Acta Oceanol. Sin., 2018, Vol. 37, No. 5, P. 54–66
DOI: 10.1007/s13131-017-1101-4
http://www.hyxb.org.cn
E-mail: hyxbe@263.net

Differential gene expression in the body wall of the sea cucumber (*Apostichopus japonicus*) under strong lighting and dark conditions

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Received 25 January 2017; accepted 9 March 2017

Abstract

Sea cucumber, *Apostichopus japonicus* is very sensitive to light changes. It is important to study the influence of light on the molecular response of *A. japonicus*. In this study, RNA-seq provided a general overview of the gene expression profiles of the body walls of *A. japonicus* exposed to strong light ("light"), normal light ("control") and fully dark ("dark") environment. In the comparisons of "control" vs. "dark", "control" vs. "light" and "dark" vs. "light", 1 161, 113 and 1 705 differentially expressed genes (DEGs) were identified following the criteria of $|\log_2 \text{ratio}| \geqslant 1$ and FDR$\leqslant$0.001, respectively. Gene ontology analysis showed that "cellular process" and "binding" enriched the most DEGs in the category of "biological process" and "molecular function", while "cell" and "cell part" enriched the most DEGs in the category of "cellular component". And the DEGs were mapped to 214, 41 and 229 pathways in the Kyoto Encyclopedia of Genes and Genomes database, and 51, 2 and 57 pathways were significantly enriched, respectively. Light-specific DEGs identified in this study will be important targets for further investigation to establish the biochemical mechanisms involved in the adaption of this sea cucumber to changes in the level of environmental light.

Key words: sea cucumber, *Apostichopus japonicus*, gene expression, dark, light, body wall

Citation: Zhang Libin, Feng Qiming, Sun Lina, Fang Yan, Xu Dongxue, Zhang Tao, Yang Hongsheng. 2018. Differential gene expression in the body wall of the sea cucumber (*Apostichopus japonicus*) under strong lighting and dark conditions. Acta Oceanologica Sinica, 37(5): 54–66, doi: 10.1007/s13131-017-1101-4

1 Introduction

Light is an important ecological factor. The photoperiod, light intensity and light color can influence the distribution (Thorson, 1964), movement (Ringelberg, 1995), behavior (Naylor, 1999), physiology (Lambert and Brandt, 1967), feeding (Volpato et al., 2013), reproduction (Lambert and Brandt, 1967; West and Lambert, 1976), and growth of aquatic animals (Zhou et al., 2000).

Apostichopus japonicus, a common temperate species of sea cucumber, is distributed in the northwest Pacific (Liao, 1980; Sloan, 1984). *Apostichopus japonicus* is a very important maricultural species and is considered to be one of the most flavorful species in markets in East and Southeast Asian countries (Zhou et al., 2014). In 2015, the area occupied by *A. japonicus* culture was 216 508 hm^2 for a total output that reached 205 791 t in China (Fisheries Bureau of Ministry of Agriculture, 2016). It generates the highest single-species output value and profit of mariculture in northern China (Zhang et al., 2015b).

Light is a very important factor in the culture of *A. japonicus*, especially during the stages of brood stock and larval culture and juvenile rearing. A dark environment is usually maintained in the hatchery (Zhang et al., 2015b). To optimize the light conditions is a key technique in *A. japonicus* culture. Therefore it is important to study the influence of light on the behavior and physiology of *A. japonicus*.

Recently, the effects of light intensity on the daily activity rhythm of juvenile *A. japonicus* (Dong et al., 2010a), and on the daily feeding rhythm and movement, and the behavior and physiology of the species (Sun et al., 2015; Pan et al., 2015) have been investigated and quantified. The influence of light on the growth (Xue et al., 2007; Lin et al., 2013), respiration and excretion (Sui et al., 2010), and energy budget (Bao et al., 2014) of *A. japonicus* have also been investigated. In addition, some of the molecular responses of differentially expressed genes in *A. japonicus* have been studied during the aestivation stage (Zhao et

Foundation item: The National Natural Science Foundation of China under contract No. 41676136; the Strategic Priority Research Program of the Chinese Academy of Sciences under contract No. XDA11020703; the Science and Technology Major Project of Shandong Province (Industry Transformation and Upgrading) under contract No. 2015ZDZX05002.
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al., 2014a; Chen and Storey, 2014), the intestine regeneration stage (Sun et al., 2013) and between different growth stages (embryo and larva) (Yang et al., 2010), under thermal (Shao et al., 2015) and osmotic stress (Dong et al., 2008), and even suffered from pathogen challenge (Zhang et al., 2013; Shao et al., 2013; Zhang et al., 2014). However, the molecular responses of differentially expressed genes in *A. japonicus* exposed to strong light and darkness, and the molecular basis of these adaptations, is poorly understood.

Generally speaking, *A. japonicus* is more like a nocturnal animal, and the feeding and activity peak mostly occurs late at night (Sun et al., 2015). Therefore, these organisms may be sensitive when exposed to different intensities of light. Some species of echinoderms have photosensory organs related to illumination, such as the brittle star *Ophiocoma wendtii* on the backs of the arms (Aizenberg et al., 2001) and the purple sea urchin *Strongylocentrotus purpuratus* on the tube feet (Raible et al., 2006). Some photosensory cells are composed of specialized structures of the ossicles (Aizenberg et al., 2001).

