Combining Transcriptomics and Proteomics Reveals Potential Post-transcriptional Control of Gene Expression After Light Exposure in *Metarhizium acridum*

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**ABSTRACT**

Light is an important stimulus for fungi as it regulates many diverse and important biological processes. *Metarhizium acridum* is an entomopathogenic fungus currently used for the biological control of insect pests. The success of this approach is heavily dependent on tolerance to environmental stresses. It was previously reported that light exposure increases tolerance to ultraviolet radiation in *M. acridum*. There is no information in the literature about how light globally influences gene expression in this fungus. We employed a combination of mRNA-Sequencing and high-throughput proteomics to study how light regulates gene expression both transcriptionally and post-transcriptionally. Mycelium was exposed to light for 5 min and changes at the mRNA and protein levels were followed in time-course experiments for two and four hours, respectively. After light exposure, changes in mRNA abundance were observed for as much as 1128 genes or 11.3% of the genome. However, only 57 proteins changed in abundance and at least 347 significant changes at the mRNA level were not translated to the protein level. We observed that light downregulated subunits of the eukaryotic translation initiation factor 3, the eIF5A-activating enzyme deoxyhypusine hydroxylase, and ribosomal proteins. We hypothesize that light is perceived as a stress by the cell that responds to it by reducing translational activity. Overall, our results indicate that light acts both as a signal and a stressor to *M. acridum* and highlight the importance of measuring protein levels in order to fully understand light responses in fungi.

**KEYWORDS**

light

transcriptomics

proteomics

*Metarhizium*

stress

*Metarhizium acridum* (Ascomycota: Sordariomycetes) is a soil-inhabiting entomopathogenic fungus currently used for the biological control of Orthoptera insects, mostly locusts and grasshoppers (Lacey *et al.* 2015). The success of biological control is heavily dependent on survival under harsh environmental conditions. Among these, heat and ultraviolet-B radiation (UV-B, 280-315 nm) are among the most stressful. The effects of UV-B radiation range from delayed conidia germination to complete inactivation (Braga *et al.* 2001; Braga *et al.* 2015). In this scenario, methods increasing *M. acridum* tolerance to UV-B radiation are highly sought after. Previous studies have shown that many physical and chemical factors can modulate stress tolerance in *Metarhizium* and other fungi (Rangel *et al.* 2011; Rangel *et al.* 2015; Dias *et al.* 2019). One of such factors is exposure to visible light.

Light is an important stimulus that regulates many biological processes in fungi. Depending upon the organism, light can regulate processes as diverse as development, secondary metabolite production, entrainment to circadian oscillators, and phototropism (Yu and Fischer 2019). Importantly, light responses are normally fast and transient with hierarchical signaling (Chen *et al.* 2009). Fungi respond to light by using photoreceptors capable of sensing mostly blue (phototropins), green (opsins), and red (phytochromes) light, although distinct
fungi will differ in their ability to sense each of these wavelengths (Yu and Fischer 2019). In *Metarhizium robertsii*, growth under white light results in the production of conidia that germinate faster and are more virulent when compared to conidia produced in the dark (Oliveira et al. 2018). Also, using blue light during growth resulted in increased conidia yield (Oliveira et al. 2018). Regarding stress tolerance, we have previously reported that exposing *M. acridum* mycelium to white or blue light leads to increased tolerance to UV-B radiation (Brancini et al. 2016). We have also shown that light induces the expression of a photolyase gene and we and others have reported that photoreactivation is probably involved in UV-B radiation tolerance (Fang and St Leger 2012; Brancini et al. 2018). Nevertheless, we have no information about how light regulates gene expression genome-wide.

Genome-wide regulation after light exposure was evaluated in the ascomycete model *Neurospora crassa* and light was found to modulate the expression of as much as 24% of all predicted genes (Wu et al. 2014). However, the authors did not measure protein levels and therefore the number of changes at the mRNA level that are effectively translated to the protein level is still unknown. In this regard, a recent study focused on combining mRNA-Seq and high-throughput proteomics to study clock-controlled genes in *N. crassa* (Hurley et al. 2018). The authors observed that circadian output is influenced by post-transcriptional regulation, especially translational control, thus emphasizing the need to measure protein levels. Here we combined mRNA-Seq and Tandem Mass Tag (TMT)-based high-throughput proteomics to study how light regulates gene expression both transcriptionally and post-transcriptionally in *M. acridum*.

**MATERIALS AND METHODS**

**Strains and growth conditions**

*Metarhizium acridum* ARSEF 324 was obtained from the USDA-ARS Collection of Entomopathogenic Fungal Cultures (Ithaca, NY, USA). The culture was maintained in Potato Dextrose Agar (Difco) supplemented with 0.5% yeast extract (Difco). Conidia were obtained by growing at 28°C in complete darkness for 12 days.

