Title: RNAseq analysis of *Arabidopsis thaliana* exhibiting novel positive blue-light phototropic root response in microgravity.

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0. **Abstract**:

Premise of the study: Growth-mediated movements, termed “tropisms”, are one of the primary mechanisms for plants to respond to their external environment. However, little is known about the interactions among these plant growth responses. Here, we utilize the condition of microgravity on board the International Space Station (ISS) to identify genes involved in the interaction between light responses (phototropism) and gravity responses (gravitropism) in *Arabidopsis thaliana* seedlings.

Methods: Seedlings were grown on the ISS, and RNA was extracted from 7 samples (pools of 10-15 plants) grown in microgravity (µg) or Earth gravity conditions *(*1.0*g).* Transcriptomic analysis of differential gene expression was performed using the HISAT2-Stringtie-DESeq2 RNASeq pipeline. Differentially expressed genes were further characterized by using Pathway Analysis as well as enrichment for Gene Ontology classifications.

Key Results: 296 genes were found to be significantly differentially expressed between microgravity when compared to 1-*g* controls ($p < 0.05$). In addition, Pathway Analysis identified 8 molecular pathways that are significantly affected by reduced gravity conditions. Specifically, light-associated pathways (e.g. photosynthesis-antenna proteins, photosynthesis, porphyrin and chlorophyll metabolism) show significant downregulation.

Conclusions: *Arabidopsis thaliana* seedlings grown in conditions of microgravity show significant alterations to gene expression when compared to the 1-*g* control. Understanding the interactions between these two essential tropistic responses not only furthers our fundamental understanding, but may also help to efficiently grow plants during long-range space missions. In addition, this study is the first to use RNAseq tools to study the interactions between tropisms in a spaceflight study.

1. Introduction

Due to their sessile nature, plants need to be able to integrate multiple external stimuli to direct their growth and development. These growth-mediated movements (or "tropisms") were first scientifically characterized by Darwin and Darwin (1880), who theorized the movement was modified circumnutation. Multiple external stimuli affect plant growth and development such as water availability (Kiss, 2007), mechanical forces (Braam, 2005), gravity (Chen, Rosen, and Masson, 1999; Kiss, 2000), as well as light (Briggs, 2014; Liscum et al., 2014; Vandenbrink et al., 2014b; Kutschera and Briggs, 2016), among others. Taken together, these tropisms play a large role in determining the overall architecture of the plant (Correll and Kiss, 2002). In addition, sensing and responding to these external cues are important for the plant to be able to adapt to changing environmental conditions.

Two tropistic responses that play a substantial role in determining plant growth direction and overall plant architecture are phototropism (directed response to light) and gravitropism (directed response to gravity). Typically, plants orient their roots towards the gravity vector (positive gravitropic response), and away from blue/white light exposure (negative phototropic response), whereas aerial organs orients away from the gravity vector (negative gravitropic response) and towards a blue/white light source (positive phototropic response; Chen et al. 1999; Kiss et al. 2003; Briggs 2014; Kutschera and Briggs 2016).

In addition, plants utilize blue-light cues to influence lateral root growth and other root architectural characteristics (Moni et al., 2015). Light irradiation also has been shown to affect root length (Laxmi et al., 2008; Silva-Navas et al., 2015). The effect of light on roots is significant, as light has been shown to penetrate several millimeters into the soil (Woolley and Stoller, 1978; Tester and Morris, 1987), with red and far-red light penetrating to greater depths than the remaining spectrum (Mandoli and Briggs, 1984).

This light exposure has led roots to evolve blue-light receptors in the upper portion of the roots and red-light receptors more distal in the root due to the more penetrative quality of red light (Mo et al., 2015).

The plant gravitropic response is divided into three stages: perception, transduction and response. During the perception phase, starch-filled statoliths interact with cytoplasmic objects in the specialized gravity-sensing columella cells (Sack, 1991; Salisbury, 1993; Kiss, 2000; Vandenbrink et al., 2014a). Once the gravity signal is perceived, a differential auxin gradient is sent along opposing sides of the root to the root elongation zone (transduction stage), where differential plant growth occurs and leads to reorientation of the root in the direction of the gravity vector (reviewed in Vandenbrink et al., 2014).

While the gravitropic and phototropic responses are well characterized, little is known about their interaction, which determines the ultimate direction of growth. Growth of plants in conditions of real or simulated microgravity have allowed for the study of phototropic response in the absence of significant gravitational influences (Kiss, 2015). Recently, studies have aimed to characterize the link between the two tropistic responses. For instance, our previous study found that light perception by the roots had an effect on shoot gravitropic response in *Arabidopsis thaliana* (Hopkins and Kiss, 2012). In addition, phototropic curvature of roots in response to blue light illumination was shown to be intimately tied to the magnitude of the gravity vector (Vandenbrink et al., 2016).

This spaceflight study also identified an association between red-light phototropic response in roots and the gravity vector (Vandenbrink et al., 2016). In terms of cell growth and cell proliferation, it has been shown that when seedlings are grown in darkness, there is a lack of balance between these key plant development functions in microgravity (Matía et al., 2010). Further evidence for this observation was provided by analyzing a dark-grown, synchronized cell culture grown in simulated microgravity (Kamal et al., 2018). Recent spaceflight results show that red-light can compensate for this effect (Valbuena et al., 2018), particularly increasing cell growth (measured by means of ribosome biosynthesis in the nucleolus) that was depleted without light stimulation.

In this study, we used RNA expression profiling to begin to elucidate the molecular mechanisms underlying tropistic interactions. Thus, in the present set of space experiments, we use transcriptomic techniques to characterize gene expression related to tropisms in conditions of microgravity. Our most significant results show a relationship between gravity, blue-light based phototropism and the pathways associated with photosynthesis.

2. Materials and Methods

Spaceflight Experiment

Seeds of *Arabidopsis thaliana* ecotype *Landsberg erecta* (Ler) were flown to the International Space Station (ISS) via the SpaceX Dragon. Spaceflight experiments were conducted utilizing the European Modular Cultivation System (EMCS) in the Columbus Module of the ISS. The EMCS facility provides two centrifuges for the production of gravity vectors, as well as atmospheric, temperature and hydration monitoring and control (Brinckmann and Schiller, 2002; Brinckmann, 2005; Kiss et al., 2014). In addition, the EMCS contains a video camera for image acquisition as well as visual monitoring of the experiment. The Seedling Growth series of experiments was conducted in two parts. The first set of experiments (termed SG1) was uploaded on to the ISS via SpaceX CRS-2 (March 2013) followed by return via CRS-3, and the second set of seedlings (termed SG2) was carried to the ISS on SpaceX CRS-4 (September 2014) and returned on CRS-5 (September 2015).

Spaceflight Procedures

Experimental containers (ECs) were sent to the ISS and loaded into the EMCS as previously described (Kiss et al., 2014; Vandenbrink and Kiss, 2016). Experimental conditions were controlled remotely from the Norwegian User Support and Operations Centre (N-USOC; Trondheim, Norway). The experiments were initiated via hydration of the seeds. Plants were grown in microgravity and 1.0-*g*.

Seedlings were illuminated under white light (30-40 µmol m^{-2} s^{-1}) for 96 h, followed by 48 hours of unidirectional photostimulation with red light, blue light, or a 1 hour red-light illumination followed by continuous blue-light illumination. Light sources were LEDs as previously described (Kiss et al., 2014). RNAseq analysis was only conducted on seedlings exposed to unidirectional blue light. After the conclusion of the experiments, seedlings were frozen and stored at -80°C in the General Laboratory Active Cryogenic ISS Experiment Refrigerator (GLACIER) freezer of the ISS. Upon return of frozen seedlings to Earth, samples were transported on dry ice, and promptly preserved with RNA*later*® (1.5 ml; ThermoFisher Scientific cat # AM7021) for subsequent RNAseq analysis.

RNA-Sequencing and Differential Gene Expression Analysis

RNA was extracted individually for each EC cassette for each of the samples. A plant specific RNA extraction NucleoSpin kit (MACHEREY-NAGEL, Catalog # 740949.250) including a DNase treatment was used to isolate whole plant mRNA. The quantity and quality of the extracted RNA was determined by a Nanodrop 2000 (Thermo Scientific). RNA remained frozen at -80C until delivery. Extracted RNA was shipped on dry ice to the David H. Murdoch Research Institute in Kannapolis, North Carolina, USA. During sequencing, pooled RNA samples were used to generate sequencing libraries using the Illumina TruSeq RNA Library Preparation Kit (Illumina, USA). Samples were individually indexed. The samples were then combined at equimolar proportions into three pools with 6 samples per pool. Each pool was loaded onto a single lane of a flow cell. A 125bp paired end sequencing run was performed on the Illumina HiSeq2500.

Paired-end 125bp reads were aligned to the Arabidopsis TAIR10 genome using the PBS-GEM workflow (https://github.com/wpoehlm/PBS-GEM) on the Clemson University Palmetto Cluster (Kim, Langmead, and Salzberg, 2015). Fragments with a Phred score below 33 were filtered using Trimmomatic (Trimmomatic, 2013). The program HISAT2 (*v2.1.0*) was used to align sequencing reads to

138 the reference genome using a minimum intron length of 60 and a maximum intron length of 2000.
139 Annotated reference gene abundances were quantified using StringTie (*v1.3.4*). Annotated reference
140 genes were identified using the TAIR10 genome GFF3 annotation file (www.arabidopsis.org).
141 Statistical analysis of differential gene expression was conducted with DESeq2 (*v1.18.1*; Anders and
142 Huber, 2010). A multiple-test corrected p-value (q-value; Benjamini and Hochberg; 1995) of 0.05 was
143 employed. The samples were pooled to reduce the *g*-level interval within biological replicates so the
144 following groups were established: microgravity (4 replicates) and 1*g* control (0.99 ± 0.06g, 3 replicates).
145 Genes identified as differentially expressed between the µ*g* and 1*g* condition were subsequently used
146 for Generally Applicable Gene-set Enrichment (GAGE) for Pathway Analysis to identify genetic pathways
147 enriched for differentially expressed genes (Luo et al., 2009). Lastly, gene ontology annotation of
148 differentially expressed genes (µ*g* vs 1*g and low g* vs 1*g*) was conducted utilizing Protein ANalysis
149 THrough Evolutionary Relationships (PANTHER) Classification System version 13.1
150 (http://www.pantherdb.org; Thomas et al., 2003; Mi et al., 2012). The PANTHER statistical
151 overrepresentation test was performed using the default settings.
152
153 **3. Results**
154 ***Identification of Differentially Expressed Genes (DEGs)***
155 We performed transcriptomic analysis of *Arabidopsis thaliana* seedlings that were grown on the
156 International Space Station (Figure 1). Dry seeds were hydrated to initiate our spaceflight experiment as
157 previously described (Kiss, 2015). Differential expression analysis was conducted via DESeq2 (Anders and
158 Huber, 2010) between µ*g* and Earth's gravity (1*g*), using 1*g* as the reference group. A q-value false-
159 discovery rate (Benjamini and Hochberg) of 0.05 was used to identify differentially expressed genes
160 (DEGs). Comparison between µ*g* and 1*g* revealed 296 differentially expressed genes significantly
161 differentially expressed between microgravity and Earth's 1*g* (Supplemental Table 1).