The body wall of *A. japonicus*, containing undeveloped bones with tiny scattered ossicles, which is in contact with the light directly by day and night, may be a sensitive photosensory tissue and influenced by light intensity. It may generate differential gene expression under strong light and in fully dark environments. However, the regulatory and response mechanisms are unclear. With the increasing availability of sequence data in recent years, expression profiling has been used to identify genes involved in the adaptive responses to environmental factors. Recently, RNA sequencing has been used to quantify, discover and profile RNAs. This is an effective and popular approach, for high throughput sequencing, with high sensitivity. The aim of this study was to develop a better understanding of the molecular responses of *A. japonicus* when exposed to strong ambient light and dark environments and to provide a theoretical basis for the development of healthy breeding conditions for *A. japonicus*.

2 Materials and methods

2.1 *Ethics statement*

It is not applicable in this study. Human or vertebrate species or relating samples were not involved. The sea cucumber, *A. japonicus* is not endangered or protected. No special permission was needed for the collection of *A. japonicus*.

2.2 *Animals*

Thirty-six fresh and healthy *A. japonicus* (80–130 g body weight) were collected from the coast of Qingdao, China and acclimated in tanks containing aerated sand-filtered seawater (salinity 31, pH 8.1) at (15±0.5)°C for one week before being exposed to different light conditions. They were fed once a day during this period. The animals were then divided randomly into three groups (12 individuals in each group). One group of sea cucumbers was maintained as the control group with natural light ("control") with the light intensity around 100 lx; the other two groups were exposed separately to strong light exposure with the light intensity around 2 000 lx ("light"), and in a fully dark tank covered by a shade cloth ("dark") for two hours. Six individuals randomly selected from each group were then dissected promptly and the body walls were sampled to be preserved for RNA extraction and sequencing. A brief overview of the rearing conditions of the sampled sea cucumbers and summarizes the key characteristics of the project are listed in Table 1.

2.3 *RNA extraction and sequencing*

RNA samples were extracted from the body walls of sea cucumbers in three different groups ("control", "light" and "dark") with an RNeasy mini kit, including DNase treatment with a RNase-free DNase (Qiagen Inc., Germany), according to the protocols of the manufacturer. Agilent 2100 bioanalyzer was used to determine the concentration and quality of the samples. Equal amount of the RNA samples from six individuals per group was pooled to prepare the sequencing library. The preparation of Libraries from the three RNA pools was carried out by Beijing Genomics Institute (BGI, Shenzhen, China), including mRNA enrichment, fragmentation, ligation of adapters, PCR amplification, and sequencing was conducted using an IlluminaHiSeq™ 2000 (BGI, Shenzhen). The sequencing was for single end read, and its read length was 200 bp.

2.4 *Read mapping and sequence quality control*

After quality control, which is applied for raw reads from primary sequencing to determine if resequencing is needed, the filtration of raw reads was involved to get clean reads through removing reads with adaptors, more than 10% unknown bases, and

Table 1. Gene expression profiles and environmental feature

Item	Description
Investigation type	sea cucumber, *Apostichopus japonicus*
Project name	gene expression response to strong lighting and dark
Collection date	April 15, 2013
Geographic location	35.97°N, 120.28°E
Country	China (Qingdao)
Environment (biome)	marine-subtidal area
Environment (material)	sea cucumbers maintained in seawater at laboratory
Temperature	(15±0.5)°C
Salinity	31
pH	8.1
Light	100 lx (control), 2 000 lx (light), fully dark (dark)
Sequencing method	pyrosequencing
Sequencing technology	IlluminaHiSeq™2000
Mapping method	Soap(2.21)
Annotation method	Blast(2.2.23), Blast2GO(2.2.5)
Reference database	Sun et al. (2011) (NCBI accession No. SRA020994), Du et al. (2012) (NCBI accession No. SRA046386)

low quality reads. Clean reads were then mapped to the reference databases using SOAP aligner/soap2 from large scale transcriptome profiling of sea cucumber, *A. japonicus* (Sun et al., 2011; Du et al., 2012). QC of alignment was also involved, including the calculation of the distribution of reads on reference genes and mapping ratio. Quality assessment of reads, sequencing saturation analysis, and randomness assessment were involved to confirm the quality of sequencing.

2.5 *Gene expression analysis, real-time PCR validation and functional classification*

Differentially expressed genes (DEGs) were detected by the RPKM method (reads per kb million reads), based on the normalized number of clean tags mapped exclusively for each gene (Mortazavi et al., 2008). The deviation of gene expression brought about by sequencing difference and gene length preference can be removed effectively by the standardization. In this study, the false discovery rate (FDR) not greater than 0.001 and an absolute value of $\log_2$ratio not less than 1 were set to determine DEGs, as described previously by Audic and Claverie (1997).

To validate RNA-seq results, the top five up-regulated genes and top five down-regulated genes were taken to perform real-time PCR. Primers were designed for optimal performance with primer3 (Table 2). The input RNA used in the synthesis of cDNA was run in triplicate of each group. The synthesis of the first strand cDNA was in 25 μL reaction system, as described in our earlier research (Sun et al., 2013). The SYBR Green® real-time PCR assay with an Eppendorf Mastercycler®eprealplex (Eppendorf, Hamburg, Germany) was used to determine the mRNA expression levels. The amplification volume (25 μL) contained 10.5 μL of RNase-free water, 12.5 μL of SYBR GreenMasterMix (Takara), 0.5 μL (each) of forward and reverse primer, and 1 μL of diluted cDNA. Thermal cycling procedure was 95°C for 5 s, and followed by 40 cycles at 95°C for 10 s, 60°C for 20 s and 72°C for 30 s. The specificity of the amplification products was confirmed by melting curve analysis. All the data were given as mean±SD (N=3) and $P<0.05$ was set for the statistical significance. And the analysis was performed with SPSS18.0 software.

