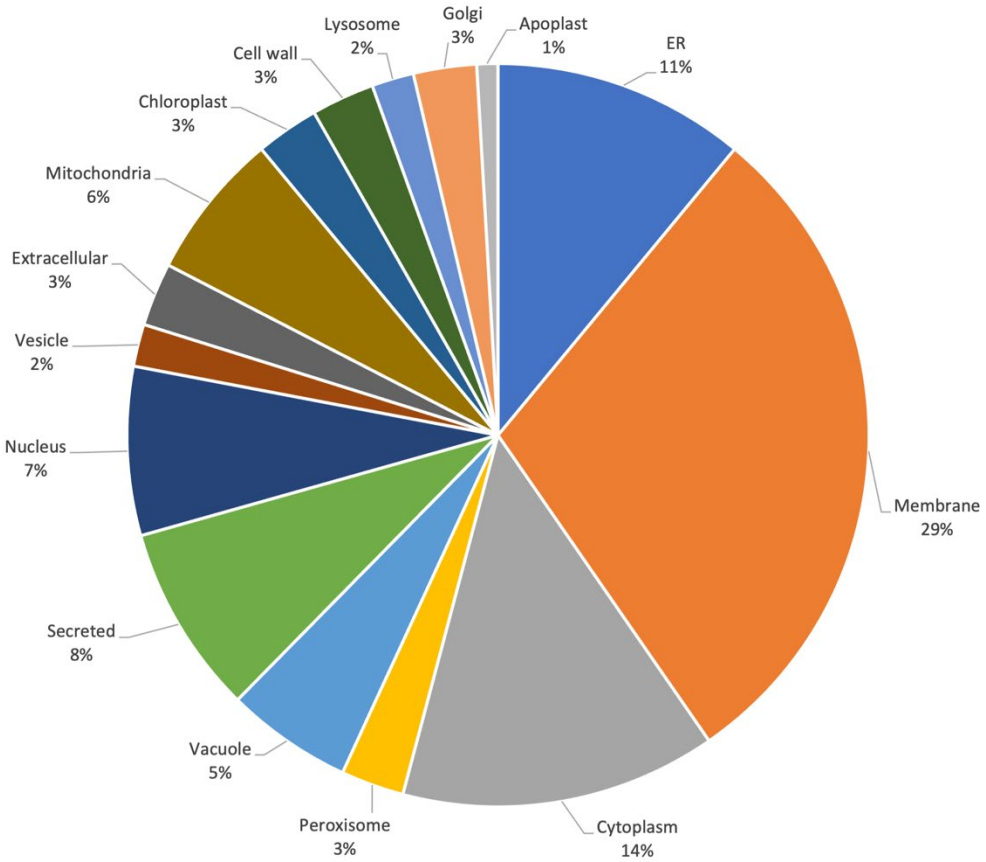

Figure 3. Localization of Repeated Protein Families.

75 families occurred in our dataset at least three times. Out of these 75 families, as shown in Figure 2, 29% were localized to the membrane, 3% to the cell wall, and 2% to vesicles, highlighting the importance of inositols to membrane function. In addition, 11% of the families were localized to the ER and 3% to the Golgi apparatus, indicating that protein synthesis and quality control were also key targets. Although the most frequent protein families were involved in redox stress management, only 6% of the 75 families were localized to the mitochondria, 3% to the chloroplast, and 3% to the peroxisome.

Table 3. Molecular Function Terms.