Enrichment analysis of gene ontology caused by microgravity

After identification of differentially expressed genes, a Generally Applicable Gene-Set (GAGE)-Pathway Analysis was conducted between µ*g* and 1*g* gravity conditions to determine if any molecular pathways were found to be significantly enriched with genes (identified as differentially expressed) to give a more holistic view of the effects of reduced gravity on plant molecular pathways (Luo et al., 2009). GAGE analysis revealed that 8 pathways were found to be significantly enriched (2 upregulated, 6 downregulated; FDR < 0.1) with genes identified as differentially expressed in the µ*g* vs 1*g* group (Table 1). These pathways included photosynthesis (Figure 2) and photosynthetic antenna proteins (Figure 3) as being significantly downregulated. In addition, porphyrin and chlorophyll metabolism, starch and sucrose metabolism, carotenoid biosynthesis, and protein processing in the endoplasmic reticulum were all shown to be downregulated (Supplemental Figures S1-S4). Only two pathways, ribosome synthesis and oxidative phosphorylation were found to be upregulated (Supplemental Figures S5 & S6).

Gene Ontology classifications of genes identified as differentially expressed between µ*g* and 1*g* was conducted via PANTHER (http://www.pantherdb.org; Thomas et al., 2003; Mi et al., 2012). The statistical overrepresentation test identified 4 molecular processes as over-represented in the list of differentially expressed (DE) genes. In addition, analysis revealed 6 biological processes, 5 protein classes, and 5 cellular components showing statistical overrepresentation (Table 2). In regard to molecular function, catalytic activity accounted for 42% of genes differentially expressed, while binding and structural molecular activity accounted for 25% and 19% respectively. Smaller contributing ontologies included receptor activity (2.0%), signal transducer activity (1.0%) and translation regulation activity (1.0%). Further division into categories of these ontologies is provided in Figure 4.

The ontological breakdown of biological processes affected by microgravity was also conducted. Metabolic processes and cellular processes each accounted for 37.8% of gene classifications, accounting for the majority of genes identified as differentially expressed (Figure 5). This was followed by response to stimulus (9.2%), cellular component organization of biogenesis (7.2%), localization (4.1%), biological replication (2.0%) and single-multicellular organismal processes and death at 1% respectively. Further categorization of gene ontology classifications for cellular component and protein class are shown in supplemental figures S7 & S8.

4. Discussion

Image analysis of seedlings grown during the Seedling Growth suite of experiments previously characterized a novel blue-light phototropic response in roots of *Arabidopsis thaliana* grown in conditions of microgravity (Vandenbrink et al., 2016). This relationship was shown to be linearly related to the magnitude of the gravity vector. To achieve a better understanding of the molecular mechanisms that underlying this unique physiological response, RNA-seq analysis was done to characterize changes in gene expression that may be associated with this novel phototropic response. Interestingly, GAGE-Pathview results indicated that genes constituting major pathways associated with light perception, photosynthesis and biosynthesis of the photosynthetic complexes showed reduced expression in conditions of microgravity.

Photosynthesis and Antenna Proteins

The two pathways which show the greatest statistical significance for down-regulation in this study were the photosynthesis – antenna protein pathway and the photosynthesis pathway. Acclimation to changing light conditions is achieved through changes in expression to the antenna protein genes (Masuda and Fujita, 2008). Antenna proteins, which aid in plant acclimation to different light

environments, had all but one light-harvesting chlorophyll protein complex (LHC) gene significantly downregulated (Supplemental Figure S1). In regard to the photosynthesis pathway, there was significant downregulation of genes associated with photosystem I and photosystem II (Figure 3). This observation confirms a previous study which detailed an overall reduction in photosystem I (PSI) complexes, as well as a 30% reduction in photochemical activity on *Brassica rapa* plants grown aboard the space shuttle (Jiao et al., 2004). The reduction in photosystem I was accompanied by an overall reduction in biomass of the samples, correlating with a reduction in photosynthetic processes. The observed reduction in photosystem I activity was supported by a study which grew *Oryza sativa* on a Random Positioning Machine (RPM) and detailed a reduction in PSI activity which was attributed to an overall reduction in the biosynthesis of PSI proteins (Chen et al., 2013).

In our present study, four genes associated with the photosynthetic electron transport (petE - plastocyanin, petF - ferredoxin, petH -ferredoxin--NADP+ reductase, petJ - cytochrome c6) showed significant downregulation. This observation suggests that there is an interaction between gravity perception and light harvesting machinery at a genetic level. This proposed interaction between gravity and light-related pathways may help explain the novel blue-light phototropic response observed in the roots of *Arabidopsis* grown in microgravity (Vandenbrink et al., 2016).

Chlorophyll Metabolism and Chloroplast Function

In addition to photosynthesis pathways, multiple pathways that are associated with light perception and photosynthesis showed significant down-regulation. The porphyrin and chlorophyll metabolism pathway, which is responsible for the biosynthesis of chlorophyll pigment, also shows significant down-regulation of genes (Supplemental Figure S2). Control of chlorophyll metabolism has been shown to be regulated by phytohormones, environmental signals, organ specificity, developmental

stage, gene expression, proteolysis, among others (Masuda and Fujita, 2008). However, the relationship between gravity and chlorophyll biosynthesis is poorly understood.

When analyzing gene expression patterns, we found significant down-regulation observed in the genes responsible for heme biosynthesis (*HemA*, *HemL*, *HemB*, *HemC*, *HemD*, *HemE*, *HemF* and *HemH*). These heme series of proteins are important for the biosynthesis of chlorophylls. In addition to the heme proteins, multiple enzymes associated with the final processing of chlorophyll a and chlorophyll b, such as chlorophyll b reductase (Kegg # 1.1.1.294) and chlorophyll synthase (Kegg # 2.5.1.62), show downregulation (Supplemental Figure S2). This observation suggests that reduction of gravity from 1*g* to μ*g* potentially reduces the ability of the plant to synthesis mature chlorophyll.

In addition to down-regulation of genes associated with the production of chlorophyll, chloroplasts of plants grown in conditions of microgravity show significant structural changes. These changes include more ovoid shaped chloroplasts than plants grown in 1*g*, and the thylakoid membranes of space-grown plants exhibited denser packing, with each of the membranes roughly 0.9 nm closer than those plants grown in 1*g* (Stutte et al., 2006). In addition, a study of clinorotated *Arabidopsis* plants showed an alteration in chloroplasts, as the number of grana per chloroplast was reduced as was the overall size of the organelle (Adamchuk, 1998). An increase in proton permeability of the thylakoid membrane was also observed in seedlings grown on a 2-D clinostat (Mikhaylenko, Sytnik, and Zolotareva, 2001). Taken together, these observations suggest that the synthesis of chlorophyll and the formation of plant chloroplasts are significantly altered in conditions of microgravity.

Previous spaceflight research has considered the effects of microgravity environments on the biosynthesis of chlorophyll. *Chlorella*, single-celled photosynthetic algae, showed alterations to their thylakoid membrane as well as a 35-50% decrease in chlorophyll content (Moleshko et al., 1991). Similarly, the chlorophyll content of wheat grown in microgravity for 19 days showed a reduction in chlorophyll content (Rumyantseva et al., 1990). The reduction in chlorophyll content was also observed

in pea plants grown in space (Laurinavichius et al., 1986). In addition to spaceflight studies, experiments utilizing simulated microgravity devices, such as clinostats and random positioning machines (RPMs), have also shown a decrease in chlorophyll content (Miyamoto et al., 2001). Also, Yamada et al. (1993) predicted that carbon metabolism was affected by microgravity when it was observed that starch granules in chloroplasts were reduced in clinorotated plants.

The Effects of Experimental Hardware on Photosynthetic Rates

There have been multiple studies of photosynthetic rates in plants exposed to microgravity, which have produced a wide range of results. Numerous reports (detailed above) have reported reductions in photosynthetic apparatuses, photosynthetic rates, chloroplast function and morphology, among others. However, in contrast, other studies have indicated no changes in chloroplast density (Stutte et al., 2006), chloroplast structure (Musgrave et al., 1998), or photosynthetic rate (Stutte et al., 2005) to name a few. When comparing results of these experiments, it is important to acknowledge the existence of spaceflight hardware effects when interpreting the results. There are multiple stresses and environmental stimuli that are encountered when growing plants in microgravity (Kiss, 2015). These include (but are not limited to) lack of convection, reduced CO_2 levels, improper temperature, elevated ethylene, spacecraft vibrations, increased radiation exposure, among others. Hardware exists that strive to mitigate many of these environmental factors that are present on the International Space Station, but no perfect hardware exists for the growth of plants in space.

A study conducted by Stutte et al. (2006) grew plants in a facility (the Biomass Production System, BPS) that aimed to limit the confounding hardware effects present in some spaceflight studies such as lack of convection, improper lighting, reduced CO_2 levels, temperature fluctuation, low humidity, and elevated ethylene. In BPS, they found no apparent changes in photosynthetic rate, and attributed previous findings of photosynthetic reduction in a microgravity environment to improper ventilation.

However, our present study was conducted utilizing the European Modular Cultivation System, which contains an air scrubbing/filtration system designed at removing excess ethylene from the seedlings during the growth phase (Kiss et al., 2014; Kiss, 2015). Thus, even with proper ventilation of plants grown in space, a reduction in gene expression of photosynthetic genes was observed.

Interestingly, there have been a few studies that have shown an increase in chlorophyll content in space-grown pea plants (Abilov, 1986; Aliyev, 1987). This increase in chlorophyll content was also observed in clinorotated rice seedlings (Jagtap et al., 2011). However, these studies all observed this increased chlorophyll content in young seedlings. Jagtap et al. (2011) noted that the increase in chlorophyll content in clinorotated rice seedlings is observed up to 5 days, and then, the trend was reversed, and chlorophyll content started to decrease. This observation suggests a temporal component to the effects of microgravity on chlorophyll production, with longer durations causing a decrease in chlorophyll biosynthesis.