To identify functional classifications of DEGs, all DEGs were mapped to gene ontology terms with the gene ontology (GO) database (http://www.geneontology.org/) and Kyoto Encyclopedia of Genes and Genomes (KEGG) (http://www.genome.jp/kegg/). Subsequently, the significantly enriched GO terms and pathways were determined by the calculation of the gene numbers related to each term and the application of a hypergeometric test applied to the DEGs. Q value was involved to determine the threshold of P value in multiple tests by the Bonferroni method (Abdi, 2007), and $P<0.05$ was set as the threshold to determine significantly enriched GO terms and KEGG pathways.

3 Results

3.1 *Analysis of sequencing data quality*

Libraries of three different groups ("control", "dark" and "light") were constructed from sea cucumber body walls. After filtering out low quality reads (adaptor reads, 10% unknown bases, and low quality reads) (Table 3), 5 919 823, 5 921 246 and 6 227 616 clean reads corresponding to the three groups have been deposited at GEO under the accession No. GSE87803. The reference transcriptome used in this work was from a 454 sequence transcriptomic database including different tissues, different developmental stages and different physiological conditions of *A. japonicus* (NCBI accession No. SRA020994 and No. SRA046386) (Sun et al., 2011; Du et al., 2012), which is currently the most thorough sea cucumber transcriptomic database. After alignment to the reference transcriptome, a total of 2 538 654 (42.88%), 2 723 388 (45.99%) and 2 633 299 (42.28%) reads were mapped to the "control", "dark" and "light" libraries as shown in Table 3. Of these, 1 777 034, 1 915 744 and 1 842 939 reads were uniquely aligned in one of the three libraries, accounting for 30.02%, 32.35% and 29.59% of all the mapped reads. Rigid data quality control and conservative matching ensured the effectiveness and accuracy of our results (Fig. S1). Saturation analysis indicated that 5×10^6 clean reads were already nearly saturated at

Table 2. Primers designed for optimal performance

Gene	Primer F	Primer R	Product size/bp
SAPA	GTACCACTGGGCGTGAGTTT	CGTTGCCCTATCGTTGCTAT	173
DSI	GGCAGGTGTTGGAAACAAAT	TCTGTCCTTCCGTCTGTGTG	186
TRA	GTGGACGGGGAAAACTTGTA	AGCTCATCCACACCTTTTGG	203
LRCP	GAGGTCGAGTGGACAGAAGC	TGTCACGAACAGCTCCAAAG	240
ASF	GCTCTGTGCATCCATCTGAA	AGCTTTCTACGGTGCGTTGT	241
AM	GCCTGAAGTTCGACCAAGTC	AATTTGAAGGATGGCGTGTC	171
PAP	CCATCCTTTTGCTCCATTGT	CCTCCGGACAATCCTGAATA	249
LRBAP	CCCCGATGGAATGAAGAGTA	CGATGGCAAGTTGACTCAGA	188
PGT	CGTTCCAAGTCAAGCGTACA	ATCATTGCCTCCATCCTGAG	201
AAPK	GTGTGCAAGTCTGCAAGGAA	ATGGTGAATTTTCCGCTCTG	202

Note: SAPA represents serum amyloid protein A; DSI dynactin subunit 5-like isoform 1; TRA transcobalamin I-like; LRCP leucine-rich repeat-containing protein; ASF ATP synthase-coupling factor 6, mitochondrial-like; AM alpha-2-macroglobulin-like; PAP papilin-like; LRBAP lipopolysaccharide-responsive and beige-like anchor protein-like, partial; PGT procollagen galactosyltransferase 1-like; and AAPK AP2-associated protein kinase 1-like.

Table 3. Summary of mapping result (mapping to reference genes)

Sample ID	Total reads	Total base pairs	Total mapped reads	Perfect match	≤2 bp mismatch	Unique match	Multi-position match	Total unmapped reads
Control	5 919 823 (100.00%)	290 071 327 (100.00%)	2 538 654 (42.88%)	1 777 034 (30.02%)	761 620 (12.87%)	1 570 290 (26.53%)	968 364 (16.36%)	3 381 169 (57.12%)
Dark	5 921 246 (100.00%)	290 141 054 (100.00%)	2 723 388 (45.99%)	1 915 744 (32.35%)	807 644 (13.64%)	1 698 268 (28.68%)	1 025 120 (17.31%)	3 197 858 (54.01%)
Light	6 227 616 (100.00%)	305 153 184 (100.00%)	2 633 299 (42.28%)	1 842 939 (29.59%)	790 360 (12.69%)	1 644 816 (26.41%)	988 483 (15.87%)	3 594 317 (57.72%)

this platform stage (Fig. S2). Assessment of sequencing randomness indicated the distribution of the reads and, in this study, the aligned reads seemed to be evenly distributed in the three libraries (Fig. S3).

3.2 *Differential gene expression analysis*

RNA-seq provided a general overview of the gene expression profiles of the body walls of *A. japonicus* exposed to strong light, normal light and fully dark environment. As a result, 1 161, 113 and 1 705 DEGs were identified following the criteria of $|\log_2 ratio| \geqslant 1$ and FDR$\leqslant$0.001, respectively, in comparisons of "control" vs. "dark", "control" vs. "light" and "dark" vs. "light" (Fig. 1).

