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Tumor Necrosis Factor-α -and Interleukin-1-Induced Cellular Responses: Coupling Proteomic and Genomic Information

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Abstract

The pro-inflammatory cytokines, Tumor Necrosis Factor-alpha (TNFα) and Interleukin-1 (IL-1) mediate the innate immune response. Dysregulation of the innate immune response contributes to the pathogenesis of cancer, arthritis, and congestive heart failure. TNFα- and IL-1-induced changes in gene expression are mediated by similar transcription factors; however, TNFα and IL-1 receptor knock-out mice differ in their sensitivities to a known initiator (lipopolysaccharide, LPS) of the innate immune response. The contrasting responses to LPS indicate that TNFα and IL-1 regulate different processes. A large-scale proteomic analysis of TNFα- and IL-1-induced responses was undertaken to identify processes uniquely regulated by TNFα and IL-1. When combined with genomic studies, our results indicate that TNFα, but not IL-1, mediates cell cycle arrest.

Keywords

Tumor Necrosis Factor-alpha; Interleukin-1; Cell Cycle Arrest; Permeability Transition Pore; Protein Interaction Network; Mass Spectrometry; Microarray

Introduction

The innate immune response functions as an important line of defense against pathogenic agents and is an integral component of the wound repair process. Both tumor necrosis factor-alpha (TNFα) and Interleukin-1 (IL-1) are cytokines required for activating the innate immune

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Supporting Information Available: Table S1, MSPlus and isoform resovler output; Table S2, proteins with GO terms and protein ratios; Figure S1, reproducibility of spectral counts; Figure S2, GO percentages; Figure S3, GO hierarchy; Figure S4, protein interaction network (http://www.biochemistry.iu.edu/personnel/Goebl/goebllab/Publications.htm). This material is available free of charge via the Internet at http://pubs.acs.org.

response,[1] mediating the recruitment, activation, and adherence of circulating phagocytic cells (macrophages and neutrophils), and terminating the innate immune response.2–4 Sustained activation and/or dysregulation of the innate immune response may contribute to the underlying pathology of chronic inflammatory diseases (ulcerative colitis,5,6 Crohn's Disease,7 and rheumatoid arthritis8) and life-threatening complications associated with diabetes[9] and cancer.[10]

In response to activation by either bacterial products (lipopolysaccharide, LPS) or foreign particles, circulating macrophages secrete TNFα, an initiator of the innate immune response. In a paracrine manner, TNFα induces surrounding cells to produce interleukin-8 (to elicit phagocyte recruitment)[11] and E-selectin (to promote phagocytic cell adhesion to the surrounding endothelium).12 Autocrine action by TNFα promotes additional TNFα production13 as well as the production and release of IL-1 by macrophages.14 In a paracrine manner, IL-1 induces the local production of Interleukin-615 and the expression of Intercellular Adhesion Molecule-1 (ICAM-1).[16] Together, these and other protein products induced by TNFα and IL-1 mediate the innate immune response and lead to activation of the adaptive immune response.

The TNFα and IL-1 signaling pathways lead to the activation of similar transcription factors (NF-κB, activator protein-1, AP-1, and CCAAT/enhancer-binding protein beta, C/EBPβ). Yet, mice with genetic deletions in TNFα or IL-1 or in their cognate receptors respond differentially to known activators of the innate immune response, such as bacterially derived LPS.[17,18] Wild-type and type I IL-1 receptor knock-out mice show similar mortality rates to low doses of LPS; while type I TNFα receptor knock-out mice are resistant to low doses of LPS. Data derived from studies with receptor and ligand knock-out mice raise the question as to how TNFα- or IL-1-specific processes are elaborated.

Proteomic and genomic analyses each have the potential to monitor multiple intracellular processes simultaneously. We hypothesized that a large-scale analysis combining proteomic and genomic information would provide insight into processes uniquely regulated by TNFα and IL-1. In this study, we provide proteomic data that in combination with existing genomic data demonstrate that TNFα, but not IL-1, specifically induces a cell cycle arrest. The generation of a protein interaction network provides a model outlining a possible mechanism responsible for the cell cycle arrest.

Methods and Materials

Materials

Cell culture media and supplements were purchased from Invitrogen (Carlsbad, CA). Sample preparation reagents and HPLC solvents were purchased from Sigma-Aldrich (St. Louis, MO). Sequence grade trypsin was purchased from Promega (Madison, WI). Recombinant human TNFα and IL-1α were purchased from Sigma-Aldrich (St. Louis,MO) and Santa Cruz Biotechnology (Santa Cruz,CA), respectively.

Cell Culture

The 293 human embryonic kidney (293 HEK) cell line immortalized with Epstein-Barr nuclear antigen was maintained in Dulbecco's modified Eagle's media supplemented with 10% fetal bovine serum, penicillin/streptomycin, and glutamine at 37 °C in a 95% air/5% CO_2 mixture.

Preparation of Protein Lysates

In all experiments, cell cultures (grown in 100 mm dishes to 90% confluence) were left untreated or were treated with either recombinant human TNFα (10 ng/mL) or recombinant

human IL-1α (10 ng/mL) for 6 h. Cell monolayers were washed with ice-cold phosphate-buffered saline (PBS), harvested on ice by scraping with a rubber policeman, and collected by centrifugation (2.3*g*, 5 min, 4 °C). PBS was removed by aspiration, and cell pellets were flash-frozen in liquid nitrogen and stored at −80 °C.

Protein Isolation

Ice-cold lysis buffer (8 M urea and 100 mM ammonium bicarbonate) and acid-washed glass beads were added to frozen cell pellets followed by thawing on ice. To ensure complete lysis, cell pellets were subjected to 10 cycles of 30 s vortexing followed by 30 s of incubation on ice. Cell lysates were separated from glass beads by piercing a hole in the bottom of a microcentrifuge tube followed by centrifugation to elute the lysates into a fresh microcentrifuge tube. Protein concentrations of the soluble lysates were quantified using a Bradford Assay (Bio-Rad, Hercules, CA). Proteins were reduced with dithiothreitol (40:1 dithiothreitol to protein molar ratio) and alkylated with iodoacetamide (20:1 iodoacetamide to protein molar ratio) to prevent unwanted side reactions involving sulfur atoms on cysteine residues. Excess iodoacetamide was neutralized using L-cysteine (40:1 L-cysteine to protein molar ratio). Reduced, alkylated proteins were then subjected to overnight incubation with trypsin at 37 °C (1:70 trypsin to protein molar ratio). Samples were dried under vacuum and resuspended in mobile phase A [5% acetonitrile (ACN), 0.025% formic acid (FA), and 0.001% heptafluorobutyric acid (HFBA)].