Starch and Sucrose Metabolism

The relationship between starch and sucrose metabolism and conditions of microgravity has been explored in previous space flight and space flight analog studies. For example, soybean seedlings grown for 6 days under simulated microgravity conditions (clinorotation) were shown to have a decrease in starch concentration in cotyledons (Brown and Piastuch, 1994). In addition, these scientists found that ADP-glucose pyrophosphorylase had reduced enzymatic activity. This observation corresponds with our own results, which show a down regulation of ADP-glucose pyrophosphorylase in the starch and sucrose metabolism pathway (Supplementary Figure S3). In addition, wheat leaves grown in conditions of altered gravity (clinorotation) were shown to have reduced sucrose and starch accumulation in leaves. However, this study did not observe a reduction in ADP-glucose pyrophosphorylase activity (Obenland and Brown, 1994).

In contrast to the above reports, sweet potato stem cuttings flown in the Space Shuttle for 5 days showed substantially greater accumulation of soluble sugars, glucose, fructose and sucrose as well as total starch concentration. The space-flown sweet potato were exposed to ~50% higher concentrations of CO_2, which likely would account for the increase in sugar accumulation (Mortley et al., 2008). Similarly, soybean and potato plants grown for 5 days onboard the Space Shuttle observed compositional changes in starch granules, however, changes in starch content were attributed to ethylene effects (Kuznetsov et al., 2001). In addition, the reported ethylene effects were not observed in the sweet potato study (Mortley et al., 2008) nor in the present study, which both used methods of ethylene removal. Thus, reduction in starch and sucrose metabolism may be species or tissue specific.

Carotenoid Biosynthesis

The carotenoid pathway also is associated with photosynthesis and light perception (Supplementary Figure S4). Carotenoids have been shown to be linked to chlorophyll production, where carotenoid content is increased in conjunction with chlorophyll biosynthesis (Howitt and Pogson, 2006). In our present spaceflight experiment, we observed a reduction in xanthophyll production of carotenoid biosynthesis, which are essential components of the plant photosynthetic apparatus (Lokstein et al., 2002). Xanthophylls are important for the formation of stable pigment-protein complexes (Paulsen, 1995), as well as act as ancillary light-harvesting pigments (Siefermann-Harms, 1987). In addition, multiple genes associated with the lutein biosynthesis arm of the carotenoid pathway are shown to be downregulated. Lutein has been shown to be the predominant carotenoid in photosynthetic tissue, playing a large role in the bulk antenna complex, or LHC II (Pogson et al., 1996). In addition, lutein has been shown to optimize antenna structure and organization to increase the efficiency of light harvesting (Lokstein et al., 2002).

Carotenoids, the second most abundant pigment in nature, play an important protective role in light sensing. For instance, these photoreceptors act as photoprotective compounds which quench triplet chlorophyll and radical oxygen species derived from excess light absorption (Demmig-Adams, Gilmore, and Adams, 1996). This nonphotochemical quenching also prevents damage to the thylakoid membrane, to which the chlorophyll and carotenoids are bound (Niyogi, 1999). Previous research on spaceflight-flown photosynthetic organisms have observed reduction in carotenoid content in organisms. For instance one study observed a 50% reduction in carotenoid contents of the alga *Chlorella* in space (Moleshko et al., 1991), while others observed a similar reduction in carotenoid content of maize flown aboard the space station *Mir* (Rumyantseva et al., 1990). These past studies further support our current findings of a reduction in gene expression associated with carotenoid biosynthesis in space-grown seedlings of *Arabidopsis*.

Ribosome Biogenesis and Oxidative Phosphorylation

In contrast with the down-regulation of phototropism and related biosynthesis and metabolic pathways, we have found two very clearly up-regulated functions in microgravity-grown seedlings when compared to 1*g* spaceflight samples, namely ribosome biosynthesis and oxidative phosphorylation (Supplemental Figure S6 and S7). Previous research using both true microgravity (Matia et al., 2010) as well as different simulated microgravity facilities using both seedlings and cell cultures (Manzano et al., 2013; Kamal et al., 2018), have shown that ribosome biogenesis was reduced in conditions of microgravity. Our recent spaceflight results shown that red light can compensate this effect (Valbuena et al., 2018) , particularly increasing cell growth (measured by means of ribosome biosynthesis in the nucleolus) that was depleted without light stimulation. The results of our present space studies support that removing the gravitropic stimuli in combination with photostimulation can lead to higher levels of protein production and metabolism rates (oxidative phosphorylation).

Conclusions

The results of this study help to detail the intricacies of interactions between gravitropic and phototropic responses. Removal of the influence of gravity on blue-light-illuminated seedlings showed a reduction in gene expression in multiple pathways associated with photosynthesis, suggesting shared molecular pathways between the two tropistic responses. In addition, pathways previously associated with light perception and response, such as carotenoid biosynthesis and starch metabolisms, were also identified as being down-regulated. Analysis of the affected gene ontologies revealed that catalytic activity and binding activity were the most significantly affected molecular functions, while cellular processes and metabolic processes were the most significantly affected biological processes. These findings in concert suggest an intricate connection between gravity and light perception in *Arabidopsis thaliana*.

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511 Tables

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Table 1: GAGE-Pathview overrepresentation test of genes identified as differentially expressed between μ*g* and 1*g* gravity conditions.

Pathway	KEGG Pathway Identifier[1]	Gene Set Size	Geometric Mean[2]	Stat Mean[3]	p-value	FDR[4]
Upregulatd Pathways						
Ribosome	ath03010	307	2.34E-25	8.08	2.34E-15	2.62E-13
Oxidative phosphorylation	ath00190	120	1.73E-03	2.95	1.73E-03	9.80E-02
Downregulated Pathways						
Photosynthesis - antenna proteins	ath00196	22	1.42E-05	-4.70	1.42E-05	1.61E-03
Photosynthesis	ath00195	45	2.08E-03	-2.98	2.08E-03	5.87E-02
Porphyrin and chlorophyll metabolism	ath00860	51	1.97E-03	-2.96	1.97E-03	5.87E-02
Protein processing in endoplasmic reticulum	ath04141	190	4.37E-03	-2.64	4.37E-03	8.23E-02
Starch and sucrose metabolism	ath00500	140	1.18E-03	-3.07	1.18E-03	5.87E-02
Carotenoid biosynthesis	ath00906	27	2.86E-03	-2.88	2.86E-03	6.46E-02

[1] Kyoto Encyclopedia of Genes and Genomes; [2]geometric mean of the individual p-values from multiple single array based gene set tests; [3]mean of the individual statistics from multiple single array based gene set tests; [4]FDR q-value adjustment of the global p-value using the Benjamini & Hochberg procedure

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Table 2: PANTHER overrepresentation test of genes identified as differentially expressed between μ*g* and 1*g* gravity conditions.

PANTHER Category	number of reference genes[1]	number of mapped genes[2]	expected number of genes[3]	Fold Enrichment[4]	+/-[5]	p-value[6]	FDR[7]
Biological Process							
generation of precursor metabolites and energy	263	21	2.89	7.27	+	6.61E-12	1.24E-09
oxidative phosphorylation	58	10	0.64	15.7	+	3.28E-09	3.09E-07
respiratory electron transport chain	153	13	1.68	7.74	+	3.70E-08	2.32E-06
response to abiotic stimulus	175	13	1.92	6.76	+	1.60E-07	7.51E-06
translation	308	14	3.38	4.14	+	1.32E-05	4.95E-04
biosynthetic process	2090	41	22.95	1.79	+	4.26E-04	1.33E-02
cation transport	67	5	0.74	6.8	+	1.13E-03	3.03E-02
Protein Class							
ribosomal protein	322	27	3.54	7.64	+	1.90E-15	3.35E-13
RNA binding protein	1115	33	12.24	2.7	+	4.03E-07	3.54E-05
nucleic acid binding	1771	38	19.45	1.95	+	9.01E-05	3.96E-03
ligand-gated ion channel	11	3	0.12	24.84	+	4.23E-04	1.49E-02
winged helix/forkhead transcription factor	31	4	0.34	11.75	+	5.48E-04	1.61E-02
anion channel	16	3	0.18	17.07	+	1.08E-03	2.38E-02
ATP synthase	47	4	0.52	7.75	+	2.28E-03	4.46E-02
ion channel	57	3	0.63	4.79	+	2.76E-02	4.04E-01
helix-turn-helix transcription factor	161	5	1.77	2.83	+	3.53E-02	4.78E-01
cation transporter	140	4	1.54	2.6	+	7.24E-02	8.49E-01
transporter	953	6	10.46	0.57	-	2.03E-01	1.70E+00
receptor	74	1	0.81	1.23	+	5.60E-01	2.19E+00
transcription factor	690	8	7.58	1.06	+	8.52E-01	2.42E+00
Cellular Component							
ribosome	325	23	3.57	6.44	+	6.44E-12	3.22E-10
macromolecular complex	1919	52	21.07	2.47	+	2.46E-09	6.16E-08
ribonucleoprotein complex	643	25	7.06	3.54	+	9.64E-08	1.61E-06
mitochondrial inner membrane	128	11	1.41	7.83	+	3.77E-07	4.72E-06
proton-transporting ATP synthase complex	25	5	0.27	18.21	+	1.67E-05	1.67E-04
protein complex	1375	29	15.1	1.92	+	8.61E-04	7.17E-03
cytoplasm	3618	60	39.73	1.51	+	1.12E-03	7.99E-03
cytosol	776	18	8.52	2.11	+	4.35E-03	2.72E-02
intracellular	5893	85	64.71	1.31	+	5.94E-03	3.30E-02
membrane	2139	37	23.49	1.58	+	6.71E-03	3.36E-02
cell part	6166	87	67.71	1.28	+	1.02E-02	4.25E-02
thylakoid	46	2	0.51	3.96	+	9.58E-02	3.68E-01
organelle	4563	59	50.11	1.18	+	1.86E-01	6.64E-01
mitochondrion	417	3	4.58	0.66	-	6.35E-01	1.18E+00
Molecular Function							
structural constituent of ribosome	264	25	2.9	8.62	+	1.66E-15	2.60E-13
structural molecule activity	530	28	5.82	4.81	+	2.28E-11	1.79E-09
proton-transporting ATP synthase activity, rotationa	25	5	0.27	18.21	+	1.67E-05	6.55E-04
hydrogen ion transmembrane transporter activity	102	8	1.12	7.14	+	2.77E-05	8.71E-04
ligand-gated ion channel activity	10	3	0.11	27.32	+	3.35E-04	8.76E-03
anion channel activity	77	5	0.85	5.91	+	2.02E-03	4.52E-02
ion channel activity	93	5	1.02	4.9	+	4.36E-03	8.57E-02
transmembrane transporter activity	858	11	9.42	1.17	+	6.15E-01	1.97E+00
transporter activity	996	11	10.94	1.01	+	8.78E-01	2.03E+00

[1] number of genes in reference genome mapped to the annotation category; [2] number of identified DE genes mapped to annotation category; [3] number og DE genes predicted to be in annotation category; [4] Fold Enrichement of genes identified as DE (# of DE genes/expected); [5] overrepresentation (+) or underrepresntation (-) when compared to expected; [6] p-value determined by Fisher's exact test; [7] False Discovery Rate determined by Benjamini-Hochberg Test.