Specifically, 145 up-regulated DEGs (0.23%, 145/62 000) and 1 016 down-regulated DEGs (1.64%, 1 016/62 000) were produced in the "control" vs. "dark" comparison, 46 up-regulated DEGs (0.07%, 46/62, 000) and 67 down-regulated DEGs (0.11%, 67/62 000) were identified when "light" was compared with "control", but 1 468 up-regulated DEGs (2.37%, 1 468/62 000) and 237 down-regulated DEGs (0.38%, 237/62 000) were detected in the "dark" vs. "light" comparison.

The top 20 up- or down-regulated DEGs from these three comparisons are listed in Table S1, S2 and S3. We found genes with light-specific expression. Compared with "dark", some DEGs, including neurogenic locus notch homolog protein 2, 60S ribosomal protein L8, phosphoinositide-3-kinase, lysine-specific demethylase 4C isoform 1, C2 domain-containing protein 3, rho GTPase-activating protein 18 isoform 2, and kinesin light chain isoform 1, were only expressed in the "light" group. Compared with "control", some DEGs, such as angiopoietin-1 receptor, were only expressed in the "light" group. However, because of the limitation of the reference databases, many DEGs were not annotated accurately.

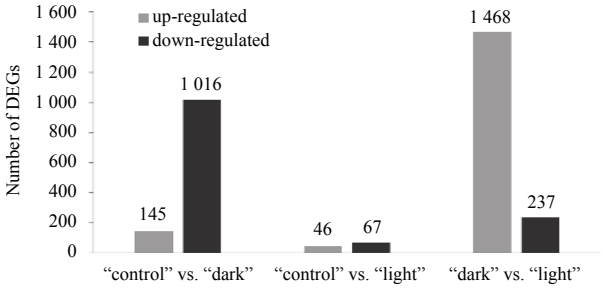

Fig. 1. Overview of differential expression (light representing strong light, dark fully dark, and control normal light).

3.3 *Classification of gene ontology (GO)*

Gene ontology analysis can provide complete functional information by assigning DEGs to three major sections ("cellular component", "molecular function" and "biological process"). In order to determine the function of the DEGs, DGEs were conducted by GO analysis. The GO term enrichment analysis detected a total of 27, 1 and 38 significantly overrepresented GO terms enriched in the comparisons of "control" vs. "dark", "control" vs. "light" and "dark" vs. "light", respectively, with corrected *P*-value less than 0.05 (Table 4). In the three comparisons, "cellular pro-

Table 4. Gene ontology (GO) terms enriched of differentially expressed genes correlated with strong light, natural light and fully dark exposure

Gene ontology term	GO No.	Reference frequency (N/M)	Cluster frequency (n/m)	Corrected *P* value
"Dark" vs. "control"				
Biological process				
Regulation of biological process	GO: 0050789	156/365 (42.7%)	1 530/5 347 (28.6%)	1.59×10^{-6}
Regulation of cellular process	GO: 0050794	138/365 (37.8%)	1 331/5 347 (24.9%)	9.76×10^{-6}
Biological regulation	GO: 0065007	167/365 (45.8%)	1 804/5 347 (33.7%)	0.000 54
Cell communication	GO: 0007154	89/365 (24.4%)	821/5 347 (15.4%)	0.001 99
Signaling	GO: 0023052	85/365 (23.3%)	775/5 347 (14.5%)	0.002 25
Single organism signaling	GO: 0044700	85/365 (23.3%)	775/5 347 (14.5%)	0.002 25
Negative regulation of cellular process	GO: 0048523	37/365 (10.1%)	251/5 347 (4.7%)	0.004 86
Signal transduction	GO: 0007165	76/365 (20.8%)	682/5 347 (12.8%)	0.004 99
Epithelial cell differentiation	GO: 0030855	10/365 (2.7%)	27/5 347 (0.5%)	0.005 68
Regulation of localization	GO: 0032879	21/365 (5.8%)	112/5 347 (2.1%)	0.016 45
Negative regulation of biological process	GO: 0048519	40/365 (11.0%)	304/5 347 (5.7%)	0.032 40
Response to stimulus	GO: 0050896	135/365 (37.0%)	1 478/5 347 (27.6%)	0.035 12
Cell surface receptor signaling pathway	GO: 0007166	41/365 (11.2%)	316/5 347 (5.9%)	0.035 85
Appendage development	GO: 0048736	13/365 (3.6%)	54/5 347 (1.0%)	0.047 49
Cellular component				
Anchoring junction	GO: 0070161	13/306 (4.2%)	48/4 541 (1.1%)	0.001 68
Cell-substrate adherens junction	GO: 0005924	10/306 (3.3%)	31/4 541 (0.7%)	0.003 39
Cell-substrate junction	GO: 0030055	10/306 (3.3%)	32/4 541 (0.7%)	0.004 64
Adherens junction	GO: 0005912	10/306 (3.3%)	36/4 541 (0.8%)	0.014 36
Cell junction	GO: 0030054	17/306 (5.6%)	91/4 541 (2.0%)	0.015 12
Neuron part	GO: 0097458	21/306 (6.9%)	137/4 541 (3.0%)	0.045 31
Molecular function				
Phosphotransferase activity, alcohol group as acceptor	GO: 0016773	45/348 (12.9%)	253/5 067 (5.0%)	2.31×10^{-7}
Protein kinase activity	GO: 0050222	41/348 (11.8%)	238/5 067 (4.7%)	3.55×10^{-6}