**Light exposure**

Conidia were scraped from plates and used to prepare a suspension at 2.5 × 10⁹ cells ml⁻¹ in TWEEN 80 0.05% (Sigma). Four milliliters of this suspension were used to inoculate 100 ml of Potato Dextrose Broth (Difco) in 250 ml Erlenmeyer flasks. For each experiment, a total of six cultures were prepared. These cultures were grown in complete darkness at 28°C under agitation (125 rpm) for 24 h to produce mycelium. Then, five of the six culture flasks were exposed to white light from fluorescent lamps (irradiance = 5.3 W m⁻²; photon flux = 24.7 μmol m⁻² s⁻¹) for 5 min. Flasks were moved back to dark for different lengths of time depending on experiment type. For transcriptomics, dark incubations after light exposure were for 0 (5L 0D), 10 (5L 10D), 25 (5L 25D), 55 (5L 55D), and 115 (5L 115D) min. For proteomics, these incubations were for 10 (5L 10D), 25 (5L 25D), 55 (5L 55D), 115 (5L 115D), and 235 (5L 235D) min. In both cases a control was always kept in the dark (DD). After the incubation was over, mycelium was vacuum filtered, washed with distilled water, and immediately frozen in liquid nitrogen. Frozen mycelia were stored at −70°C until RNA or protein extraction. Three independent experiments were performed for mRNA-Seq and three independents experiments for high-throughput proteomics.

**Effects of light on the transcriptome**

Frozen mycelia were ground with mortar and pestle under liquid nitrogen to obtain a fine powder. Approximately 50 mg of frozen powder were added to 450 μl RLT buffer from the RNeasy Plant Mini Kit (Qiagen). Purification was performed following manufacturer’s instructions and total RNA was eluted with nuclease-free water. Quality assessment was performed on an Agilent Bioanalyzer 2100 and all samples presented with RNA Integrity Number ≥ 7. Libraries were constructed with the TruSeq Stranded mRNA v4 (Illumina) following manufacturer’s instructions. Library quantification was performed via quantitative PCR and sequencing was run on HiSeq 2500 equipment. Three independent experiments were performed separately and sequenced together in the same lane. Because each experiment consisted of six samples, a total of 18 samples were sequenced yielding approximately 20 million reads per sample.

Sequencing data were aligned to *M. acridum* genome (Gao et al. 2011) with Hisat2 (Kim et al. 2015). The alignments were then analyzed with Cufflinks (Trapnell et al. 2010) using the -g option (no Reference Annotation Based Transcript assembly). Differential expression and statistical testing were performed with Cuffdiff 2 (Trapnell et al. 2013). Finally, Cuffdiff output was analyzed with cummeRbund (Trapnell et al. 2012). Differences between light treatments and DD were considered significant if they could satisfy P < 0.01 and a twofold cutoff. Gene clustering by expression pattern was performed with clust (Abu-Jamous and Kelly 2018), heat maps were built with TM4 MeV (Saeed et al. 2003), and principal component analysis was achieved with ClustVis (Metsalu and Vilo 2015). Gene ontology analyses were performed on the Blast2GO suite (Gotz et al. 2008).

Validation of mRNA-Seq data were performed for photolyase (MAC_05491) and UV-endonuclease (MAC_07337) coding genes with quantitative reverse transcription PCR (qRT-PCR). Total RNA extraction was performed exactly as described for mRNA-Seq and the downstream protocol for cDNA synthesis and gene quantification was as previously described (Brancini et al. 2018).

**Effects of light on the proteome**

Frozen mycelia were ground with mortar and pestle under liquid nitrogen to obtain a fine powder. Approximately 50 mg of frozen powder were added to 500 μl of extraction buffer [7M urea, 2M thiourea, 4% CHAPS (Sigma)] and the mixture was vortexed for 2 min. Samples were then centrifuged at 10,000 × g and 4°C for 5 min. The supernatant was collected and total protein was quantified with the 2-D Quant Kit (GE Healthcare). Protein purification was performed with a methanol/chloroform protocol as previously described (Wessel and Flugge 1984).

Proteins were reduced with dithiothreitol, alkylated with iodoacetamide, and finally digested with trypsin. Resulting peptides were labeled with TMT 10-plex (Thermo Scientific) with one tag for each condition according to manufacturer’s instructions. After isobaric tagging, the six conditions in each experiment were pooled and fractionated by reverse phase chromatography (C₁₈, 1 × 100 mm, 3.5 μm, 130 Å, Waters). Elution was performed at 0.1 ml/min using a gradient of A (20 mM pH 10 ammonium formate) and B (acetonitrile) from 1 to 37.5% over 61 min. A total of 12 fractions were collected. These were dried in a vacuum centrifuge and solubilized in 0.1% formic acid.