High Performance Liquid Chromatography (HPLC) Conditions

All samples were analyzed using the Paradigm MG4 HPLC system (Michrom Bioresources, Inc.) which allowed for fully automated desalting and two-dimensional (Strong Cation Exchange (SCX) and reverse phase (RP)) separation of complex peptide mixtures. Tryptic peptides were loaded (150 μg; 50 μL/min) directly onto a C-18 microtrap (Michrom Bioresources, Inc.) and desalted by flushing the trap with 5 vol of mobile phase A. Peptides were then eluted with 98% ACN/0.025% FA/0.001% HFBA (50 μL/min) directly onto an SCX microtrap (Michrom Bioresources, Inc.). Increasing concentrations of Ammonium formate (4 mM to 1 M) were used to elute the peptides in a stepwise manner onto a C-8 nanotrap (Michrom Bioresources, Inc.) coupled directly with an analytical C-18 column (100 μm i.d., 10 cm of resin) with a pulled tip. Peptides were eluted (500 nL/min) using a 95 min gradient of 98% ACN/0.025% FA (5%–50%) and sprayed directly into an LTQ Mass Spectrometer (Thermo Scientific).

Mass Spectrometry and Data Analysis

For each treatment group ($n = 3$), an MS scan (m/z 450–1500; 1 microscan) was collected followed by MS/MS scans on the two most abundant ions from the full scan. Dynamic exclusion duration was set to 4 min with early expiration enabled when the signal-to-noise threshold fell below 2 during four or more consecutive full scans. Default instrument settings for maximum trap fill time and target values were used. Collected MS/MS spectra were searched against the Human IPI database (Version 3.08) using Sequest (v. 27 rev. 12) and Mascot (v. 1.9) packages.[19] Peptide identifications were validated using XCorr and MOWSE score filters set in MSPlus.20 The false discovery rate of peptide identification was determined by searching the Human IPI database with protein sequences that have been reversed.21

The spectra for each validated peptide were counted, summed, and used as an estimate of protein abundance. In previous studies, spectral counting has been shown to be a good estimator of protein abundance.[20] In this study, spectral counting also showed good reproducibility among treatment group replicates as measured by the correlation coefficient (IL-1: Replicate 1 versus Replicate 2 $r = 0.92$, Replicate 1 versus Replicate 3 $r = 0.76$, Replicate 2 versus Replicate 3 $r = 0.75$; TNFα: Replicate 1 versus Replicate 2 $r = 0.94$, Replicate 1 versus Replicate

3 $r = 0.76$, Replicate 2 versus Replicate 3 $r = 0.77$; Untreated: Replicate 1 versus Replicate 3 $r = 0.88$, Replicate 1 versus Replicate 3 $r = 0.73$, Replicate 2 versus Replicate 3 $r = 0.68$). Two-dimensional plots of replicate versus replicate are shown in Figure S1 in Supporting Information.

MSPlus-validated peptides were grouped into protein groups using IsoformResolver[20] (MSPlus and IsoformResolver outputs are presented in Table S1, Supporting Information). Protein $\log_2$ ratios were determined using spectral counting for each treatment group ($n = 3$), [20] and the mean ratios were fit to a normal distribution. Protein ratios that were greater than 2 standard deviations from the sample mean have a p-value < 0.05 and were considered statistically significant. Even though biologically significant proteins may be excluded using this approach, it does provide a means for examining the most pronounced protein level changes induced by TNFα and IL-1.

Gene Ontology (GO) Term Analysis

IPI numbers of proteins that significantly changed in response to either TNFα or IL-1 treatment compared with respective controls were loaded into an MS ACCESS database, along with additional information including protein ratios and statistical confidence. We grouped the proteins according to GO categories, excluding proteins detected in only one treatment group identified with a single peptide. We also calculated the mean TNFα and IL-1 protein differential change ratio for each GO category. Text files containing IPI number, their associated GO identification numbers, and GO terms were downloaded from the Gene Ontology Web site (http://www.geneontology.org) and integrated into the MS ACCESS database.

Network Analysis

Saccharomyces cerevisiae homologues to human proteins that changed significantly ($p < 0.05$) upon TNFα or IL-1 treatment were identified using NCBI's Homologene database (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Homologene) and performing additional BlastP analysis.[22] Since the yeast and human homologues share common protein interaction and functional domains, the homologues likely interact with similar proteins. Using these homologues, we compiled a human protein interaction database based upon a yeast protein interaction database developed in-house. This database integrates the yeast protein interaction databases from SGD (ftp://ftp.yeastgenome.org/yeast/) and BIND (http://bond.unleashedinformatics.com/). Our in-house yeast database contains genetic and physical interactions on all characterized ORFs and excludes interactions characterized by experiments with potentially high false-positive rates (i.e., yeast two-hybrid experiments). Here, we used the yeast protein interaction database and transferred this knowledge into human for network analysis primarily due to incomplete publicly available human protein interaction databases. To construct the human protein interaction network, we filtered the protein interaction database to include only proteins among or interacting with differentially changed proteins. To visualize the network, Proteolens (http://bio.informatics.iupui.edu/Proteolens), a freely accessible software developed to visualize large protein interaction networks, was used.

Analysis of Gene Expression Omnibus Data Sets (GEO-datasets)

Affymetrix microarray GEOdatasets generated from TNFα (unpublished GDS690, unpublished GDS85223) or IL-1 (GDS64924) treated cell cultures were downloaded from the NCBI Web site (http://www.pubmed.gov). The mRNA expression data from each data set were downloaded, normalized using the justgcrma() function of Bioconductor (http://www.bioconductor.org),[25–27] and fit to a normal distribution to determine statistical significance.