Figure Legends

Fig. 1: Images of seedlings of *Arabidopsis thaliana* growing on the International Space Station. A) image of 5 seedling cassettes positioned within the European Modular Cultivation System (EMCS). B) Higher magnification view of seedlings growing within a seed cassette.

Fig. 2: Photosynthesis pathview of differentially expressed genes identified in conditions of microgravity when compared to 1*g* control. Differentially expressed genes were identified using the HISAT2-Stringtie-DESeq analysis pathway (p = 5.87E-02). Genes highlighted with green indicate reduced expression when compared to 1*g* control.

Fig. 3: Photosynthesis – Antenna Proteins pathview of differentially expressed genes identified in conditions of microgravity when compared to 1*g* control. Gene were identified using the HISAT2-Stringtie-DESeq analysis pathway (p = 1.61E-03). Genes highlighted with green indicate reduced expression when compared to 1*g* control.

Fig. 4: PANTHER Molecular Function of Differentially Expressed Genes. Classification of the functions that proteins identified from differentially expressed genes perform on its direct molecular target (µ*g* vs 1*g*).

Fig. 5: PANTHER Biological Process of Differentially Expressed Genes. Classification of biological systems that identified differentially expressed genes belong to (µ*g* vs 1*g*).

Fig. S1: Porphyrin and Chlorophyll Metabolism pathview of differentially expressed genes identified from HISAT2-Stringtie-DESeq. p = 5.87E-02

Fig. S2: Starch and Sucrose Metabolism pathview of differentially expressed genes identified from HISAT2-Stringtie-DESeq. p = 5.87E-02

Fig. S3: Carotenoid Biosynthesis pathview of differentially expressed genes identified from HISAT2-Stringtie-DESeq. p = 4.99E-09

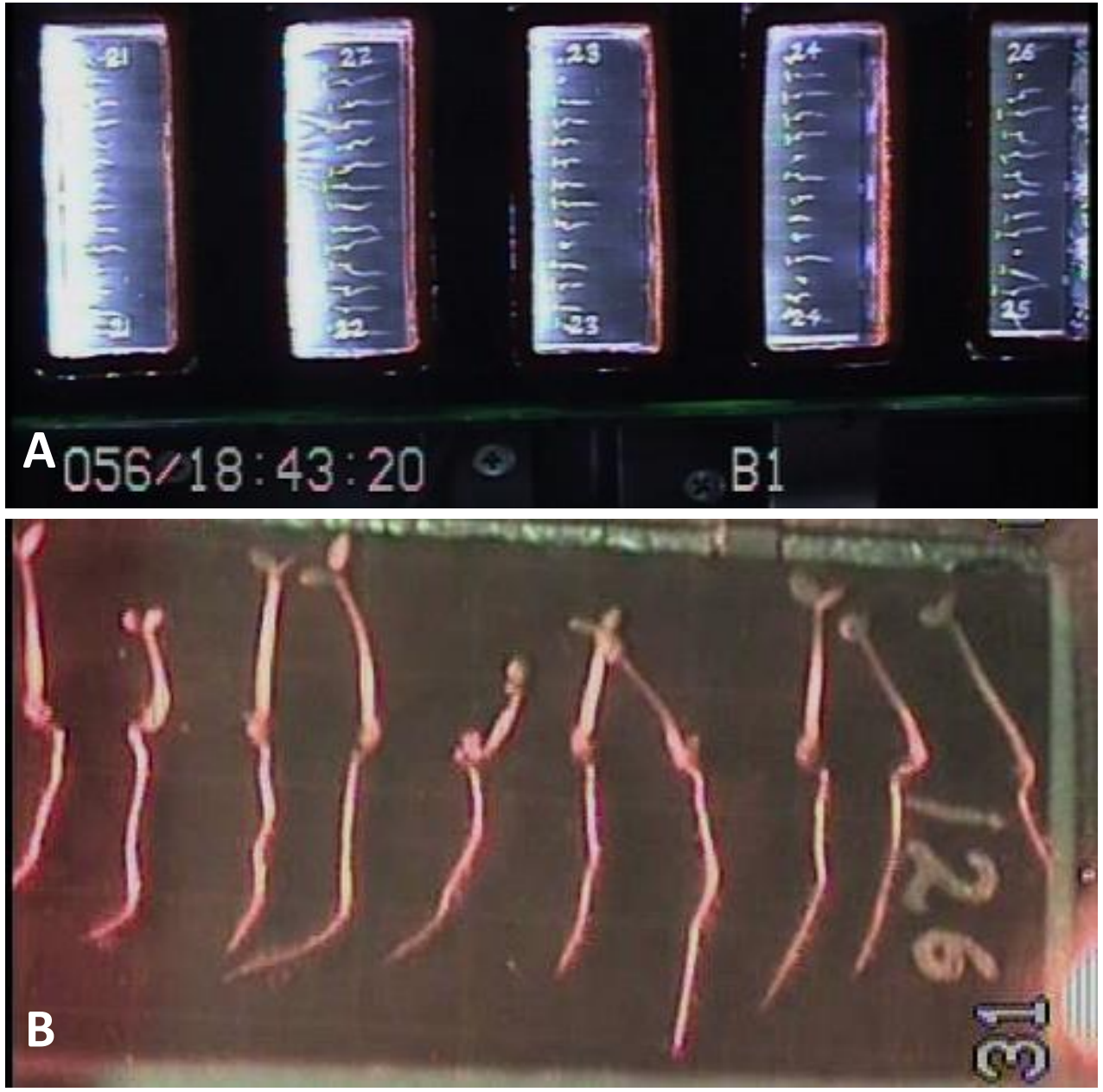
Fig. S4: Protein Processing in the Endoplasmic Reticulum pathview of differentially expressed genes identified from HISAT2-Stringtie-DESeq. p = 8.23E-02

Fig. S5: Ribosome pathview of differentially expressed genes identified from HISAT2-Stringtie-DESeq. p = 2.62E-13

Fig. S6: Oxidative Phosphorylation pathview of differentially expressed genes identified from HISAT2-Stringtie-DESeq. p = 9.80E-02

Fig. S7: PANTHER Cellular Location of Differentially Expressed Genes. Cellular localization of the protein products derived form genes identified as differentially expressed in conditions of microgravity.

Fig. S8: PANTHER Protein Class of Differentially Expressed Genes. Ontological classification of protein products derived from genes identified as differentially expressed in conditions of microgravity.



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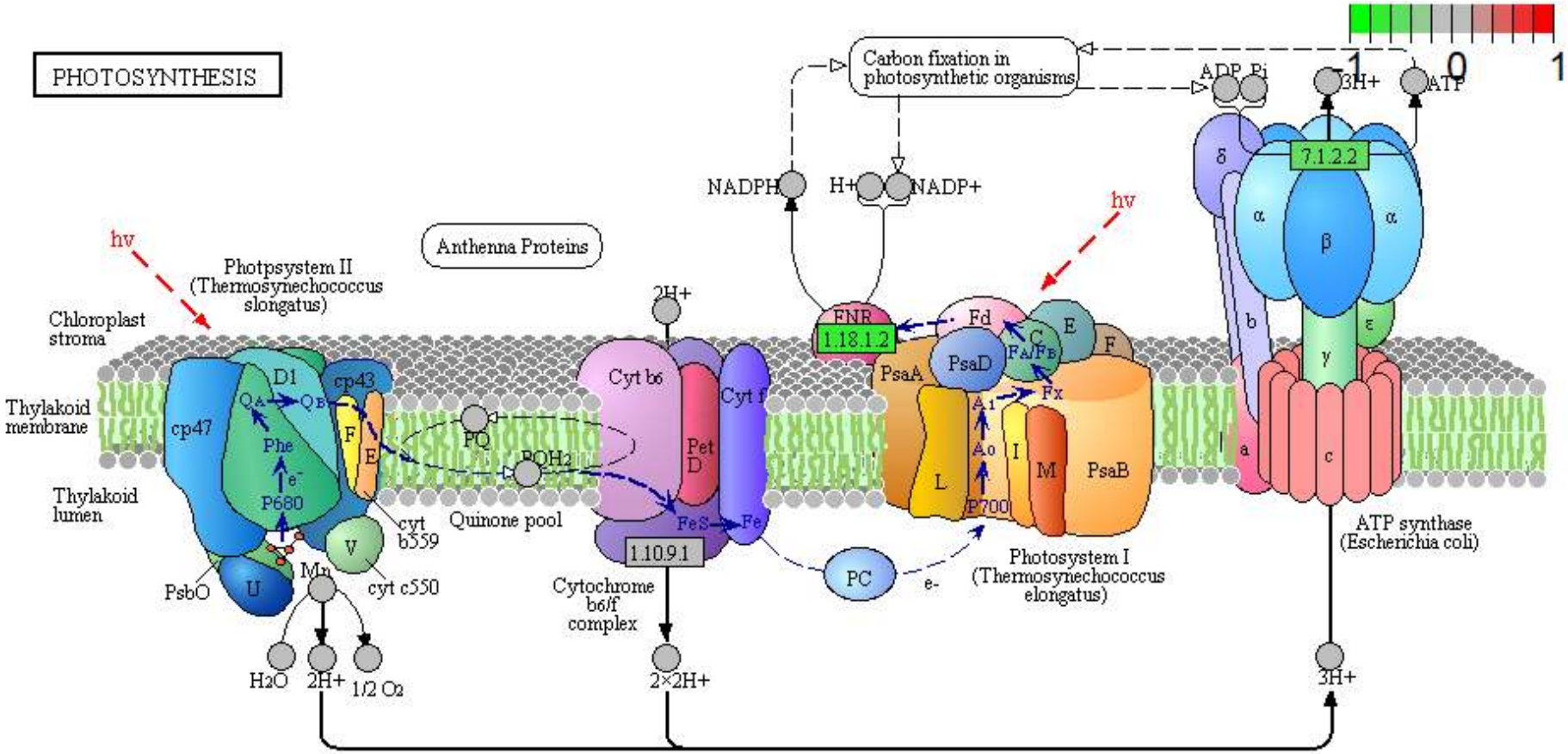


Photosystem II

D1	D2	cp43	cp47	cyt b559			
PsbA	PsbD	PsbC	PsbB	PsbE	PsbF		
						MSP	OEC
PsbL	PsbJ	PsbK	PsbM	PsbH	PsbI	PsbO	PsbP
PsbQ	PsbR	PsbS	PsbT	PsbU	PsbV	PsbW	PsbX
PsbY	PsbZ	Psb27	Psb28	Psb28-2			

Cytochrome b6/f complex

PetB	PetD	PetA	PetC	PetL	PetM	PetN	PetG

Photosynthethic electron transport

PC	Fd	FNR	cyt c6
PetE	PetF	PetH	PetJ

Photosystem I

PsaA	PsaB	PsaC	PsaD	PsaE	PsaF	PsaG	PsaH
PsaI	PsaJ	PsaK	PsaL	PsaM	PsaN	PsaO	PsaX

F-type ATPase

beta	alpha	gamma	delta	epsilon	c	a	b

Fig. 2: Photosynthesis pathview of differentially expressed genes identified from HISAT2-Stringtie-DESeq (p = 5.87E-02). Genes highlighted with green indicate reduced expression when compared to 1*g* control.