to be continued

Continued from Table 4

Gene ontology term	GO No.	Reference frequency (N/M)	Cluster frequency (n/m)	Corrected P value
Kinase activity	GO: 0016301	50/348 (14.4%)	348/5 067 (6.9%)	4.12×10^{-5}
Transferase activity, transferring phosphorus-containing groups	GO: 0016772	61/348 (17.5%)	492/5 067 (9.7%)	0.000 34
Enzyme binding	GO: 0019899	28/348 (8.0%)	201/5 067 (4.0%)	0.038 67
Protein binding	GO: 0005515	111/348 (31.9%)	1 209/5 067 (23.9%)	0.044 41
Protein domain specific binding	GO: 0019904	13/348 (3.7%)	63/5 067 (1.2%)	0.048 4
"Light" vs. "control"				
Biological process				
Cellular component				
Molecular function				
Hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds	GO: 0016810	2 out of 10 genes, 20.0%	29 out of 5 067 genes, 0.6%	0.012 45
"Light" vs. "dark"				
Biological process				
Regulation of biological process	GO: 0050789	241/555 (43.4%)	1 530/5 347 (28.6%)	2.88×10^{-12}
Regulation of cellular process	GO: 0050794	211/555 (38.0%)	1 331/5 347 (24.9%)	3.42×10^{-10}
Biological regulation	GO: 0065007	257/555 (46.3%)	1 804/5 347 (33.7%)	7.38×10^{-8}
Intracellular signal transduction	GO: 0035556	68/555 (12.3%)	320/5 347 (6.0%)	2.98×10^{-6}
Cell communication	GO: 0007154	134/555 (24.1%)	821/5 347 (15.4%)	7.75×10^{-6}
Signal transduction	GO: 0007165	116/555 (20.9%)	682/5 347 (12.8%)	9.87×10^{-6}
Signaling	GO: 0023052	127/555 (22.9%)	775/5 347 (14.5%)	1.73×10^{-5}
Single organism signaling	GO: 0044700	127/555 (22.9%)	775/5 347 (14.5%)	1.73×10^{-5}
Negative regulation of cellular process	GO: 0048523	52/555 (9.4%)	251/5 347 (4.7%)	0.000 61
Negative regulation of biological process	GO: 0048519	59/555 (10.6%)	304/5 347 (5.7%)	0.001 05
Regulation of response to stimulus	GO: 0048583	53/555 (9.5%)	270/5 347 (5.0%)	0.002 77
Cell surface receptor signaling pathway	GO: 0007166	58/555 (10.5%)	316/5 347 (5.9%)	0.008 48
Cellular response to stimulus	GO: 0051716	137/555 (24.7%)	946/5 347 (17.7%)	0.008 66
Regulation of phosphorylation	GO: 0042325	29/555 (5.2%)	119/5 347 (2.2%)	0.009 01
Actin cytoskeleton organization	GO: 0030036	24/555 (4.3%)	90/5 347 (1.7%)	0.010 83
Cellular response to chemical stimulus	GO: 0070887	28/555 (5.0%)	114/5 347 (2.1%)	0.011 04
Gastrulation	GO: 0007369	15/555 (2.7%)	42/5 347 (0.8%)	0.012 01
Actin filament-based process	GO: 0030029	24/555 (4.3%)	91/5 347 (1.7%)	0.013 30
Regulation of protein phosphorylation	GO: 0001932	27/555 (4.9%)	109/5 347 (2.0%)	0.013 48
Small GTPase mediated signal transduction	GO: 0007264	18/555 (3.2%)	58/5 347 (1.1%)	0.015 17
Positive regulation of response to stimulus	GO: 0048584	27/555 (4.9%)	111/5 347 (2.1%)	0.019 32
Cell migration	GO: 0016477	26/555 (4.7%)	105/5 347 (2.0%)	0.019 74
Regulation of metabolic process	GO: 0019222	88/555 (15.9%)	559/5 347 (10.5%)	0.025 05
Immune response-activating cell surface receptor signaling pathway	GO: 0002429	6/555 (1.1%)	8/5 347 (0.1%)	0.034 36
Antigen receptor-mediated signaling pathway	GO: 0050851	6/555 (1.1%)	8/5 347 (0.1%)	0.034 36
Immune response-regulating cell surface receptor signaling pathway	GO: 0002768	7/555 (1.3%)	11/5 347 (0.2%)	0.034 38
Positive regulation of biological process	GO: 0048518	66/555 (11.9%)	394/5 347 (7.4%)	0.044 41
Cellular component				
Dendrite	GO: 0030425	14/461 (3.0)	38/4 541 (0.8)	0.001 68
Neuron part	GO: 0097458	30/461 (6.5)	137/4 541 (3.0)	0.005 12
A band	GO: 0031672	4/461 (0.9)	4/4 541 (0.1)	0.017 84
Neuron projection	GO: 0043005	26/461 (5.6)	124/4 541 (2.7)	0.036 97
Cell junction	GO: 0030054	21/461 (4.6)	91/4 541 (2.0)	0.037 37
Molecular function				
Phosphotransferase activity, alcohol group as acceptor	GO: 0016773	58/512 (11.3)	253/5 067 (5.0)	1.49×10^{-7}
Protein kinase activity	GO: 0050222	54/512 (10.5)	238/5 067 (4.7)	9.08×10^{-7}
Kinase activity	GO: 0016301	67/512 (13.1)	348/5 067 (6.9)	1.41×10^{-5}
Protein binding	GO: 0005515	168/512 (32.8)	1 209/5 067 (23.9)	0.000 16
Transferase activity, transferring phosphorus-containing groups	GO: 0016772	79/512 (15.4)	492/5 067 (9.7)	0.002 11
Enzyme binding	GO: 0019899	39/512 (7.6)	201/5 067 (4.0)	0.007 91

Note: N is the number of all genes with GO annotation, n the number of DEGs in N, M the number of all genes that are annotated to certain GO terms, and m the number of DEGs in M.

cess" and "binding" enriched the most DEGs in the category of "biological process" and "molecular function", while "cell" and "cell part" enriched the most DEGs in the category of "cellular component" (Fig. 2). Moreover, in the comparison of "control" vs. "light", "metabolic process" was another dominant term for "biological process", and "catalytic activity" was another dominant term for "molecular function".