Results and Discussion

Physiological responses to TNFα and IL-1 have been defined using receptor and cytokine knock-out mouse models.[28–33] On the cellular level, information regarding global responses to pro-inflammatory cytokines has been restricted to inferences based upon the analysis of microarray data.[23,24,34] To gain a better understanding of the global cellular responses elicited by TNFα and IL-1, proteomic technology was used to define the proteome of TNFα- or IL-1-treated human embryonic kidney cells (293 HEK). Since the 293 HEK cell line is widely used in cytokine research, results gained from our study will be of value to other research laboratories.

Selecting Proteins for further Characterization

Between the three treatment groups (untreated, TNFα, and IL-1), 6585 proteins were validated and quantified. Approximately 55% of the protein identifications were based upon a single peptide (single hit proteins). Single hit proteins may represent low-abundance proteins, proteins with numerous post-translational modifications, and/or contain peptides that ionize weakly during an MS analysis. Despite the potential for obtaining biologically significant information from single hit proteins, the single hit proteins were excluded due to their higher false discovery rate (Figure 1). Assuming the false discovery rate equals the probability of incorrectly identifying single hit proteins, the probability for having all peptides incorrectly identified for proteins with one, two, or three identified peptides was 6%, 0.4%, and 0.02%, respectively. Since the probability associated with incorrectly identifying proteins with two or more peptides is less than 1%, only these proteins were selected for further analysis.

Another approach for lowering the false discovery rate is to choose stricter parameters. Theoretically, the number of correctly identified peptides failing to pass XCorr and Mascot filters will decrease with a lower false discovery rate. If this approach is used, it would be difficult to distinguish between the correct and incorrect peptide identifications, and the number of identified proteins may decrease. By the use of a probability-based two-peptide rule in place of lowering score thresholds, single hit proteins are merely placed aside from the current analysis until later experiments warrant further characterization of these proteins to determine biological significance.

Determining Significant Protein Abundance Changes

Spectral counts of validated peptide identifications used to calculate protein ratios (cytokine-treated versus untreated or IL-1 versus TNFα) serve as a measure of protein abundance providing insight into how the cytokines regulate protein levels. To assess the significance of changes in protein abundance, the protein ratios were fit to a normal distribution. The central limit theorem postulates that the larger a data set is, the more it will tend to approximate a normal distribution.[35] As expected, the protein ratios from the IL-1-treated versus untreated (Figure 2A) and the TNFα-treated versus untreated (Figure 2B) data sets approximated a normal distribution. On average, 70% of the protein ratios fell within 1 standard deviation (σ) of the average of the protein ratios within a given treatment group (sample mean), 92% of the ratios fell within 2σ of the sample mean, and 96% of the ratios fell within 3σ of the sample mean. When a data set follows a normal distribution, the probability of a protein ratio being significantly different from the sample mean is based upon the number of standard deviations a data point varies from the sample mean (*Z*-score). Protein ratios were considered significant (p-value < 0.05) if they were more than 2σ away from the mean (*Z*-score > 2.0 or *Z*-score < −2.0). Protein ratios at least 2σ away from the mean represent proteins that exhibited a 2.27- to 3.41-fold change in response to TNFα and a 2.17- to 3.-20-fold change in response to IL-1 (Table 1).

GO provides a hierarchy for cellular processes that increases in specificity when proceeding to the next level within the hierarchy and is commonly used to annotate proteomic data sets.[36–38] This hierarchy can be employed to gain a global perspective on the processes regulated by TNFα and IL-1. To begin the analysis, individual proteins from each treatment group were assigned a GO process, and the *Z*-scores of the proteins assigned a given process were averaged. A significance threshold of 2σ was applied to the averages, because if individual proteins follow a normal distribution, then an average of individual protein *Z*-scores should also follow a normal distribution. At first glance, it would seem that all processes should be statistically significant when the groups themselves consist of individual ratios that were statistically significant. In this data set, many processes were no longer significant in both cytokine treatment groups once grouped together (Figure 3). For example, the DNA replication GO process group was no longer significant in response to IL-1, because some proteins increased and some proteins decreased. As a result, the process fell below the 2σ threshold. In the TNFα treatment group, all proteins mapped to the DNA replication GO process group showed significant decreases upon TNFα treatment with an average standard deviation for the process of $< -2\sigma$. GO processes that are not statistically significant may be indicative of proteins that are modified (i.e., phosphorylation or ubiquitin) after translation has occurred and may serve as the basis for future studies characterizing post-translational modifications occurring in response to TNFα or IL-1 treatment. On the other hand, statistically significant processes may be indicative of proteins that have protein levels directly regulated by a particular cytokine. Since the purpose of this study was to examine protein levels that significantly changed in response to cytokine treatment, only statistically significant GO processes were further characterized. The number of proteins per GO Term expressed as percentage of the total number of proteins is presented in Figure S2 of Supporting Information. A detailed hierarchy for all processes is shown in Figure S3 of Supporting Information.

TNFα treatment leads to changes in the nuclear processes of DNA replication, mRNA export from nucleus, mRNA processing, nucleosome assembly, and protein import into nucleus (Figure 3A). As cells traverse the G1/S border, activation of E2F occurs which leads to the expression of genes necessary for DNA synthesis. During this period, increases in histone gene expression allow the synthesis of histones in order to package the newly synthesized DNA.[39] In response to TNFα, we detected a decrease in DNA replication machinery proteins and an increase in the synthesis of histone variants. The decrease in DNA replication machinery coupled with a shift in chromatin conformation due to changes in nucleosome composition may allow the expression of a distinct subset of genes. The protein levels of DNA replication machinery components (RPA1, RPA3, RFC2, and PCNA) were also significantly decreased upon TNFα treatment (Table 2). TNFα treatment also increased protein levels associated with the GO process nucleosome assembly, including the H2A variant H2A.Z (associated with the opening of chromatin to transcription factors40) and macroH2A (associated with CpG methylated DNA and heterochromatin)40 (Table S2 in Supporting Information). Decreases in the components of the DNA replication machinery and changes in nucleosome composition elicited by TNFα could prevent S phase activities leading to a cell cycle arrest.

Consistent with inducing a growth arrest response, TNFα significantly decreased cyclin dependent kinase-2 (CDK2) protein levels. Analysis of publicly available microarray data sets also revealed that TNFα significantly decreased cyclin A mRNA levels (Table 2). CyclinA/CDK2 activity is required for cells to move through S phase, while cyclin A/Cdc2 complexes regulate G2 progression.[41,42] Together, a decrease in cyclin A transcript levels and a decrease in CDK2 protein levels would also inhibit cell cycle progression. Interestingly, the TNFα-induced changes in cell cycle progression appear to be independent of changes in the levels of the cyclin-dependent kinase inhibitors (CDKIs) that mediate cell cycle arrest at various points throughout the cell cycle (Table 2).