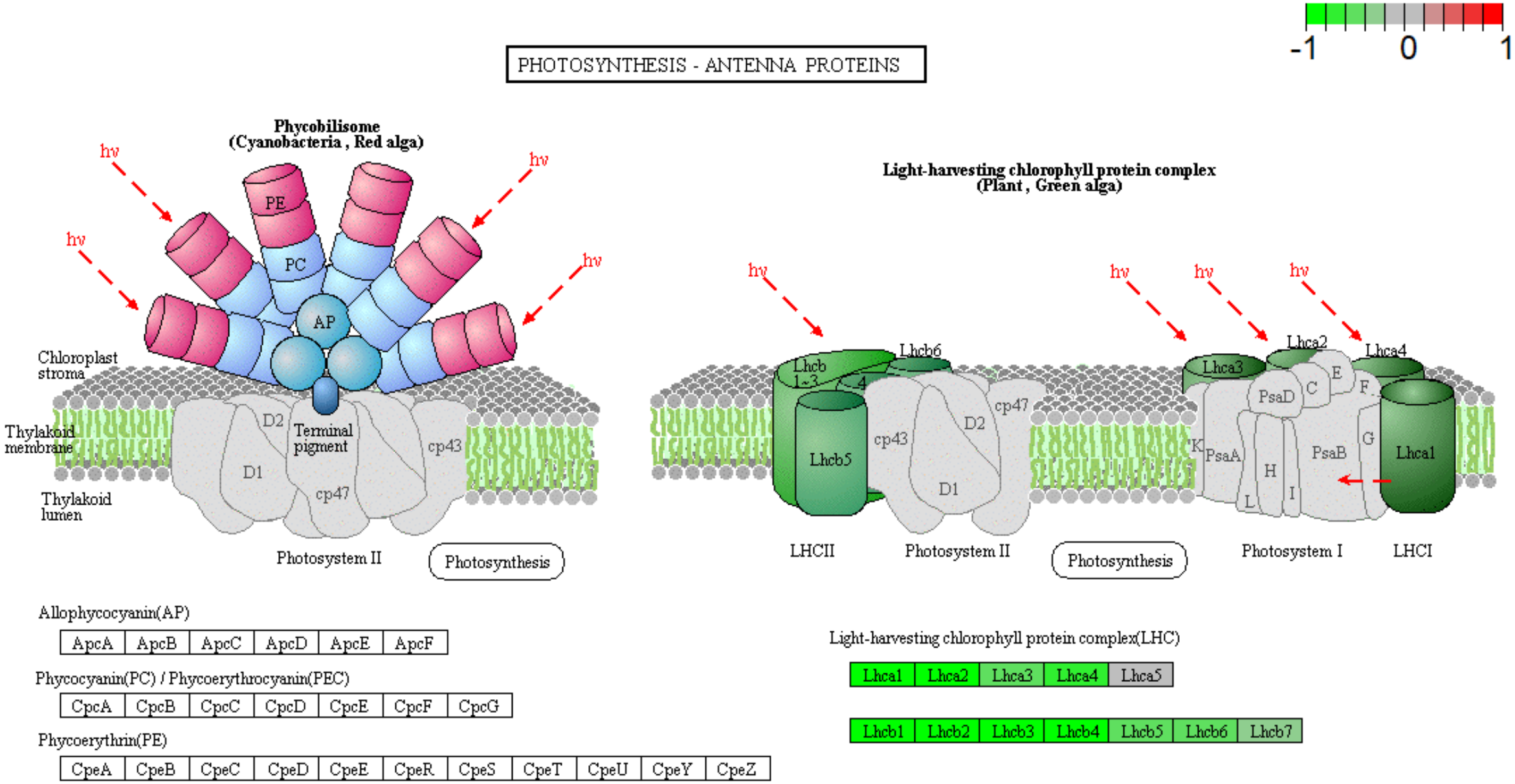
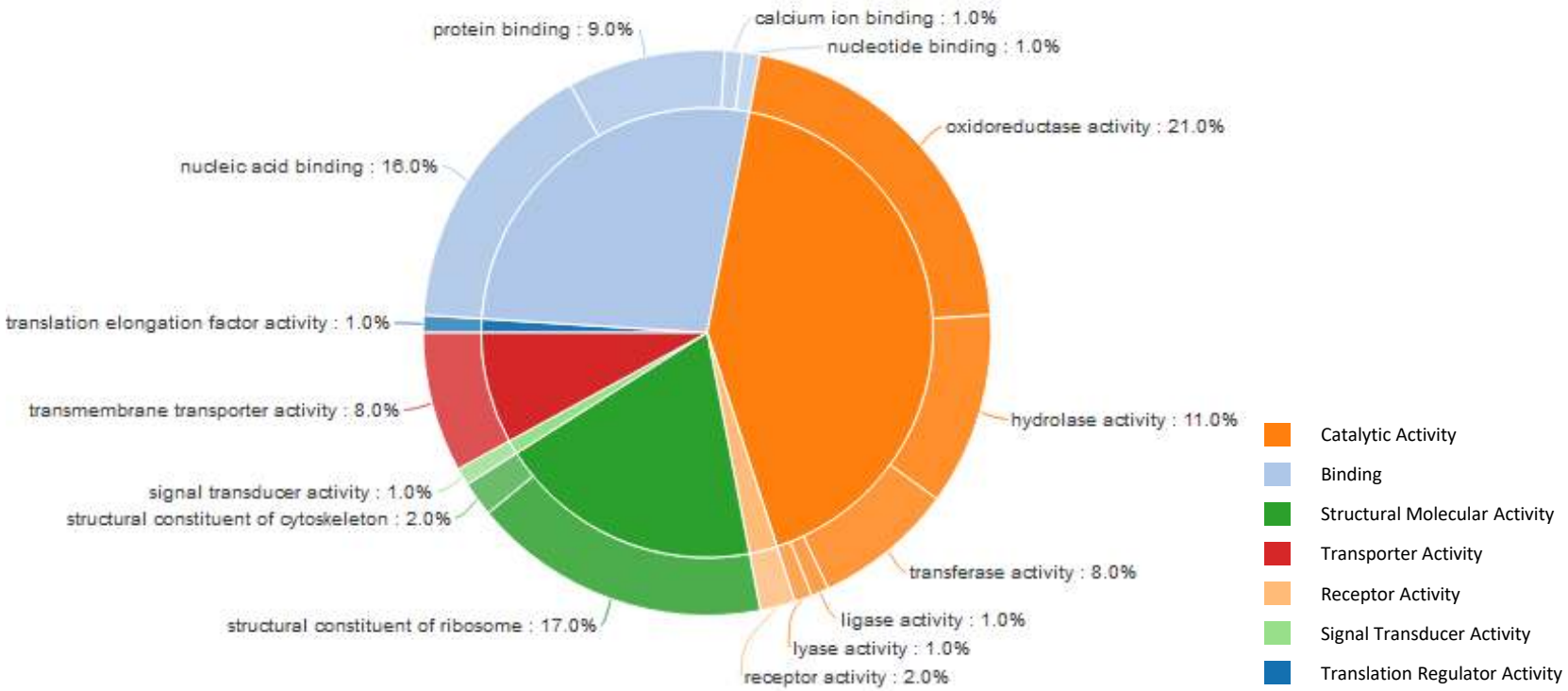


Fig. 3: Photosynthesis – Antenna Proteins pathview printout of differentially expressed genes identified from HISAT2-Stringtie-DESeq. p = 1.61E-03



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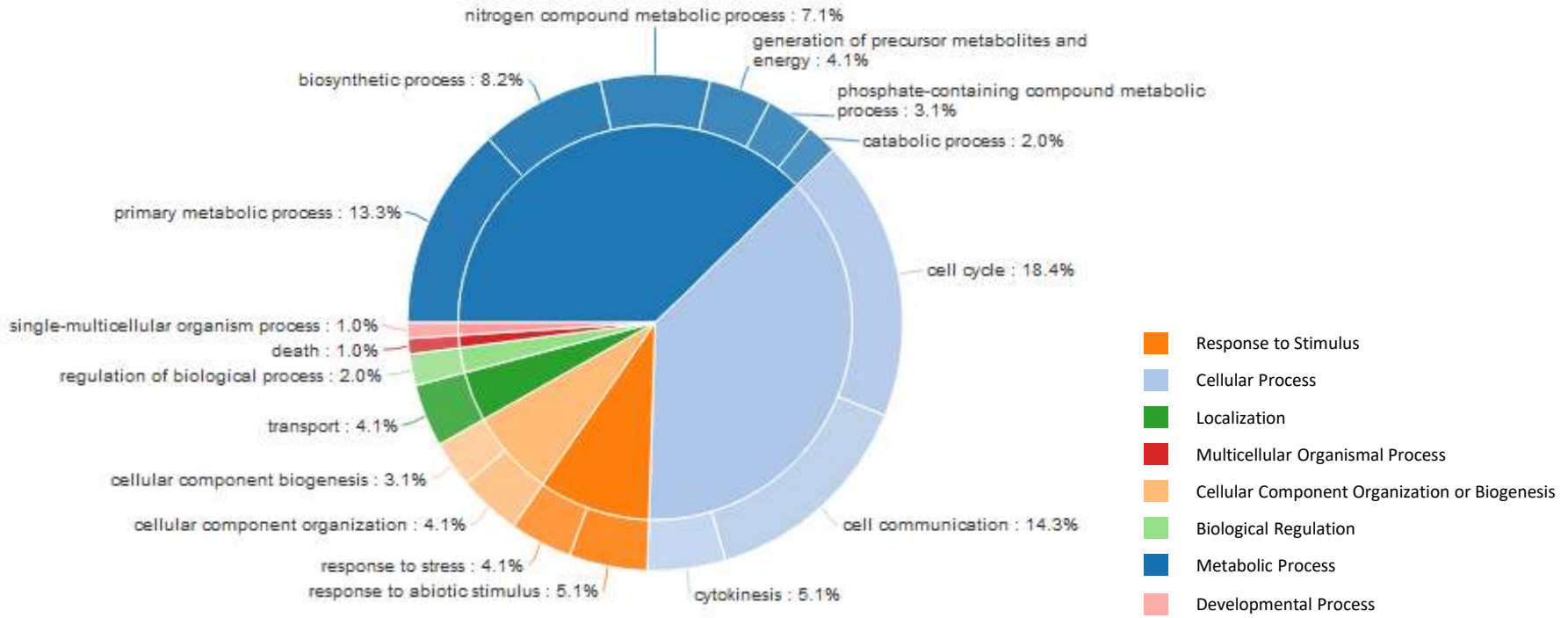


Fig. 5: PANTHER Biological Process of Differentially Expressed Genes. Classification of biological systems that identified differentially expressed genes belong to (µ*g* vs 1*g*).