3.4 *Classification according to the Kyoto Encyclopedia of Genes and Genomes (KEGG)*

The DEGs were also mapped to KEGG metabolic and regulatory pathways with a correct *P*-value cutoff of being less than 0.05. The differential gene expression in the three comparisons "dark" and "control", "light" and "control", "light" and "dark" affected a range of KEGG pathways. The top ten significantly enriched pathways for DEGs are listed in Table 5. The differentially expressed genes were mapped to 214, 41, and 229 pathways in the KEGG database, and 51, 2, and 57 pathways were significantly enriched, respectively (corrected *P*-value less than 0.05). The most representative KEGG pathways included Focal adhesion (ko04510), the ErbB signaling pathway (ko04012), and Fc gamma R-mediated phagocytosis (ko04666) in the comparison of "control" vs. "dark", ECM-receptor interaction (ko04512) and Focal adhesion (ko04510) in the comparison of "control" vs. "light", and the Chemokine signaling pathway (ko04062), ErbB signaling pathway (ko04012), and Focal adhesion (ko04510) in the comparison of "dark" vs. "light".

3.5 *Validation of DGE analysis*

Real-time PCR was carried out to confirm the expression profiles of the top DGEs identified in the RNA-seq. Top five up-regulated genes (serum amyloid protein A (SAPA); dynactin subunit 5-like isoform 1 (DSI), transcobalamin I-like (TRA), leucine-rich repeat-containing protein (LRCP), ATP synthase-coupling factor 6, mitochondrial-like (ASF)) and top five down-regulated genes (alpha-2-macroglobulin-like (AM), papilin-like (PAP), lipopoly-