TNFα and Induction of Cell Cycle Arrest Mediated by HIF1α Transcriptional Inactivation

The targets of Hypoxia Inducible Factor 1-alpha (HIF1α) include genes involved in cell proliferation, angiogenesis, apoptosis, and metabolism.43 Blocking of cell proliferation occurs in the presence of a HIF1α pharmacological inhibitor (YC-1).44 In our proteomic data sets, mitogen activated protein kinase-1 (MAPK1) and CEBPζ (a co-activator recruited to promoters by CBP/p300) are significantly decreased upon TNFα treatment (Table 2). MAPK1-mediated HIF1α phosphorylation has been shown to be essential for the formation of complexes containing HIF1α and the transcriptional co-activator CBP/p300.[43] Thus, the TNFα-mediated decreases in MAPK1 and HIF1α co-activator protein levels could indicate that TNFα mediates the transcriptional inactivation of HIF1α.

TNFα and HIF1α Stabilization

Although TNFα has been shown to inhibit HIF1α protein degradation under normoxic conditions,[45] the mechanism for this protein stabilization remains poorly understood. In response to TNFα treatment, cullin-2 (Cul2) protein levels decreased (Table 2). Reduced Cul2 protein levels may stabilize HIF1α, since following the hydroxylation of HIF1α by prolyl-hydroxylase under normoxic conditions, Cul2 in complex with Von Hippel-Lindau (VHL) mediates HIF1α ubiquitin-dependent proteasomal degradation. [43] Arginosuccinate lyase (ASL), an enzyme that catalyzes the generation of arginine through the catabolism of arginosuccinate, protein levels increased (compared to IL-1 treatment, Table S2 in Supporting Information). The observed increased protein levels of ASL may lead to an increase in intracellular arginine levels, a precursor for nitric oxide.46 Since nitric oxide acts as an inhibitor of prolyl-hydroxylase, inhibition of HIF1α hydroxylation may occur.47 Thus, on the basis of our observations, TNFα may stabilize HIF1α by inducing ASL-mediated prolyl-hydroxylase inactivation and by decreasing Cul2 protein levels.

IL-1 Induced Processes Occurred Mainly in the Cytoplasm

IL-1-induced changes in the processes of ER to Golgi vesicle-mediated transport, glycolysis, microtubule-based movement, mitochondrial transport, protein polymerization, response to unfolded protein, RNA splicing, and ubiquitin-dependent protein catabolism (Figure 3B). IL-1 treatment leads to a significant increase in enzymes that act in concert to raise intracellular pyruvate levels including pyruvate kinase (PKM2; Table 2), phosphoglucomutase (PGM1, Table S2 in Supporting Information) and increases triosephosphate isomerase-1 (TPI1) protein levels (Table 2).

TNFα and IL-1-Induced Metabolic Changes and Differential Regulation of HIF1α

Exogenous addition of pyruvate and lactate has been shown to stabilize levels of HIF1α protein through an uncharacterized mechanism.[48] TNFα treatment significantly increased the protein levels of lactate dehydrogenase (LDHLA6B) (compared to IL-1 treatment, Table S2 in Supporting Information) and the E2 of pyruvate dehydrogenase (DLAT) (compared to the untreated, Table 2). LDHLA6B stimulates the conversion of pyruvate to lactate, while DLAT converts pyruvate to acetyl-CoA.[49,50] On the basis of our proteomic data, TNFα-induced increases in LDHLA6B and DLAT could increase lactate production, which in turn might also lead to HIF1α stabilization (Figure 4A).

Studies with 293 HEK cells have shown that the addition of pyruvate leads to accumulation of HIF1α protein and induces the expression of HIF1α-dependent genes.[48] In contrast to the significant increases found in response to TNFα, IL-1 treatment did not lead to changes in the protein levels of DLAT or LDHLA6B (enzymes that catabolize pyruvate) (Table S2 in Supporting Information). Instead, IL-1 treatment leads to an increase in PKM2, which catalyzes

the conversion of phosphoenolpyruvate to pyruvate. Both PKM2, and its product pyruvate have been linked to activation of HIF1α dependent genes[43] (Figure 4).

TNFα and the Regulation of the Mitochondrial Permeability Transition Pore (PTP)

The PTP consists of the following proteins: adenine nucleotide translocase (ANT), voltage-dependent anion channel (VDAC), cyclophilin-D (PPIF), protein transporter at the outer membrane (TOMM40), and protein transporter of the inner membrane (TIMM9).[51] PTP opening allows the entry of small molecules into the lumen of the mitochondria which leads to mitochondrial swelling from osmosis and mitochondrial release of cytochrome *C* (CytC).[52] TNFα treatment increased TIMM9 protein levels, while TNFα and IL-1 decreased TOMM40 protein levels. TNFα and IL-1 treatments led to a significant increase in the level of PPIF protein, while TNFα increased PPIF transcript levels to a greater extent than IL-1 (Table 2). The necessity of PPIF and other PTP complex members for PTP opening is under debate. However, PPIF has been shown to protect cells from apoptosis resulting from the opening of the PTP.[53] Thus, the increased levels of PPIF protein and transcript may protect against TNFα-induced apoptosis during cell cycle arrest.

Network Analysis Couples PTP and HIF1α Regulation

A protein interaction network, based upon biochemical and genetic interactions in *S. cerevisiae* (described in Materials and Methods), was used to explore the interactions between HIF1α and PTP regulatory elements (Figure 5). Upon TNFα treatment, ARD1A, the human homologue of the protein Arrest Defective (ARD1), which is essential for entry into the stationary phase of *S. cerevisiae*,[54] was increased (Table 2). Mouse studies have shown that under normoxic conditions ARD1A-mediated acetylation of HIF1α on lysine 532 targets HIF1α in a VHL/Cul2-dependent manner for proteasomal degradation.[55] In human cell lines, ARD1A/HIF1α complexes can be detected; however, loss of ARD1A had no effect upon HIF1α protein stability.[56,57] It would appear that ARD1A and HIF1α proteins may be linked, however, their role in ARD1A activity in the regulation of HIF1α stability remains unclear.