Tandem mass spectrometry (MS/MS) analyses were performed as previously described (Becher et al. 2018). Briefly, peptides from each of the 12 fractions were analyzed on a nanoLC (UltiMate 300 RSLC, Thermo) equipped with a C₁₈ pre-column (Pecolumn C₁₈ PepMap 100, 300 μm × 5 mm, 5 μm, 100 Å) and an analytical column (Acclaim C₁₈ PepMap 100, 75 mm × 50 cm, 3 μm, 100 Å).
The nanoLC equipment was coupled to a Q Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer (Thermo). Elution was always performed with solvents A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile). Peptides were loaded into the column at 30 μl/min solvent A for 3 min. Peptides were eluted from the column with an elution gradient adjusted to 0.3 ml/min over 120 min. The concentration of B in the gradient was ramped to 4% over 4 min, to 8% over 2 min, to 26% over 96 min, and to 40% over 10 min. Eluted peptides were analyzed in positive mode and data-dependent method. Full scan spectra were obtained in the 375-12,000 m/z range. The top ten precursors in MS were selected for MS/MS.

Raw spectra were processed with IsobarQuant (Franken et al. 2015) and protein identification was performed with MASCOT (Matrix Science). Identification was based on the *M. acridum* genome (Gao et al. 2015). MASCOT search parameters were as follows: enzyme trypsin; up to three missed cleavages; peptide tolerance 10 ppm; MS/MS tolerance 0.02 Da; carbamidomethyl (Cys) and TMT10plex (Lys) as fixed modifications; TMT10plex on N-terminus, oxidation (Met), and N-acetylation as variable modifications. Batch effects were removed using limma (Ritchie et al. 2015) and results were normalized via the vsn strategy of variance normalization (Huber et al. 2002). Quantitative information was only analyzed when a given protein was found in two or three experiments. If the protein was identified in two experiments, missing data for the third experiment were imputed with the k-nearest neighbor algorithm. Changes at the protein level were considered significant if they could satisfy a twofold cutoff relative to DD at False Discovery Rate < 0.05. Combined mRNA/protein graphs were plotted with Origin 8.0 software (OriginLab Corporation).

**Data availability**

Supplemental material available at Figshare: https://doi.org/10.25387/g3.8115998.

**RESULTS**

**Effects of light on the transcriptome**

To evaluate light-regulated gene expression, we performed miRNA-Seq of RNA extracted from mycelia exposed to light for 5 min followed by incubation in the dark for different lengths of time (0, 10, 25, 55, and 115 min). A control was kept in complete darkness (DD). Our analysis encompassed 9514 genes corresponding to 95.4% of the genome (Table S1). A gene was considered light-regulated if significant mRNA change was observed in at least one time point relative to DD. Light regulated the expression of 4819 genes at P < 0.01. Because many genes were only weakly regulated, we applied a two-fold cutoff and observed that 1128 transcripts changed in abundance under these criteria (Table S2). Of these, 719 (64%) were upregulated and 409 (36%) were downregulated. Principal component analysis revealed that the majority of changes occurred at the initial time points (especially 5L 0D, 5L 10D, and 5L 25D) and not at later time points (Figure 1).

To understand the kinetics of gene regulation after light exposure, we clustered the 1128 light-regulated genes according to their expression profile by using *clust* (Abu-Jamous and Kelly 2018). *clust* deals with the clustering problem with a data extraction approach instead of the more traditional data partitioning. On the one hand, this generates tight clusters with little to no ambiguity in gene assignment. On the other hand, only about 50% of all genes are clustered (Abu-Jamous and Kelly 2018). For our data set, *clust* generated 13 clusters comprising 619 genes (54.9%) with an average cluster size of 47.6 genes (Figure 2 and Table S3).

The upregulated clusters (C1 through C6) showed that light can regulate gene expression at multiple time points, thus allowing us to classify genes as early- (5L 0D, 5L 10D, and 5L 25D; clusters C1 through C5) and late- (5L 55D and 5L 115D; cluster C6) regulated according to their peak expression (Figure 2). Also, this revealed a potential hierarchical model in which light initially drives the expression of genes coding for transcription factors that will then act on downstream genes. Approximately the same phenomenon was observed for downregulated gene clusters (C7 through C10), although late downregulated genes were not observed (Figure 2). Finally, some gene clusters presented an oscillatory pattern characterized by initial downregulation followed by late upregulation (C11 through C13) (Figure 2).