Supplementary Figures

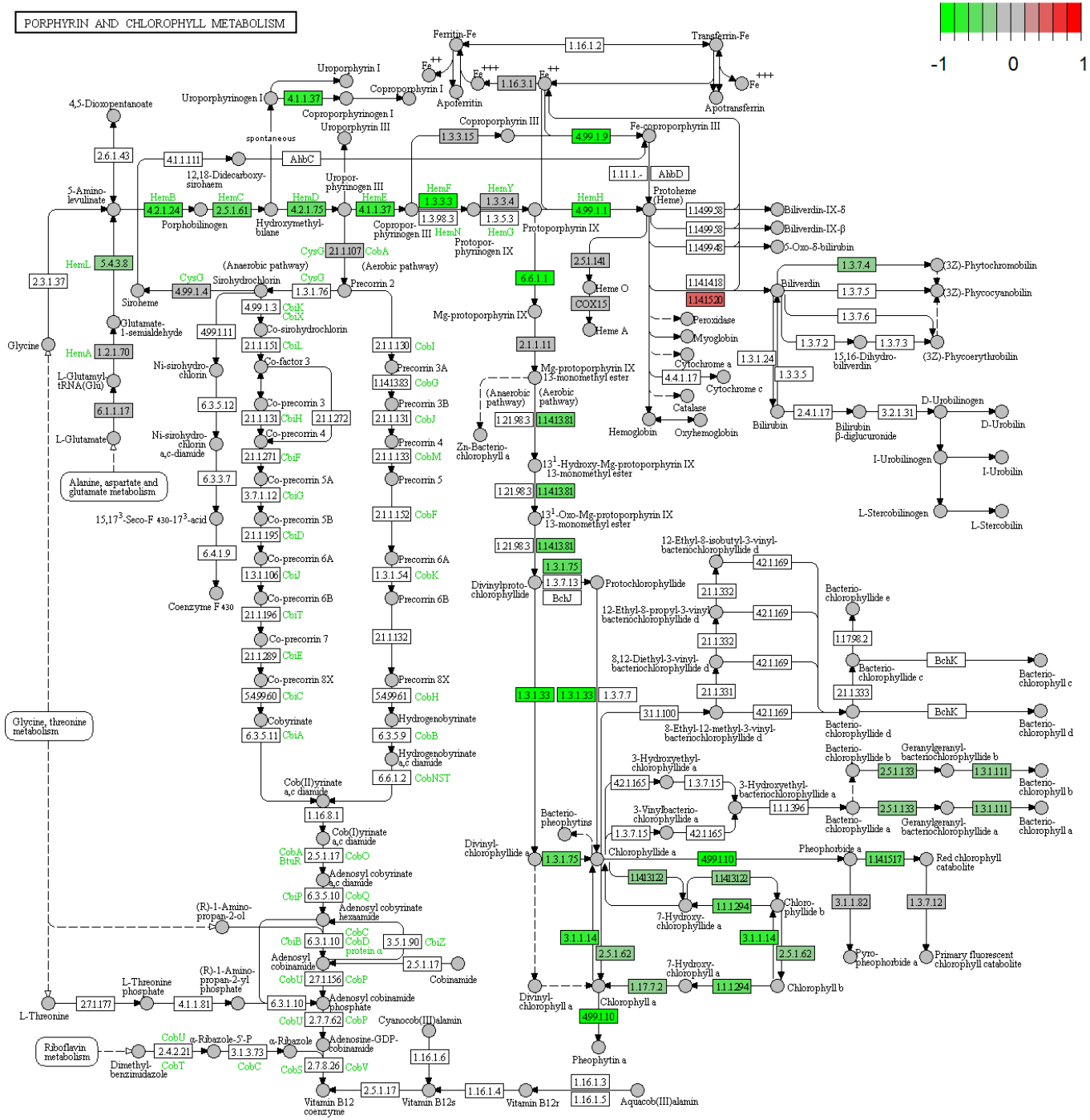


Fig. S1: Porphyrin and Chlorophyll Metabolism pathview of differentially expressed genes identified from HISAT2-Stringtie-DESeq.
p = 5.87E-02

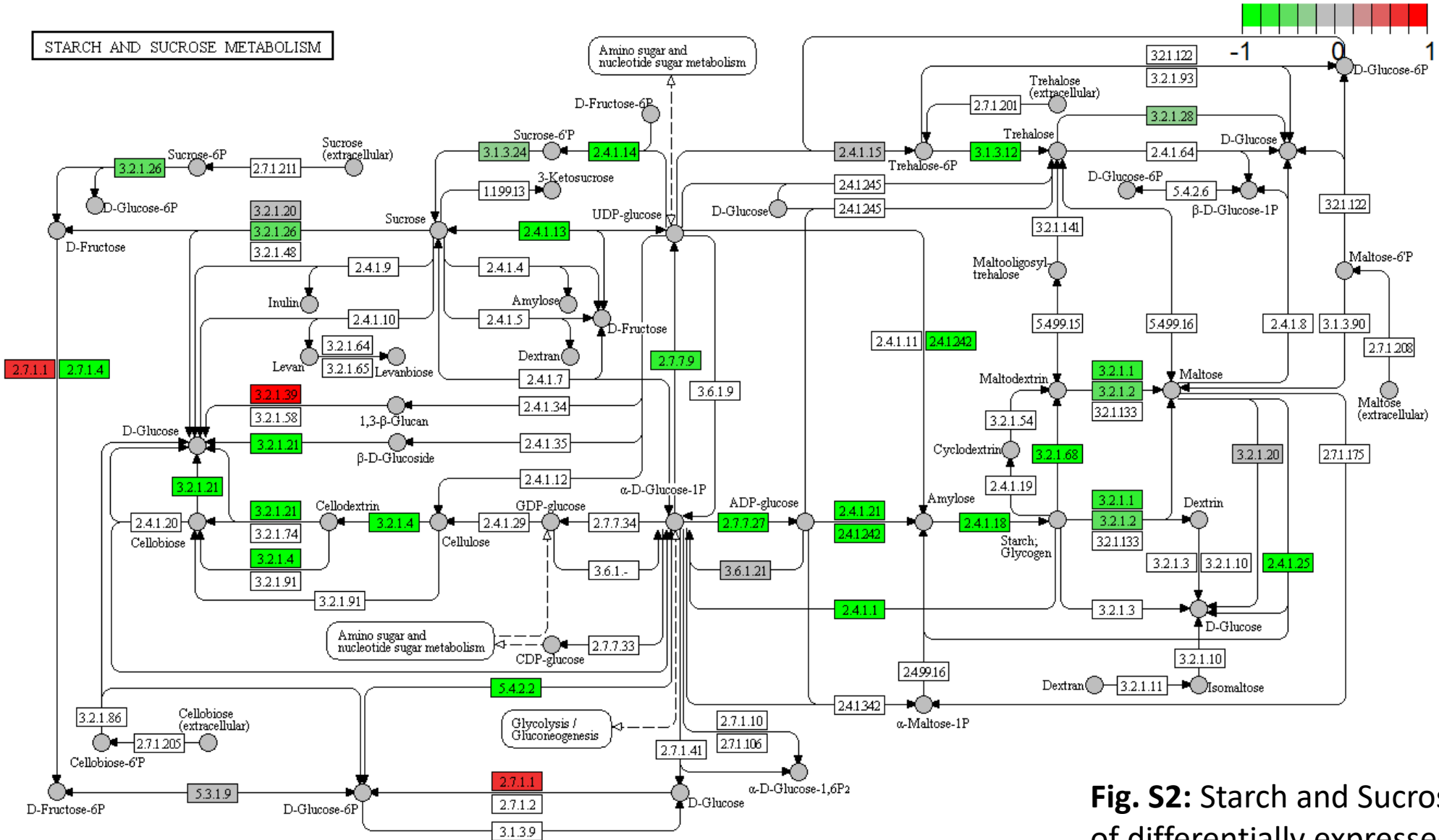


Fig. S2: Starch and Sucrose Metabolism pathview of differentially expressed genes identified from HISAT2-Stringtie-DESeq.
p = 5.87E-02