Conclusion

TNFα production often precedes the production of IL-1 during the innate immune response.[1] In 293 HEK cells, TNFα may prepare the cell to respond to IL-1 through the induction of G1 cell cycle arrest and inhibition of DNA replication. We can formulate a model in which TNFα-mediated cell cycle arrest appears to result from TNFα-dependent increases in lactate leading to stabilization of HIF1α. IL-1 appears to activate HIF1α through the induction of glycolytic enzymes that produce pyruvate. The TNFα-induced stabilization of HIF1α followed by the IL-1 dependent activation of HIF1α provides an example of how TNFα and IL-1 work together to coordinate different cellular processes to regulate the inflammatory process.

Results from this study demonstrate how a combined examination of both mRNA expression data and proteomic data can provide novel insight into the cellular processes uniquely regulated by TNFα. Data generated in this study provide a starting point for future studies aimed at examining the relationship between the cytokines TNFα and IL-1 in the regulation of the inflammatory response.

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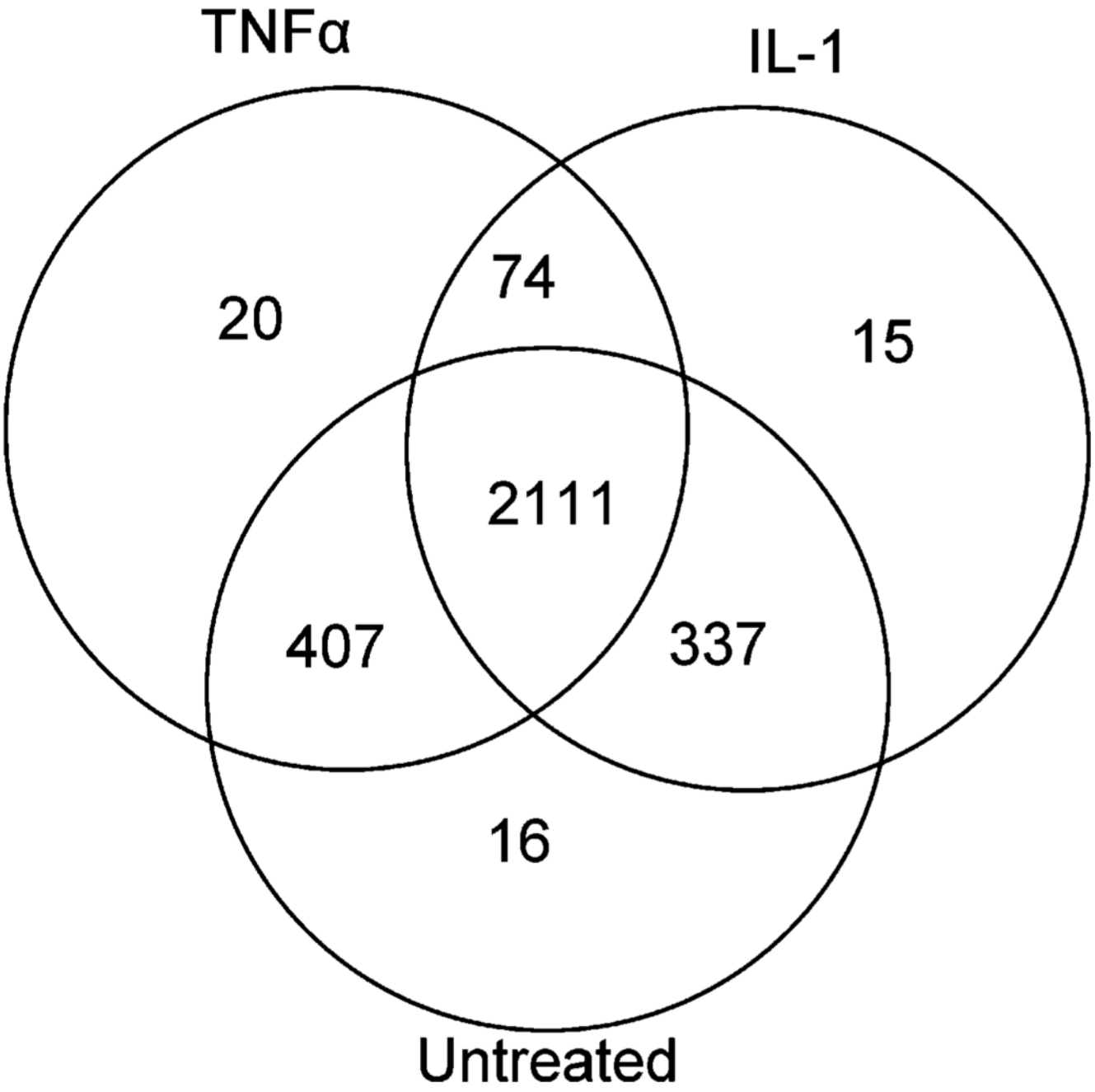

Figure 1.
Euler diagram of all identified proteins after removal of single hit proteins. A total of 2980 proteins remained after excluding single hit proteins. The circles represent the three groups: TNFα-treated, IL-1 treated, or untreated. Circle intersections represent the number of proteins common between two or more of the groups.