To gain better insight into which biological processes were regulated by light, we performed Gene Ontology analyses on clusters C2 and C8 which are the largest up and downregulated gene clusters, respectively. Overall, light upregulated genes involved in cellular response to stress and cellular protein localization (Figure 3A) and downregulated genes involved in transmembrane transport (Figure 3B). Some biological processes, such as ‘oxidation-reduction process’ and ‘regulation of transcription from RNA polymerase II promoter’, were shared by both clusters. Because response to stress and transcriptional regulation were enriched in cluster C2, we looked for genes belonging to known oxidative stress response pathways. We observed that a stress-activated MAPK gene (MAC_08084) homolog to *N. crassa as-2* and *Aspergillus nidulans hogA* was upregulated together with the bZip transcription factor *asl-1* homolog (MAC_03844).

Because transcriptional regulators were abundant in clusters C2 and C8, we performed a separate analysis for such light-regulated genes (Figure 4). Among these, we found homolog genes for the core circadian oscillator *frq* (MAC_01916) and the circadian transcriptional repressor *csp-1* (MAC_07134) both of which are also regulated by light in *N. crassa* (Froehlich et al. 2002; Sancar et al. 2011). Future experiments should elucidate whether *M. acridum* possesses a circadian clock.
For validation purposes, we have evaluated the expression of genes encoding for a photolyase (MAC_05491) and a UV-endonuclease (MAC_07337) by qRT-PCR. Similar patterns of light regulation in mRNA-Seq and qRT-PCR experiments were observed for both genes (Figure 5).

Effects of light on the proteome

For high-throughput proteomics experiments, we analyzed a longer time point (5L 235D) in order to better account for the expected delay between mRNA and protein peak. We also removed the very short 5L 0D time point from proteomics analyses. Our proteomics data showed good agreement between the three experiments and quantitative information was used only if a protein was present in at least two experiments (Figure 6).

Our analysis encompassed 3852 proteins representing 38.6% of all predicted gene products. Of these, only 57 were regulated by light at least twofold, with 41 upregulated and 16 downregulated proteins. Changes in abundance at the protein level peaked at 5L 235D for 89.5% of regulated proteins, with only six proteins changing at earlier time points (Figure 7).

Figure 2 Clustering analysis for light-regulated genes resulted in 13 clusters depicting upregulated (C1 through C6), downregulated (C7 through C10), and oscillatory (C11 through C13) genes. Values in y-axis are Z-scores.

Figure 3 Gene Ontology analysis for genes on clusters (A) C2 and (B) C8 which are the largest up and downregulated gene clusters, respectively.
We then analyzed the top 10 most up and downregulated proteins after light exposure (Table 1 and Table 2). The strongest upregulated protein, acid sphingomyelinase (MAC_02084), is involved in sphingolipid metabolism. SignalP-5.0 (Almagro Armenteros et al. 2019) sequence analysis revealed the presence of an N-terminal secretory signal peptide that could indicate the protein has a role in insect pathogenicity. Five out of the ten most upregulated proteins are currently uncharacterized and two of these (MAC_09637, MAC_02991) have no homologs in N. crassa.

One of the uncharacterized proteins (MAC_09800) is annotated as a flavin-binding monooxygenase in M. guizhouense, M. brunneum, and M. majus. We also observed the accumulation of two other flavin-binding monooxygenases (MAC_09799, MAC_09164) after light exposure (Table S4). Furthermore, MAC_09799 and MAC_09800 are neighboring genes and presented the same protein accumulation profile (Table S4). We also observed the upregulation of heat shock protein 30 (MAC_07554) and photolyase (MAC_05491), both probably involved in light-induced stress tolerance.

Among downregulated proteins, subunits E and M of eukaryotic translation initiation factor 3 (eIF3) were at least twofold regulated after light exposure (Table 2). The downregulation of two eIF3 subunits prompted us to lower the twofold cutoff in the search for other regulated eIF3 subunits. We found eIF3 subunit K to be 1.8-fold and eIF3 subunit F to be 1.4-fold downregulated (Figure 8A). This observation was specific to eIF3 as subunits for other translation initiation factors were unchanged (Table S5). However, the enzyme deoxyhypusine hydroxylase (MAC_01359) was downregulated at the protein level (Table 2 and Figure 8B). This protein is one of two enzymes required for the post-translational modification that activates eukaryotic initiation factor 5A (eIF5A) which has a role in translation elongation (Saini et al. 2009).

The decreased translation initiation/elongation caused by light exposure prompted us to look for regulated ribosomal proteins. We observed downregulation of 40S ribosomal proteins S14 and S29 (MAC_07998, MAC_09100) and 60S ribosomal protein P0 (MAC_01037), although these only satisfied a 1.5-fold cutoff (Figure 8C). Furthermore, mitochondrial ribosomal proteins S4 L12 (MAC_00638) and 40S MRP2 (MAC_09204) were 2.5- and 1.5-fold downregulated, respectively (Table 2 and Figure 8D).