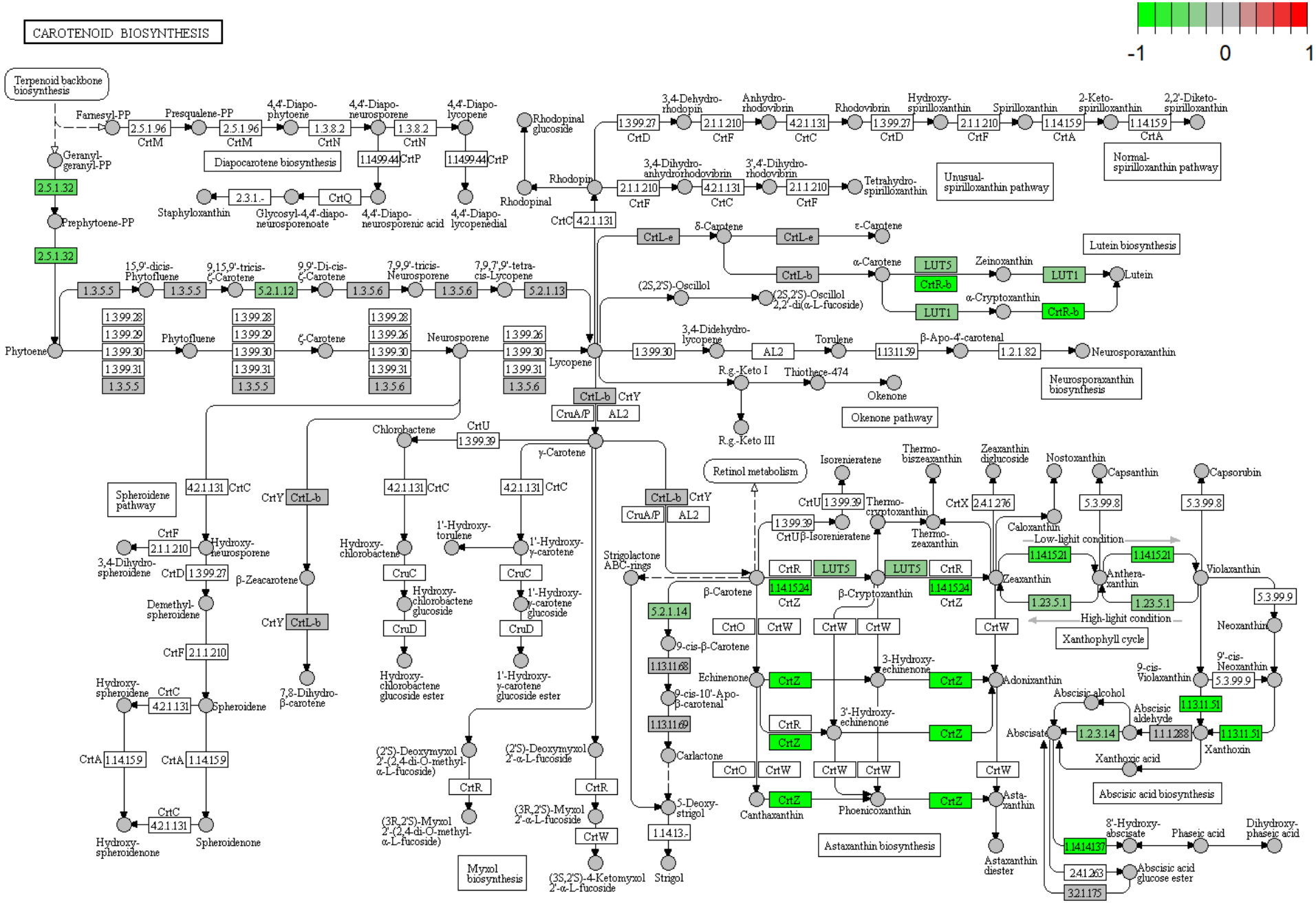


Fig. S3: Carotenoid Biosynthesis pathview of differentially expressed genes identified from HISAT2-Stringtie-DESeq. p = 4.99E-09

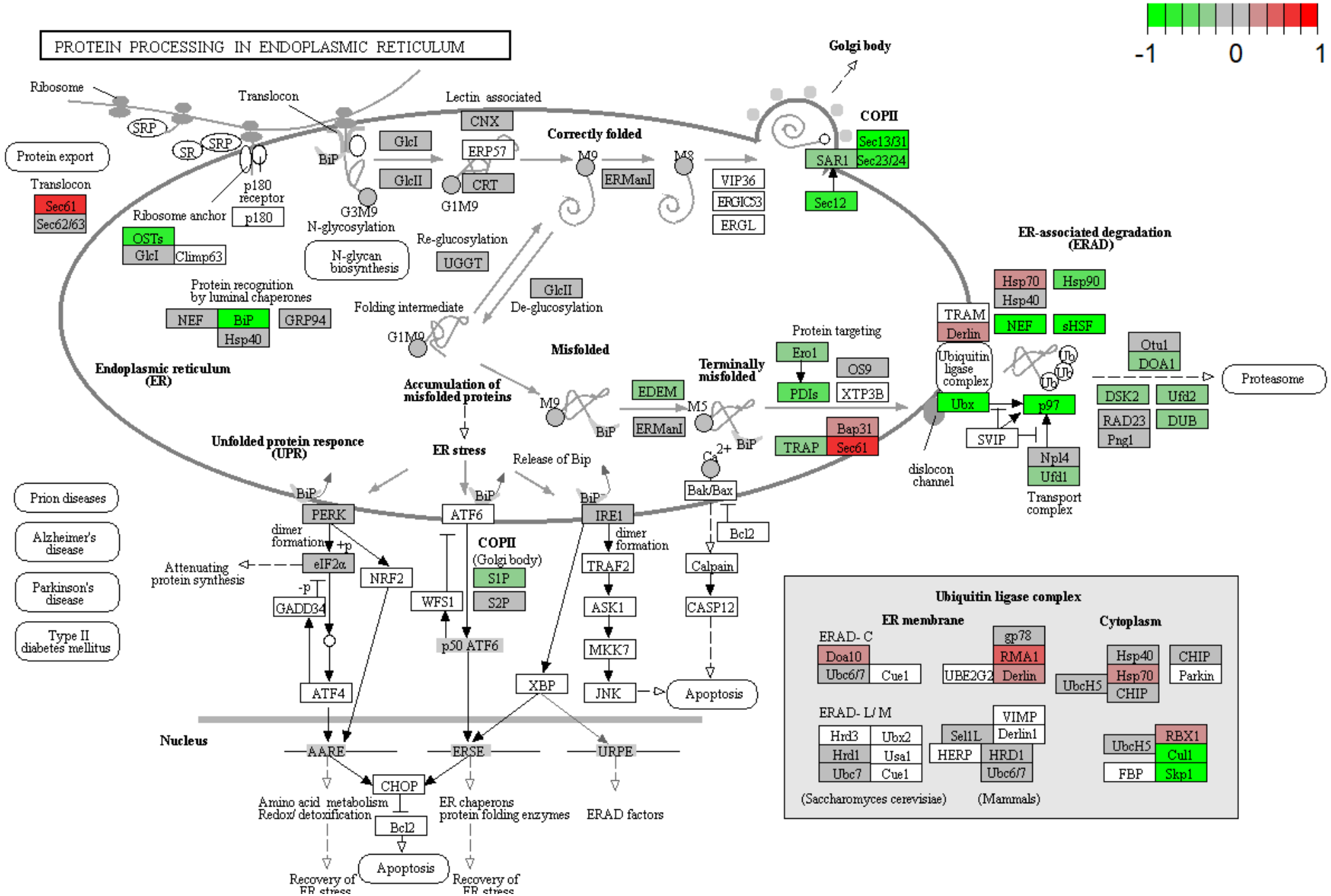


Fig S4: Protein Processing in the Endoplasmic Reticulum pathview of differentially expressed genes identified from HISAT2-Stringtie-DESeq.
p = 8.23E-02

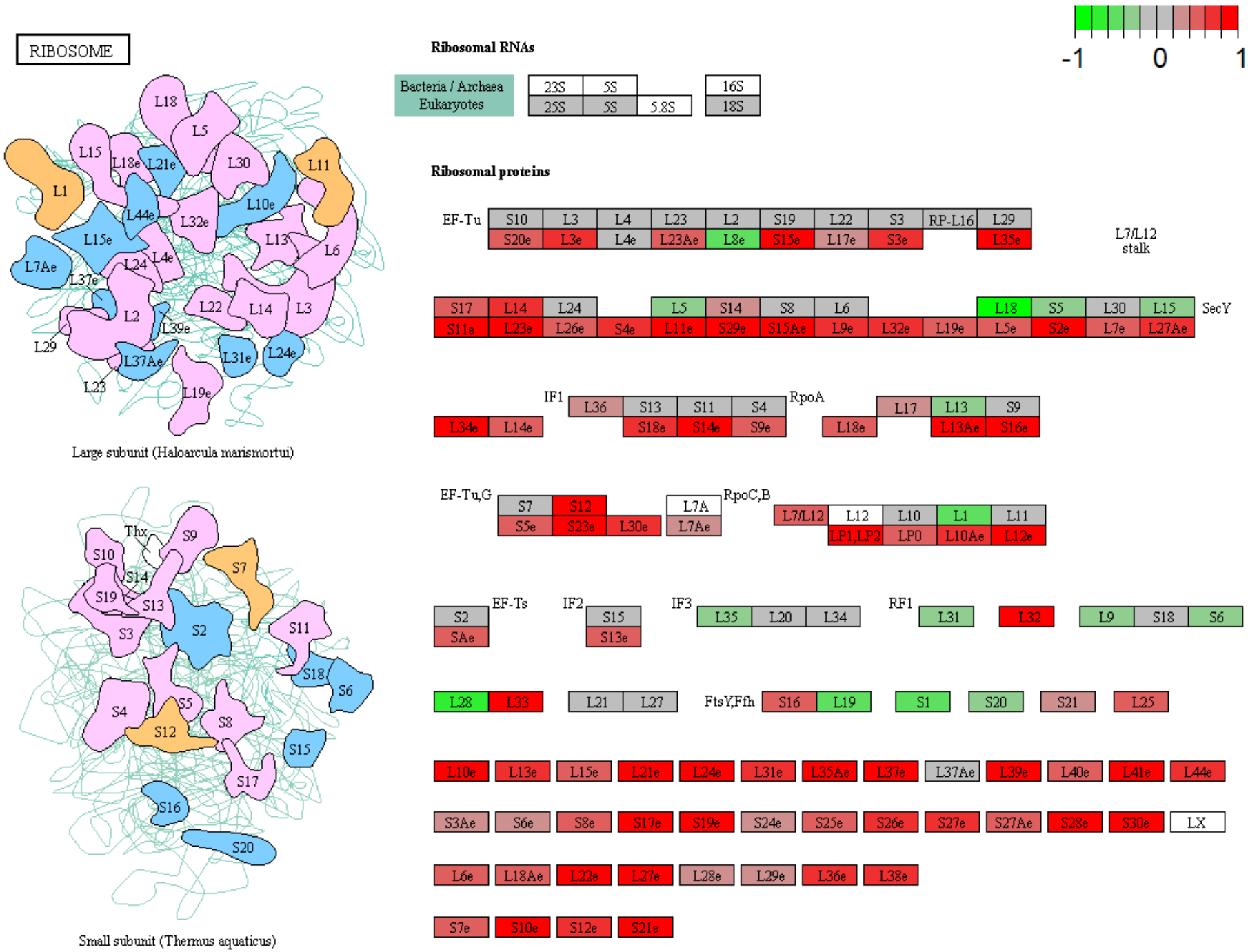


Fig. S5: Ribosome pathview of differentially expressed genes identified from HISAT2-Stringtie-DESeq.
p = 2.62E-13