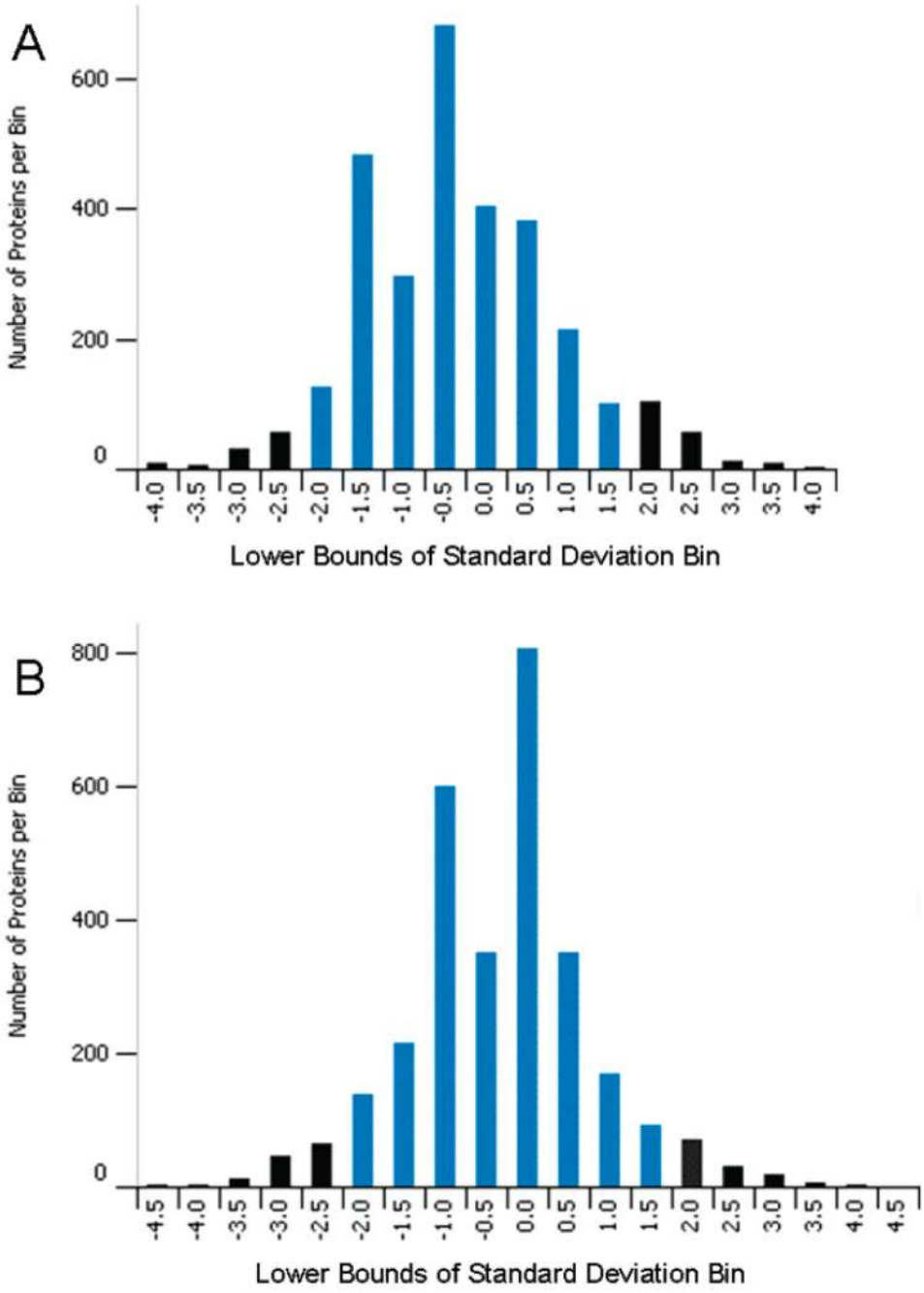

Figure 2.

Distribution of protein ratios from TNFα- and IL-1-treated data sets. (A) TNFα- or (B) IL-1-treated versus untreated protein ratios were placed into bins separated by 0.5σ. The total number of proteins for a particular bin is plotted along the *y*-axis; while the *x*-axis represents standard deviation bins. Only proteins greater than 2σ away from the sample mean (black bars) were considered for network analysis.

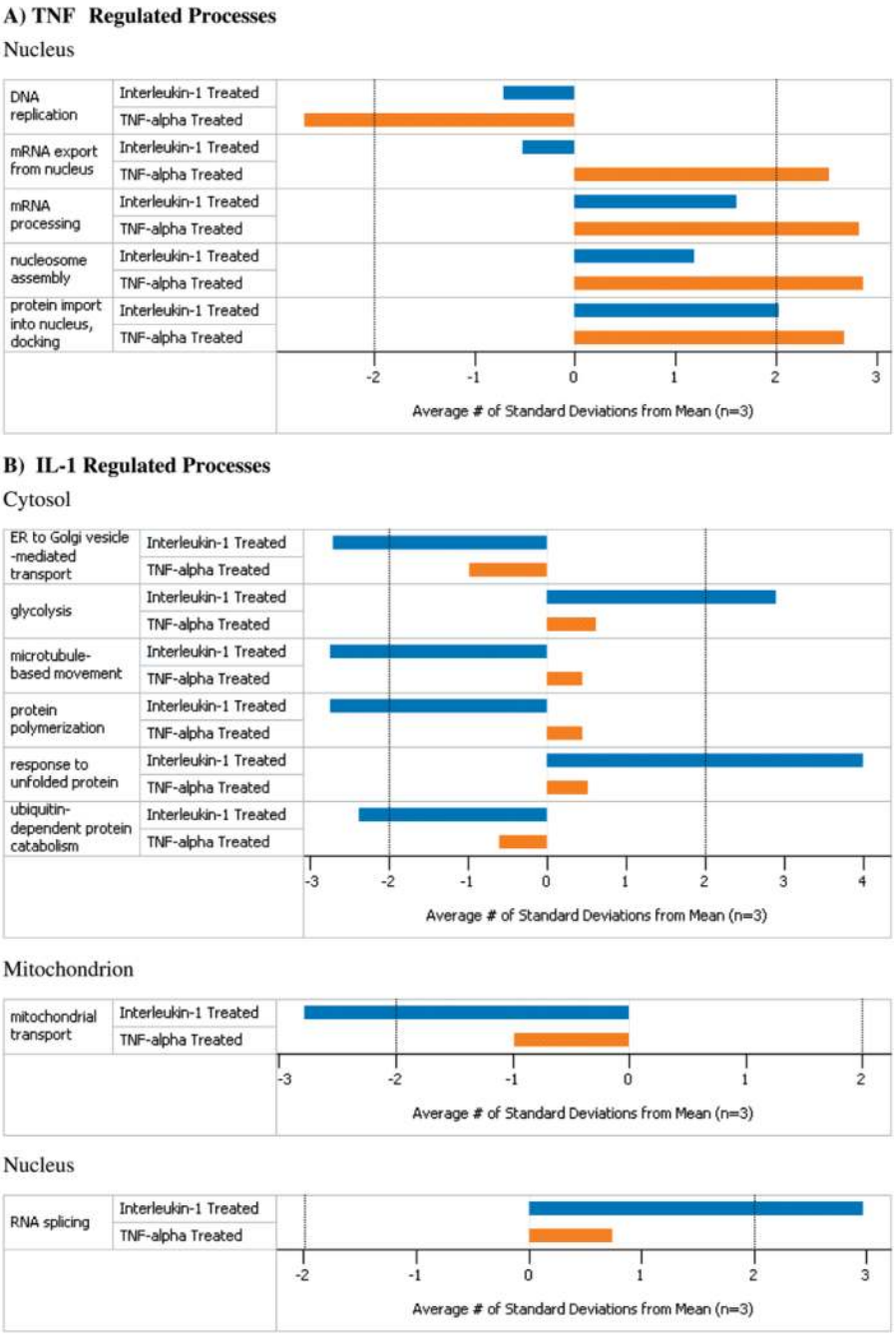

Figure 3.