Combining proteomics and mRNA-Seq data to find post-transcriptional regulatory mechanisms

After light exposure, 1128 mRNAs (out of 9514 evaluated) changed in abundance while only 57 proteins (out of 3852 evaluated) did so. Combining both data sets resulted in 34 light-regulated mRNA/protein pairs. We used these pairs to elucidate the average time required to go from peak mRNA to peak protein change. This was done by calculating R^2 for log2-log2 correlation plots. Overall, mRNA change at any time point best correlated with protein change 1-2 h later (Table 3 and Table S6).

The majority of pairs followed this 1-2 h delay as observed for the photolyase (Figure 9A). A very early regulated gene coding for a C2H2 transcription factor (= N. crassa CSP-1) presented an accompanying early protein accumulation and was one of the fastest regulated protein in the data set, perhaps a requirement to fulfill its biological role (Figure 9B). In at least two instances there was protein accumulation after gene downregulation, such as observed for a polyketide synthase (Figure 9C).

A consequence of having only 34 mRNA/protein pairs is that 23 proteins changed abundance in the absence of mRNA regulation (Table 4). In principle, this would leave us with 1094 mRNAs for which there was no protein change. However, we need to take into account that mRNA-Seq and proteomic data sets are different sizes (9514 vs. 3852, Table 4). Therefore, the number of mRNAs changing after light exposure without an accompanying protein change is actually 347, while the remaining 747 present no protein quantitative data (Table 4).
This means that a large number of changes at the mRNA level are not translated into changes at the protein level. More importantly, it was not possible to predict, based on mRNA fold change or expression pattern, whether regulation at the transcript level would lead to changes at the protein level. Some mRNAs were upregulated by as much as 18-fold and downregulated by as much as 5.7-fold without any accompanying changes in protein expression levels (Table S6).

DISCUSSION

The success of biological control with *M. acridum* depends on the fungus surviving the stresses imposed by the environment. Among these, heat and UV-B radiation can be cited as the most relevant. We previously observed that a 5-min exposure to light will increase tolerance to UV-B radiation in a time-dependent manner (Brancini et al. 2016). Therefore, we combined transcriptomics (mRNA-Seq) and high-throughput proteomics to understand how light regulates gene expression both transcriptionally and post-transcriptionally. Our experiments were performed by exposing mycelium to a 5-min pulse of light and then incubating it in the dark for different lengths of time. Conversely, most studies evaluating light responses in fungi expose mycelium to light for different lengths of time with no incubation afterward (Chen et al. 2009; Ruger-Herreros et al. 2011; Fuller et al. 2013; Schumacher et al. 2014; Wu et al. 2014). Because light is also regarded as a stress to fungi, different exposures to light would inevitably lead to varying amounts of stress based on the length of light exposure. We have therefore tried to mitigate this effect by using the same exposure for all time points in our analysis. Furthermore, the 5-min exposure was chosen based on prior work (Brancini et al. 2016) in which we observed that this exposure was sufficient to result in increased tolerance to UV-B radiation.

Light transcriptionally regulated 1128 genes or 11.3% of the genome after a 5-min exposure. Most genes were regulated in the first 30 min after light exposure with only few genes being late regulated (Figure 1 and 2). According to the hierarchical model of gene regulation by light uncovered in *N. crassa* (Chen et al. 2009), the White Collar Complex (WCC) initially drives the expression of its target genes and some of these are transcription factors that will then act downstream of the
Table 1 – Ten most upregulated proteins after light exposure

| Name | Gene ID | Protein Entry | log$_2$ fold-change$^a$ (time point) |
|------|---------|---------------|-------------------------------------|
| Acid sphingomyelinase, putative | MAC_02084 | E9DW76 | 2.40 (5L 235D) |
| Uncharacterized protein | MAC_09637 | E9EID9 | 2.09 (5L 235D) |
| Membrane protein, putative | MAC_09840 | E9EIZ2 | 2.00 (5L 55D) |
| Photolyase | MAC_05491 | E9E6J3 | 1.76 (5L 115D) |
| Heat shock protein 30 | MAC_07554 | E9ECF6 | 1.70 (5L 115D) |
| Uncharacterized protein | MAC_05425 | E9E6C7 | 1.69 (5L 235D) |
| Uncharacterized protein | MAC_09800 | E9EIV2 | 1.64 (5L 235D) |
| Uncharacterized protein | MAC_01545 | E9DVAR | 1.57 (5L 235D) |
| Lysine amidinotransferase | MAC_04410 | E9E3G2 | 1.56 (5L 235D) |
| Uncharacterized protein | MAC_02991 | E9DZE3 | 1.51 (5L 235D) |

$^a$ peak log$_2$ fold-change relative to DD.