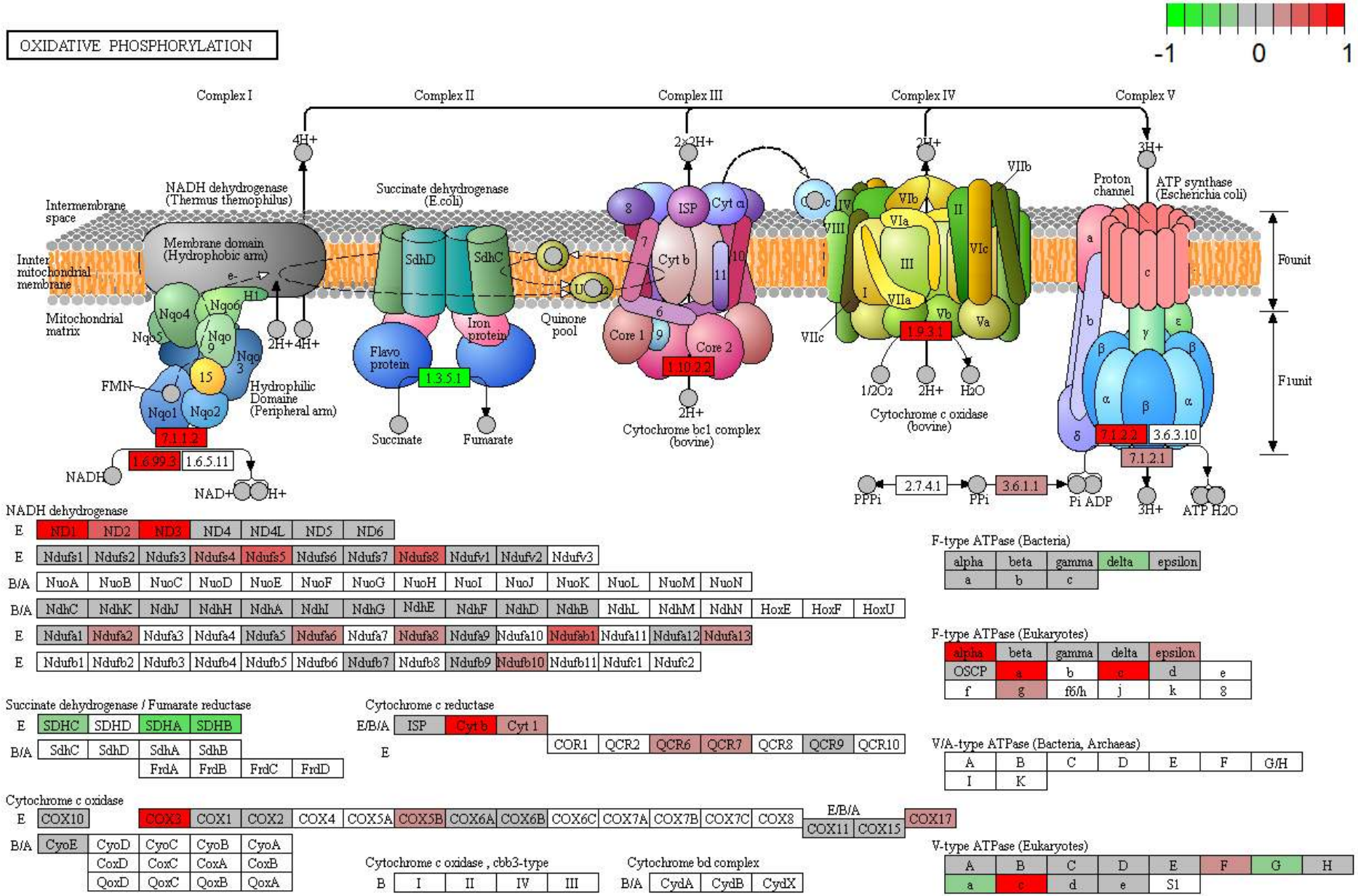
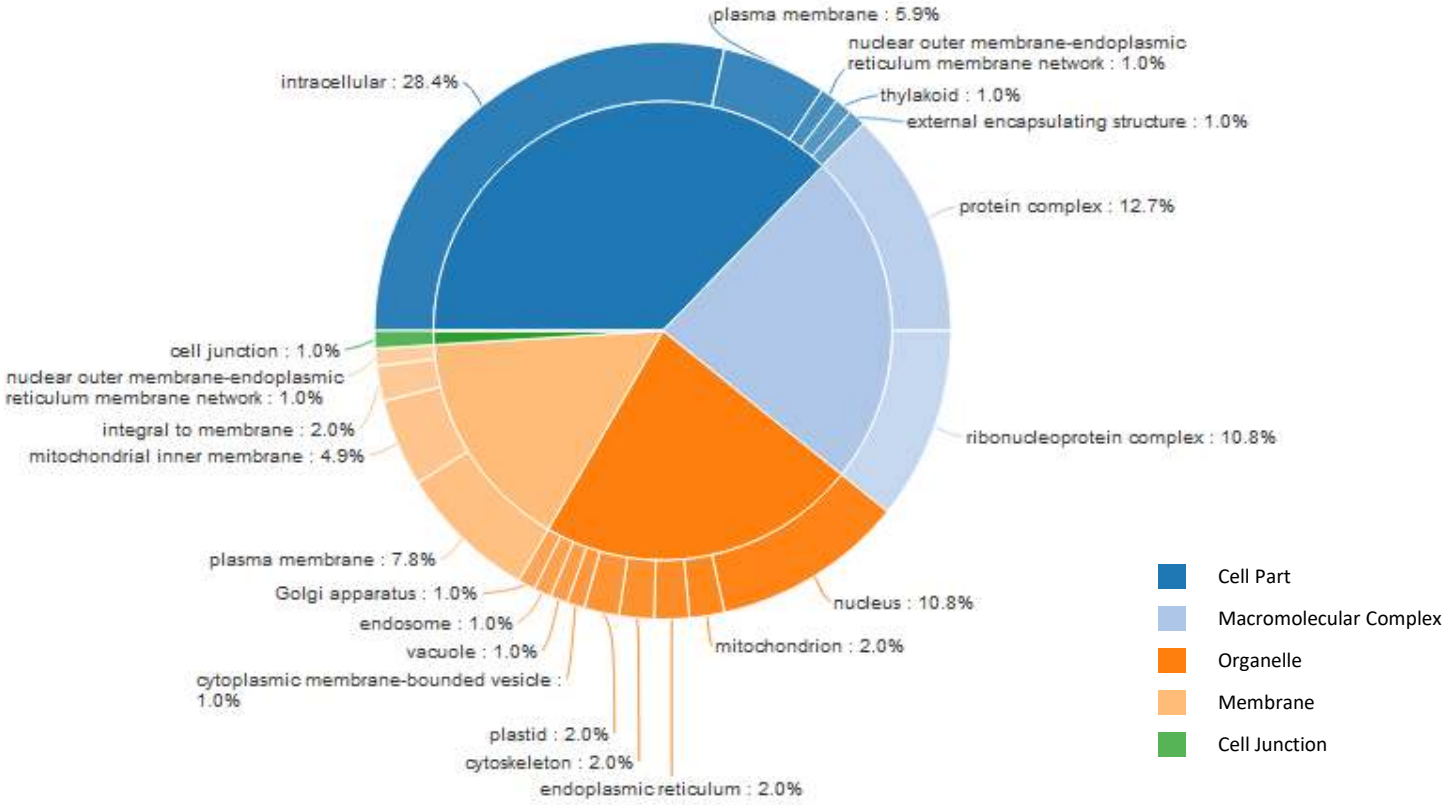


Fig. S6: Oxidative Phosphorylation pathview of differentially expressed genes identified from HISAT2-Stringtie-DESeq. p = 9.80E-02



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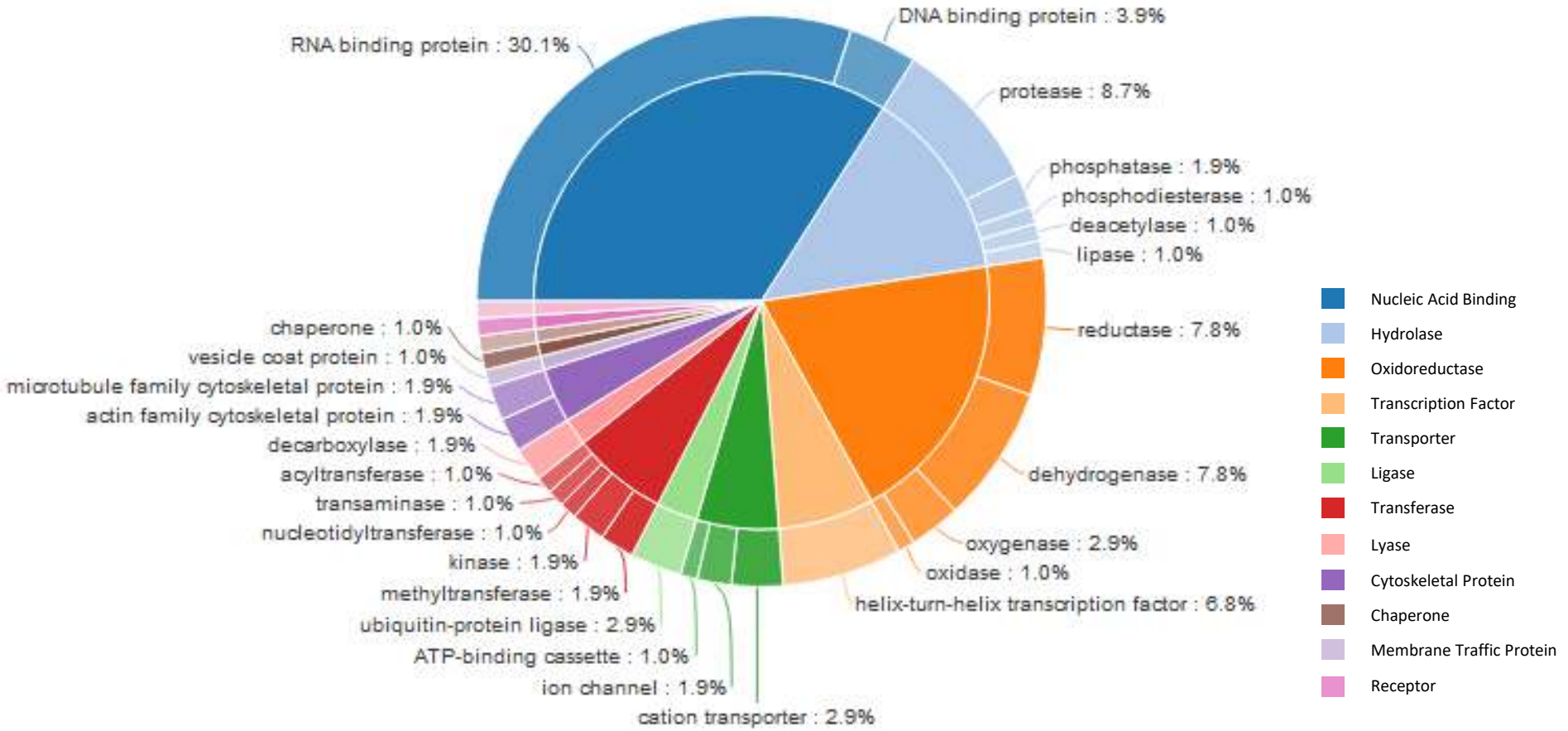

Fig. S8: PANTHER Protein Class of Differentially Expressed Genes**.** Ontological classification of protein products derived from genes identified as differentially expressed in conditions of microgravity.