GO processes changed significantly in response to TNFα and IL-1 as a function a subcellular compartment. Proteins at least 2σ away from the mean were grouped according to their processes for TNFα- and IL-1-treated cells. Each GO process was then assigned a GO component. The average number of standard deviations from the mean were calculated for each GO process. Only processes >2σ were considered significant as indicated by the dotted line.

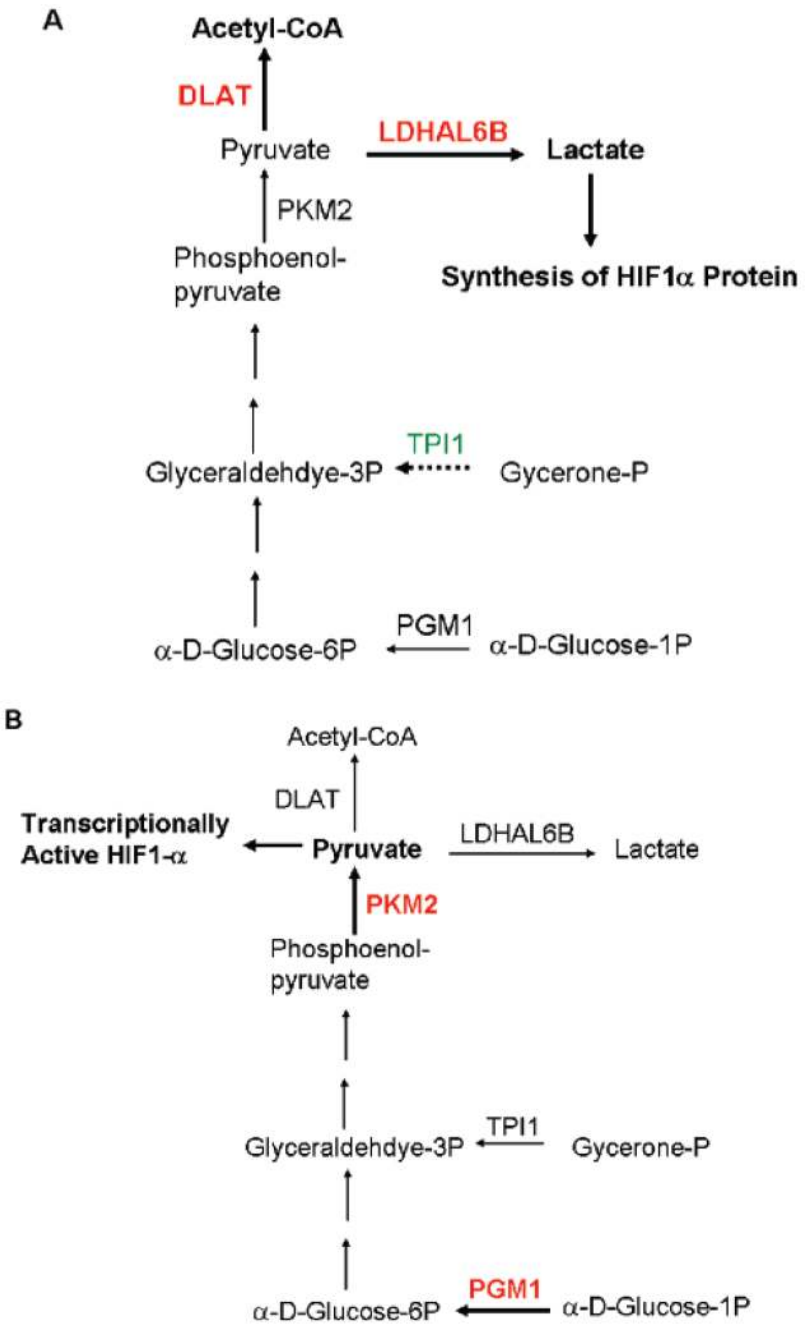

Figure 4.

TNFα and IL-1 regulation of lactate and pryruvate metabolism. (A) TNFα stimulates the production of lactate, which leads to accumulation of HIF1α protein. (B) IL-1 stimulates the production of pyruvate, which activates the transcription activity of HIF1α. Proteins increased in response to cytokine treatment are shown in red. Proteins decreased in response to cytokine treatment are shown in green.