Table 2 – Ten most downregulated proteins after light exposure

| Name | Gene ID | Protein Entry | log$_2$ fold-change$^a$ (time point) |
|------|---------|---------------|-------------------------------------|
| Cyanochrome P450 phenylacetate 2-hydroxylase, putative | MAC_06623 | E9DWS5 | −2.17 (5L 235D) |
| Amino acid transporter, putative | MAC_02949 | E9DZA1 | −1.42 (5L 235D) |
| Carboxyphosphonoenolpyruvate phosphonomutase, putative | MAC_05902 | E9E7Q4 | −1.37 (5L 235D) |
| 54S ribosomal protein L12 | MAC_00638 | E9DSP0 | −1.35 (5L 235D) |
| Eukaryotic translation initiation factor 3 subunit E | MAC_09482 | E9EHY4 | −1.30 (5L 235D) |
| GNAT family N-acetyltransferase, putative | MAC_02756 | E9DYQ9 | −1.26 (5L 235D) |
| Sphingomyelinase hydroxylase | MAC_01545 | E9DUR1 | −1.18 (5L 235D) |
| Vitamin B6 transporter, putative | MAC_05825 | E9E7H7 | −1.18 (5L 235D) |
| Rhomboid family protein | MAC_03768 | E9E1M0 | −1.15 (5L 235D) |
| Eukaryotic translation initiation factor 3 subunit M | MAC_01057 | E9DVT9 | −1.12 (5L 235D) |

$^a$ peak log$_2$ fold-change relative to DD.
day (Hurley et al. 2018), which is line with prior work reporting that translational activity is decreased at late subjective morning (Caster et al. 2016). It seems reasonable to say that light reduces translational activity in *N. crassa* by resetting the clock to subjective morning. It should be noted that the aforementioned downregulated proteins did not present downregulation of their corresponding mRNAs in our data set. This could be due to these proteins being post-transcriptionally regulated or it could be a consequence of late gene regulation that is beyond our last time point (5L 115D).

Combining our transcriptomic and proteomic data sets revealed an interesting phenomenon: while 1128 mRNAs changed in abundance in response to light, only 57 proteins did so (Table 4). These values correspond to 11.8% of all 9514 evaluated transcripts and 1.48% of all 3852 evaluated proteins. No more than 34 mRNA-protein pairs could be formed that were regulated in both data sets (Table S6). These pairs were used to calculate the 1-2 h delay required to go from mRNA peak regulation to protein peak regulation (Figure 9 and Table 3).

As mentioned above, we observed that the majority of changes at the mRNA level were not translated to changes at the protein level. Lack of protein change in the event of gene downregulation could be explained by protein stability: stable proteins will last many hours inside the cell and our experiment only encompassed the first four hours following light exposure. However, if proteins are stable, then accumulation would be expected from gene upregulation. The negative effect light apparently had on translation initiation and elongation could perhaps help explain such a phenomenon.

Under conditions of decreased translational activity, there should be a mechanism allowing specific mRNAs to bypass this overall decrease. Light downregulated eIF3 subunits E (eIF3e), M (eIF3m), K (eIF3k), and F (eIF3f) but it did not regulate any other subunit. eIF3 is thought to mediate 43S pre-initiation complex assembly and attachment to mRNA, scanning, and start codon selection (Hinnebusch 2017). In *Schizosaccharomyces pombe*, there are two distinct eIF3 complexes formed with either eIF3m or eIF3e (Zhou et al. 2005). On the one hand, the complex formed with eIF3m binds to the bulk of cellular mRNA and is responsible for overall translation. This makes eIF3m an essential gene. On the other hand, the complex formed with eIF3e is more restricted and regulates the translation of specific mRNAs (Zhou et al. 2005). In *N. crassa*, mutants for all the known eIF3 subunits were analyzed. In accordance with *S. pombe*, eIF3m was found to be an essential gene whereas eIF3e and eIF3k mutants

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**Table 3** - Person correlation coefficient for changes at the mRNA and protein levels. Correlation was calculated based on the 34 light-regulated mRNA/protein pairs.

| mRNA-Seq  | SL 10D | SL 25D | SL 55D | SL 115D | SL 235D |
|------------|--------|--------|--------|---------|---------|
| **Proteomics** |        |        |        |         |         |
| 5L 0D      | 0.29   | 0.26   | 0.74   | 0.73    | 0.10    |
| 5L 10D     | 0.15   | 0.11   | 0.71   | 0.70    | 0.02    |
| 5L 25D     | 0.11   | 0.19   | 0.79   | 0.76    | 0.16    |
| 5L 55D     | 0.18   | 0.21   | 0.78   | 0.81    | 0.36    |
| 5L 115D    | 0.04   | 0.28   | 0.55   | 0.59    | 0.65    |