Molecular Function Term	Adjusted p-value
LRR domain binding	0.0000000325
nutrient reservoir activity	0.000000537
glutathione transferase activity	0.0000257
carbohydrate binding	0.0000517
iron ion binding	0.000202
structural constituent of cell wall	0.000238
quinone binding	0.000459
scopolin beta-glucosidase activity	0.000677
FAD binding	0.000826
glutathione binding	0.00107
cation binding	0.00128
glutamate receptor activity	0.00138
endopeptidase inhibitor activity	0.00315
xyloglucan:xyloglucosyl transferase activity	0.00333
ligand-gated monatomic ion channel activity	0.00485
transmembrane transporter activity	0.0135
quercitrin binding	0.0182
camalexin binding	0.0182
ion channel inhibitor activity	0.0182
monatomic cation channel activity	0.0182
sulfate transmembrane transporter activity	0.0204
inositol 3-alpha-galactosyltransferase activity	0.0254
peptide-methionine (R)-S-oxide reductase activity	0.0345
siRNA binding	0.0345

We also conducted a Gene Ontology term enrichment analysis of the repeated genes using g:Profiler's g:GOst. The molecular function terms shown in Table 3 were driver terms significantly represented in our dataset. The majority (54.17%) of the terms are involved in oxidative stress.

Given the primacy of protein degradation, redox stress, and membrane structure both in the connection between inositol and the UPR and in our dataset, we classified the 146 repeated protein families into four categories: 41 involved in oxidative stress, 22 involved in transportation, 19 involved in protein degradation/synthesis, and 21 involved in membrane structure.

Discussion

Inositol is an essential sugar highly involved in signal transduction (Balla, 2013). Inositol depletion has been associated with UPR activation, the dysregulation of which has been associated with several diseases (Snapp, 2012). With findings suggesting novel or intertwined mechanisms of UPR activation and uncertainty surrounding inositol's connection to the UPR, analyzing the transcriptomic consequences of inositol metabolite depletion could help elucidate the mechanisms of UPR activation and thus its role in several diseases. Focusing on RNA control of inositol metabolic enzymes, we establish a transcriptional profile of inositol metabolite depletion. We find that while inositol metabolite depletion assisted in proteolytic function, disrupted the membrane, and targeted ROS, it did not upregulate the canonical UPR targets.

Inositol metabolism disruption did affect protein degradation. 19 out of our 84 classified repeated protein families were involved in protein homeostasis, including several peptidases. The g:Profiler functional analysis of our filtered genes found that endopeptidase function was significantly increased ($p=0.00316$). However, the effect on protein homeostasis was nuanced: 10 of the upregulated protein families (including the HSP70 protein family, which chaperones and directly degrades proteins) assist in protein degradation while 5 families are involved in protein synthesis (Mayer & Bukau, 2005). The upregulation of both protease inhibitors and ribosomal proteins points to a multifaceted modulation of protein synthesis.

We also found that inositol metabolism dysregulation disrupted membrane function, as expected. 21 out of our 84 classified repeated protein families were involved in lipid stability. Three of these protein families were the Oleosin family, OBAP family, and Caleosin family, collectively occurring 13 times in our original dataset. All three of these families are involved in the dynamics of oil bodies (triacylglyceride storage compartments), and the first and the last are components of the oil body membrane. While the first two, which both prevent oil body fusion, were on average downregulated, the latter, which contributes to oil body degradation, was on average upregulated (Shimada & Hara-Nishimura, 2010; Lopez-Ribera et al., 2014; Poxleitner et al., 2006). Just as oil body membrane integrity was disrupted, the downregulation of the Tetraspanin family, which is essential for the organization of the fluid mosaic, implicates inositol metabolism disruption in the disruption of several other cell membranes in animals, plants, and fungi (Termini & Gillette, 2017). In addition to those families clearly linked to membrane stability, a number of families merely involved in membrane dynamics were transcriptionally controlled in a way that could disrupt membrane function. For example, the upregulation of the Plant Lipid Transfer Protein (Plant LTP) family has implications for phospholipid transfer between membranes, and the upregulation of the Flotillin family has been associated with the failure of certain lipid rafts to correctly bind transmembrane receptors (Kader, 1996; Mielich-Süss et al., 2013). However, the upregulation of the Remorin and PMP3 families, which organize lipids and stabilize membrane geometry, again provides nuance to our conclusion that membrane structure was disrupted, although these families occurred the least of our lipid stability families in our original dataset and might merely be responding to membrane stress (Su et al., 2023; Block et al., 2015).