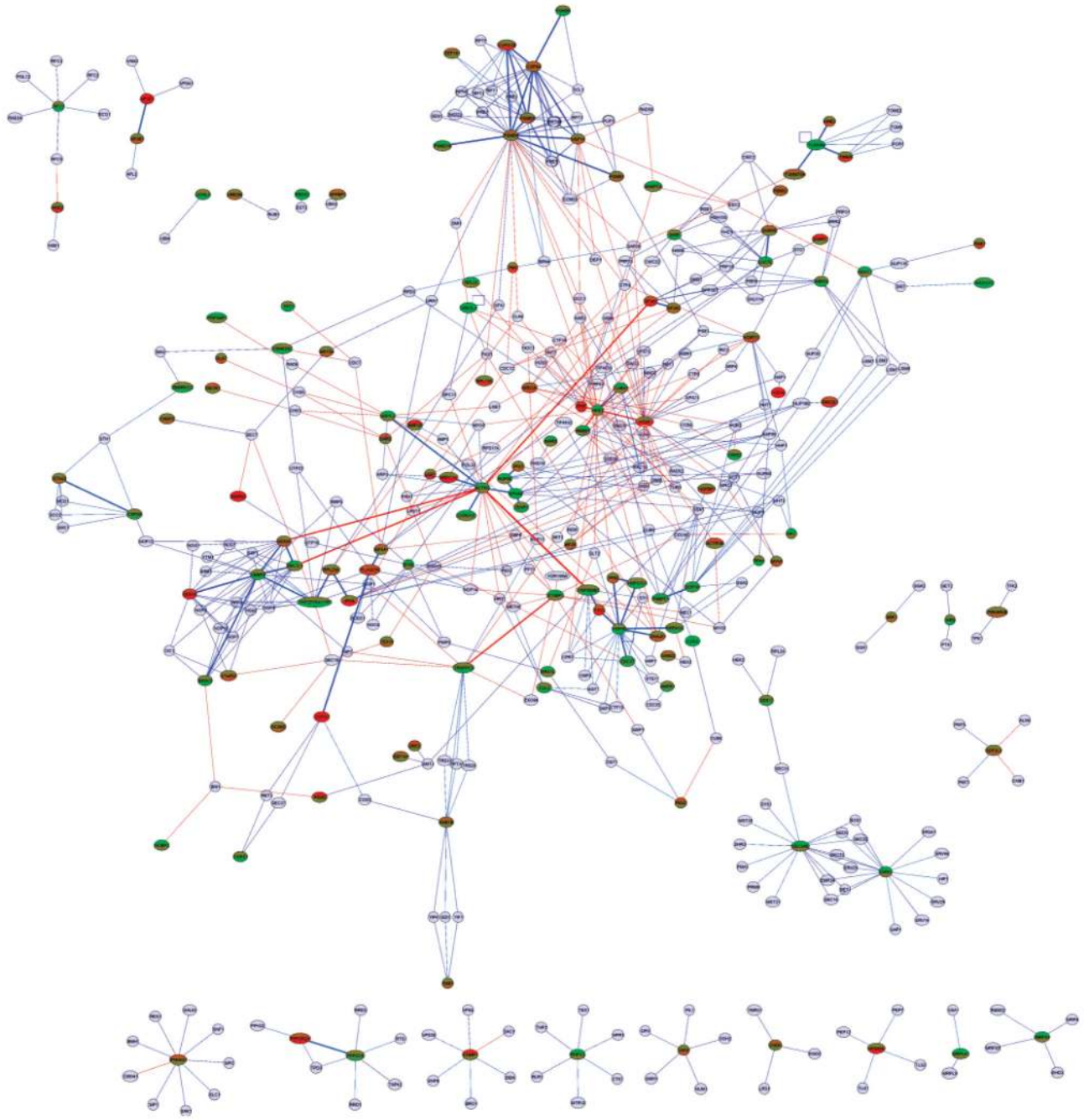

Figure 5.

TNFα and IL-1 interaction network. Each node contains two halves, where the top half represents the change seen upon IL-1 treatment, and the bottom half represents the change seen upon TNFα treatment. A color gradient of red (increased protein levels) to green (decreased protein levels) was used to show a particular protein's response to cytokine treatment. These bicolored nodes are labeled with human protein names, while light blue nodes are labeled with yeast protein names and represent proteins with no currently known homologue or no significant change in protein levels. Red edges connecting the nodes represent synthetic lethalities, and the blue edges represent physical interactions resulting from affinity capture

experiments and/or reconstituted complexes. An image (JPG) of the protein interaction network is presented in supplemental Figure S3 in Supporting Information.

Table 1

Relationships between Standard Deviations from the Mean (*Z*-Score) and Fold Differences in Protein Abundance

	TNFα	IL-1
< −3σ	−3.41 to −3.92	−3.20 to −4.37
−2σ to −3σ	−2.27 to −3.41	−2.17 to −3.20
−1σ to −2σ	−1.51 to −2.27	−1.47 to −2.17
Mean to −1σ	1.01 to −1.51	1.05 to −1.47
Mean to 1σ	1.01 to 1.51	1.05 to 1.47
1σ to 2σ	1.51 to 2.27	1.47 to 2.17
2σ to 3σ	2.27 to 3.41	2.17 to 3.20
>3σ	3.41 to 6.12	3.20 to 6.78

Table 2

Z-Scores for TNFα and interleukin-1 Regulated Proteins and mRNAs[a,b,c],

Cell Cycle Genes				
Gene	Protein IL-1	mRNA IL-1	Protein TNFα	mRNA TNFα
ARDIA	2.180	−0.340	2.572	−1.209
CCNAI	NA	−0.353	NA	−2.749
CDC37	−2.094	0.527	−2.114	1.879
CDC6	NA	−2.257	NA	−0.506
CDK2	−2.921	−0.080	−2.484	−0.472
CDKN1B	NA	−2.847	NA	0.322
CDKN3	NA	−2.281	NA	−0.167
CUL2	−1.878	−0.309	−2.326	−0.660
PIN1	0.610	NA	2.996	NA
DNA Replication Genes				
Gene	Protein IL-1	mRNA IL-1	Protein TNFα	mRNA TNFα
PCNA	−1.701	−2.293	−2.037	−0.667
RFC2	−1.812	−0.358	−3.638	0.490
RPA1	−0.498	−1.593	−2.326	−1.830
RPA3	−2.512	−0.526	−2.347	−2.622
Mitochondrial Permeability Transition Pore Genes				
Gene	Protein IL-1	mRNA IL-1	Protein TNFα	mRNA TNFα
TIMM9	0.949	−0.703	2.926	−0.421
TOMM40	−2.974	0.325	−2.659	−0.269
PPIF	3.272	0.342	3.405	1.483
Pyruvate and Lactate Metabolism Genes				
Gene	Protein IL-1	mRNA IL-1	Protein TNFα	mRNA TNFα
DLAT	0.512	−1.244	2.509	−0.353
PKM2	3.659	0.314	0.088	−0.221
TPI1	−1.845	0.619	−3.052	−0.678
HIF1-alpha Transcriptional Activity Regulatory Genes				
Gene	Protein IL-1	mRNA IL-1	Protein TNFα	mRNA TNFα
MAPK1	0.511	0.107	−2.121	−0.403
CEBPZ	−0.936	−0.653	−2.326	−0.073

[a] Red protein or mRNA levels are T least 2σ above the mean.

[b] Green protein or mRNA levels are least 2σ below the mean.

[c] NA indicates genes that were not detected during the indicated analysis.