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**Figure 8** Light downregulated proteins involved in translation, including (A) eIF3 subunits M (MAC_01057), E (MAC_09482), K (MAC_04650), and F (MAC_03887); (B) the eIF5A-activating enzyme deoxyhypusine hydroxylase (MAC_01359); (C) cytosolic ribosomal proteins P0 (MAC_01037), S14 (MAC_07998), and S29 (MAC_09100); and (D) mitochondrial ribosomal proteins L12 (MAC_00638) and MRP2 (MAC_09204). Values in y-axis are log2 fold-change relative to DD.
were viable (Smith et al. 2013). Therefore, different eIF3 subunits are probably involved in the translation of distinct mRNA molecules and their regulation constitutes an additional layer of post-transcriptional control (Genuth and Barna 2018). We speculate that light can affect the translation of specific mRNAs by regulating eIF3 subunits and therefore translation initiation.

In line with this hypothesis, light also downregulated some ribosomal proteins (Figure 8C and 8D) while the majority remained unchanged (Table S5). It was previously shown in mouse embryonic stem cells that active ribosomes are heterogeneous with respect to ribosomal proteins (Shi et al. 2017). These heterogeneous ribosomes translate different pools of mRNAs involved in different biological processes such as metabolism, proliferation, and cell survival. For instance, RPL10A was found to be required for the translation of specific mRNAs. This regulation was mediated, at least in part, by 5'UTR internal ribosome entry site (IRES) elements (Shi et al. 2017). It seems a natural consequence that regulating the abundance of ribosomal proteins could lead to differences in mRNA translation for specific genes. This “ribosome code” has been speculated and discussed for the past 60 years, but it is only recently gaining more attention (Emmott et al. 2019).

Table 4 – Combination of mRNA-Seq and proteomics data sets based on the number of regulated mRNAs/proteins

|                  | mRNA-Seq | Proteomics |
|------------------|----------|------------|
| Genome           | 9974     |            |
| Evaluated mRNAs/proteins | 9514 | 3852 |
| Light-regulated  | 1128a    | 57b        |
| Upregulated      | 719 (64%)| 41 (72%)   |
| Downregulated    | 409 (36%)| 16 (28%)   |
| mRNA/protein pairs | 34      |            |
| protein change without mRNA change | 23    |            |
| mRNA change without protein change | 347   |            |

aP < 0.01 and at least twofold regulation.
bFalse Discovery Rate < 0.05 and at least twofold regulation.

Taken together, our results indicate that light acts as both a signal and a stress in M. acridum. When acting as a signal, light regulates the transcription of as much as 11.3% of the genome. Because it is also perceived as a stress, light ultimately causes a decrease in translational activity by downregulating some eIF3 subunits, the eIF5A-activating enzyme deoxyhypusine hydroxylase, and ribosomal proteins. We hypothesize the downregulation of these proteins buffers the changes at the mRNA level and ultimately results in the small number of regulated proteins observed. Therefore, our results show that changes at the mRNA level are not necessarily translated to changes at the protein level and highlight the importance of analyzing the proteome in order to fully understand light responses in fungi.

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LITERATURE CITED

Abu-Jamous, B., and S. Kelly, 2018 Clust: automatic extraction of optimal co-expressed gene clusters from gene expression data. Genome Biol. 19: 172. https://doi.org/10.1186/s13059-018-1536-8