Redox stress was also targeted by the transcriptome following inositol metabolic dysfunction. Our Gene Ontology term enrichment analysis found that many redox binding reactions involved in ROS production, including iron binding and quinone binding, were significantly enriched in our restricted gene dataset (Ying et al., 2021; Paranagama et al., 2010). Of our protein family classifications, redox stress was the largest with 41 families out of the 84 we could classify. The protein families with more than 10 occurrences in the original dataset included Cytochrome P450, oxidoreductases, and peroxidases, all heavily involved in redox stress Among those families was Cytochrome P450, the most frequent family in our original dataset with 29 occurrences. As heme monooxygenases are mostly present in the ER, Cytochrome P450s can generate large amounts of ROS as a byproduct of regular function (Veith & Moorthy, 2018). Thus, their upregulation on average due to the disruption of inositol metabolism could create the same redox stress in the ER that has been associated with the UPR. However, while 22 of the redox stress families promoted ROS proliferation, 19 scavenged ROS

to reduce redox stress. One of those upregulated scavengers was MsrB, a methionine sulfoxide reductase, which has been shown to detoxify ROS (Dhandayuthapani et al., 2009). To explain the contradiction in upregulating ROS producers and scavengers, we speculate that the disruption to inositol's metabolic pathways and the subsequent stress upregulated ROS producers, and the ROS scavengers were upregulated by the cell in response to the redox stress. Ultimately, we conclude that inositol metabolic disruption modulated redox homeostasis.

Our observations about the transcriptome following the knockout of inositol metabolic enzymes align with the expectations for UPR activation. The upregulation of protein chaperones and proteolytic enzymes along with the modulation of ribosomal proteins is normal in UPR activation (Higgins et al., 2015). As discussed in the Introduction, both membrane stress and redox stress have been associated with UPR activation. However, our dataset did not resemble the canonical set of UPR gene expression targets. Of the 371 genes previously identified as UPR targets by studies not using inositol depletion for UPR activation, none were significantly up- or down-regulated by the knockout of an inositol metabolic enzyme.

The presence of physiological hallmarks and proposed activators of the UPR combined with the lack of canonical UPR target gene modulation in response to inositol metabolite depletion raises the possibility of confounding transcriptional and physiological noise in inositol depletion studies. Accounting for the 62 unclassified protein families and the far-reaching genes in the original dataset that we could not single out, we speculate that such noise could create certain markers of ER stress without actually activating the UPR pathway and have numerous uncertain other physiological consequences, upsetting researchers' attempts to elucidate the link between membrane aberrancy and the UPR and thus the root causes of many prevalent, costly diseases.

In an attempt to gather as much useful data as possible, we used datasets from a number of different studies using several different species and cell types, from Bacteroides thetaiotaomicron to HeLa cells. This variation in experimental conditions inevitably calls into question our conclusions. Furthered with the uncertainty of making physiological inferences from transcriptional data and the deficiencies of gene annotation datasets for most species, we cannot conclusively report a confounding noise caused by inositol metabolite depletion in inositol depletion studies. Rather, this study aims to create a foundation for further, more targeted research into the transcriptional consequences of inositol depletion's depletion of inositol metabolites.

Beyond merely confirming these speculations, future research into inositol metabolite depletion should advance in two directions: (1) attempting to discern the presence of inositol metabolite noise in inositol depletion studies and (2) elucidating the character of the redox and membrane stress. If our conclusions are confirmed, determining the extent to which inositol depletion studies are affected by inositol metabolite noise will be essential to continue investigating membrane aberrancy's role in the UPR. With the same assumption, understanding why the redox and membrane stress did not trigger the UPR will be essential to confirming inositol's link to membrane aberrancy and will provide a starting point to understanding inositol depletion's link to the UPR. Ultimately, our results bring to light questions about the UPR literature's underlying assumptions about inositol that must be answered to elucidate the critical uncertainties in UPR regulation.

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