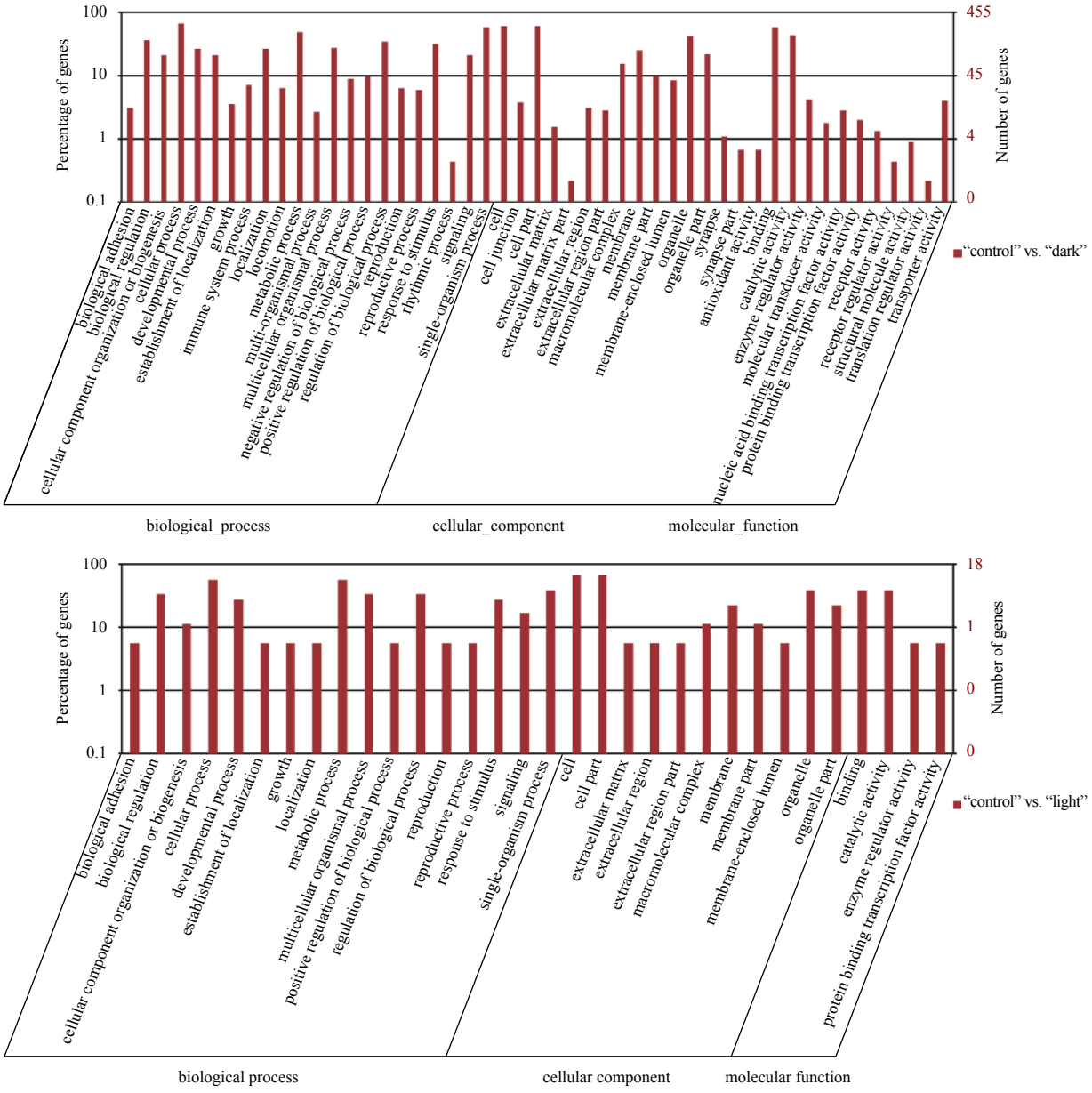

Fig. 2

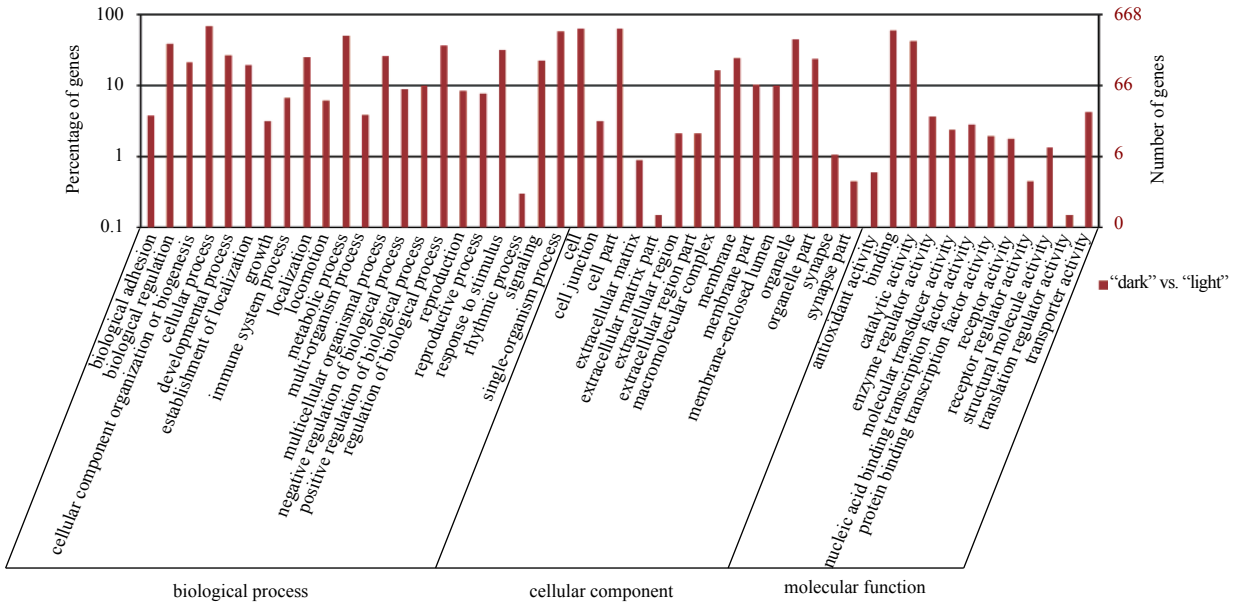

Fig. 2. Distribution of gene ontology (GO) terms of DEGs from the body wall of *A. japonicus* exposure to different light environment ("control" vs. "dark", "control" vs. "light", and "dark" vs. "light") (light representing strong light, dark representing fully dark, control representing normal light). The percentage of GO terms in the categories “cellular component”, “molecular function” and “biological process” are shown.

saccharide-responsive and beige-like anchor protein-like, partial (LRBAP), procollagen galactosyltransferase 1-like (PGT), AP2-associated protein kinase 1-like (AAPK)) were applied to real-time PCR at “dark” vs. “control” (Fig. 3). β-actin was taken as a reference gene to normalize gene expression data. Real-time PCR results showed that five up-regulated genes and four out of the five down-regulated genes showed exact correlations in their expression profiles between real-time PCR and RNA-seq, which implied our results were credible.

3.6 *Key DEGs responding to different light conditions*

Based on the primary results, genes whose functions appear to be important for understanding the response of *A. japonicus* when its body wall is exposed to different light conditions are shown in Table 6. The key DEGs associated with light density were classified into five groups. Generally speaking, when compared with “control”, the change (-fold) in gene expression varied less in “light” than in “dark”. For example, when compared with “control”, some key DEGs associated with movement of cells or subcellular components, such as dynein light chain roadblock-type 2, exhibited up-regulation in both “dark” and “light”. Some DEGs, such as dynactin subunit 1-like, showed down-regulation in “dark” but no significant change in “light”.

4 Discussion

Most organisms exhibit daily physiological and behavioral rhythms that are regulated by molecular circadian clocks. Light is the most common signal that entrains these rhythms (Reitzel et al., 2010). *Apostichopus japonicus* is nocturnal, sensitive to light and tends to keep away from light. The animals usually hide during daylight hours and feed during the dark of night (Sun et al., 2015). *Apostichopus japonicus* prefers habitats with low light intensity. Lin et al. (2013) found that *A. japonicus* moved quickly to the low light area after being placed in the center of the flume within 90 min. They prefer to spawn in the dark and have distinct rhythms, most individuals retreating to shelter during the daytime and emerging and feeding during the night (Zhang et al., 2015a; Dong et al., 2011). They usually inhabit and are attached to the shadow area of reefs when exposed to strong light (Zhang et al., 2006; Zhang et al., 2009; Chen et al., 2007). As a result, when *A. japonicus* is exposed to different light conditions, specific behavioral and physiological characteristics may be observed and molecular regulatory mechanisms may be affected.