Almagro Armenteros, J. J., K. D. Tsirigos, C. K. Sonderby, T. N. Petersen, O. Winther et al., 2019 SignalP 5.0 improves signal peptide predictions...
using deep neural networks. Nat. Biotechnol. 37: 420–423. https://doi.org/10.1038/s41587-019-0036-z
Bayram, Ö., C. Biesemann, S. Krappemann, P. Galland, and G. H. Braus, 2008 More Than a Repair Enzyme: Aspergillus nidulans Photolyase-like CayA Is a Regulator of Sexual Development. Mol. Biol. Cell 19: 3254–3262. https://doi.org/10.1091/mbc.e08-01-0061
Becher, I., A. Andrés-Pons, N. Romanov, F. Stein, M. Schramm et al., 2018 Pervasive Protein Thermal Stability Variation during the Cell Cycle. Cell 173:1495–1507 e1418. https://doi.org/10.1016/j.cell.2018.03.053
Braga, G. U., S. D. Flint, C. D. Miller, A. J. Anderson, and D. W. Roberts, 2001 Both solar UV and UBV radiation impair conidial culturability and delay germination in the entomopathogenic fungus Metarhizium anisopliae. Photochem. Photobiol. 74: 734–739. https://doi.org/10.1562/0031-8655(2001)74<0734:FSUAVUR>2.0.CO;2
Braga, G. U., D. E. Rangel, E. K. Fernandes, S. D. Flint, and D. W. Roberts, 2015 Molecular and physiological effects of environmental UV radiation on fungal conidia. Curr. Genet. 61: 405–425. https://doi.org/10.1007/s00294-015-0483-0
Brancini, G. T., D. E. Rangel, and G. U. Braga, 2016 Exposure of Metarhizium acridum mycelium to light induces tolerance to UV-B radiation. FEMS Microbiol. Lett. 363. https://doi.org/10.1093/femsle/fnw036
Brancini, G. T. P., L. Bachmann, M. Ferreira, D. E. N. Rangel, and G. U. L. Braga, 2018 Exposing Metarhizium acridum mycelium to visible light up-regulates a photolyase gene and increases photoreactivating ability. J. Invert. Pathol. 152: 35–37. https://doi.org/10.1016/j.jip.2018.01.007
Caster, S. Z., K. Castillo, M. S. Sachs, and D. Bell-Pedersen, 2016 Circadian clock regulation of mRNA translation through eukaryotic elongation factor eEF-2. Proc. Natl. Acad. Sci. USA 113: 9605–9610. https://doi.org/10.1073/pnas.1525269113
Castrillo, M., J. García-Martínez, and J. Aivalos, 2013 Light-Dependent Functions of the Fusarium fujikuroi CryD DASH Cryptochrome in Development and Secondary Metabolism. Appl. Environ. Microbiol. 79: 2777–2788. https://doi.org/10.1128/AEM.03110-12
Chen, C. H., C. S. Ringelberg, R. H. Gross, J. C. Dunlap, and J. J. Loros, 2015 Thermal proteome profiling of multivariate data using Principal Component Analysis and heatmap. J. Invertebr. Pathol. 132: 1–41. https://doi.org/10.1016/j.jip.2015.07.009
Clancy, L. M., R. Jones, A. L. Cooper, G. W. Griffith, and R. D. Satner, 2018 Dose-dependent behavioural fever responses in desert locusts challenged with the entomopathogenic fungus Metarhizium acridum. Sci. Rep. 8: 14222. https://doi.org/10.1038/s41598-018-32524-w
Crawford, R. A., and G. D. Pavitt, 2019 Translational regulation in response to stress in Saccharomyces cerevisiae. Yeast 36: 5–21. https://doi.org/10.1002/yea.3349
de Bekker, C., R. A. Ohm, R. G. Loreto, A. Sebastian, I. Albert et al., 2015 Gene expression during zombie ant biting behavior reflects the complexity underlying fungal parasitic behavioral manipulation. BMC Genomics 16: 620. https://doi.org/10.1186/s12864-015-1812-x
Dias, L. P., N. Pedrini, G. U. L. Braga, P. C. Ferreira, B. Pupin et al., 2019 Outcome of blue, green, red, and white light on Metarhizium robertsi during mycelial growth produces conidia with increased germination speed and virulence. Fungal Biol. 122: 555–562. https://doi.org/10.1016/j.funbio.2017.12.009
Rangel, D. E., G. U. L. Braga, and D. E. N. Rangel, 2018 Metarhizium robertsi illuminated during mycelial growth produces conidia with increased germination speed and virulence. Fungal Biol. 122: 555–562. https://doi.org/10.1016/j.funbio.2017.12.009
Rangel, D. E., G. U. L. Braga, C. A. Keyser, J. E. Hallsworth et al., 2015 Stress tolerance and virulence of insect-pathogenic fungi are determined by environmental conditions during conidial formation. Curr. Genet. 61: 383–404. https://doi.org/10.1007/s00294-015-0477-y
Rangel, D. E., K. E. Fernandes, G. U. Braga, and D. W. Roberts, 2011 Visible light during mycelial growth and conidiation of Metarhizium robertsi produces conidia with increased stress tolerance. FEMS Microbiol. Lett. 315: 81–86. https://doi.org/10.1111/j.1574-6968.2010.02168.x
Ritchie, M. E., B. Phipson, D. Wu, Y. Hu, C. W. Law et al., 2015 limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res. 43: e47. https://doi.org/10.1093/nar/gkv007
Ruger-Herreros, C., J. Rodriguez-Romero, R. Fernandez-Barranco, M. Olmedo, R. Fischer et al., 2011 Regulation of Conidiation by Light in Aspergillus nidulans. Genetics 188: 809–822. https://doi.org/10.1034/j.1574-9361.2012.130009
Saeed, A. L., V. Sharov, J. White, J. Li, W. Liang et al., 2003 TM4: a free, open-source system for microarray data management and analysis. Biotechniques 34: 374–378. https://doi.org/10.2144/03342mt01