Large scale gene expression profiling may facilitate the identification of systemic gene expression and regulatory mechanisms for the environmental tolerance of sea cucumbers. In this study, RNA-seq analysis was used to allow a comprehensive evaluation of differences in gene expression in the body wall of the sea cucumber *A. japonicus* under the influence of full darkness and strong light. The results indicated that exposure of *A. japonicus* to environments of different light intensity is associated with thousands of transcriptional variations. Numerous light-associated genes (Tables 4–6) showed different levels of expressions under the influences of full darkness and the strong light environment. These genes may be excellent candidates for future studies on the molecular mechanisms associated with the behavior of *A. japonicus* under different light conditions. In addition, based on the annotation of unigenes, the classification of DEGs was conducted by a GO process in terms of different functions, biological processes, and locations (Table 4, Fig. 2). Pathway enrichment analysis identified the most significantly affected pathways when sea cucumbers were exposed to light of different intensities (Table 5).

4.1 *Genes associated with movement of cells or subcellular components*

Molecular motors are biological machines. They are the essential agents of movement in animals. The movement of all kinds of tissues, organs, and even the whole body is ultimately attributed to the movement of molecular motors. Dynein is a molecular motor in cells which can convert the chemical energy contained in ATP into the mechanical energy of movement.

Table 5. Top ten Significant enrichment of pathways for DEGs

Number	Pathway	DEGs with pathway annotation (662)	All genes with pathway annotation (10 236)	*P* value	*Q* value	Pathway ID
"Dark" vs. "control"						
1	Focal adhesion	65 (9.82%)	463 (4.52%)	1.64×10^{-9}	2.10×10^{-7}	ko04510
2	ErbB signaling pathway	21 (3.17%)	71 (0.69%)	1.97×10^{-9}	2.10×10^{-7}	ko04012
3	Fc gamma R-mediated phagocytosis	25 (3.78%)	115 (1.12%)	5.88×10^{-8}	4.19×10^{-6}	ko04666
4	Insulin signaling pathway	26 (3.93%)	132 (1.29%)	2.60×10^{-7}	1.18×10^{-5}	ko04910
5	Bacterial invasion of epithelial cells	25 (3.78%)	124 (1.21%)	2.76×10^{-7}	1.18×10^{-5}	ko05100
6	Chemokine signaling pathway	25 (3.78%)	129 (1.26%)	6.08×10^{-7}	1.91×10^{-5}	ko04062
7	Fc epsilon RI signaling pathway	17 (2.57%)	66 (0.64%)	6.24×10^{-7}	1.91×10^{-5}	ko04664
8	Chronic myeloid leukemia	17 (2.57%)	69 (0.67%)	1.23×10^{-6}	3.29×10^{-5}	ko05220
9	Neurotrophin signaling pathway	22 (3.32%)	112 (1.09%)	2.26×10^{-6}	5.39×10^{-5}	ko04722
10	Natural killer cell mediated cytotoxicity	14 (2.11%)	53 (0.52%)	4.43×10^{-6}	9.48×10^{-5}	ko04650
"Light" vs. "control"						
1	ECM-receptor interaction	8 (20%)	245 (2.39%)	3.81×10^{-6}	0.000156	ko04512
2	Focal adhesion	8 (20%)	463 (4.52%)	0.000 353	0.007 236	ko04510
3	PPAR signaling pathway	3 (7.5%)	91 (0.89%)	0.005 295	0.064 526	ko03320
4	Rheumatoid arthritis	3 (7.5%)	101 (0.99%)	0.007 071	0.064 526	ko05323
5	Staphylococcus aureus infection	3 (7.5%)	105 (1.03%)	0.007 869	0.064 526	ko05150
6	Complement and coagulation cascades	4 (10%)	250 (2.44%)	0.015 939	0.108 916	ko04610
7	Hematopoietic cell lineage	2 (5%)	60 (0.59%)	0.022 85	0.133 834	ko04640
8	Oxidative phosphorylation	3 (7.5%)	178 (1.74%)	0.031 919	0.145 074	ko00190
9	Autoimmune thyroid disease	1 (2.5%)	9 (0.09%)	0.034 639	0.145 074	ko05320
10	Cell adhesion molecules (CAMs)	2 (5%)	76 (0.74%)	0.035 384	0.145 074	ko04514
"Light" vs. "dark"						
1	Chemokine signaling pathway	38 (3.85%)	129 (1.26%)	1.65×10^{-10}	3.77×10^{-8}	ko04062
2	ErbB signaling pathway	26 (2.63%)	71 (0.69%)	7.24×10^{-10}	8.29×10^{-8}	ko04012
3	Focal adhesion	85 (8.6%)	463 (4.52%)	2.72×10^{-9}	2.07×10^{-7}	ko04510
4	Bacterial invasion of epithelial cells	33 (3.34%)	124 (1.21%)	4.23×10^{-8}	2.42×10^{-6}	ko05100
5	Chronic myeloid leukemia	23 (2.33%)	69 (0.67%)	5.42×10^{-8}	2.48×10^{-6}	ko05220
6	Fc gamma R-mediated phagocytosis	31 (3.14%)	115 (1.12%)	7.88×10^{-8}	3.01×10^{-6}	ko04666
7	Axon guidance	40 (4.05%)	172 (1.68%)	9.57×10^{-8}	3.13×10^{-6}	ko04360
8	Neurotrophin signaling pathway	30 (3.04%)	112 (1.09%)	1.49×10^{-7}	4.25×10^{-6}	ko04722
9	Natural killer cell mediated cytotoxicity	19 (1.92%)	53 (0.52%)	2.12×10^{-7}	4.87×10^{-6}	ko04650
10	Insulin signaling pathway	33 (3.34%)	132 (1.29%)	2.13×10^{-7}	4.87×10^{-6}	ko04910

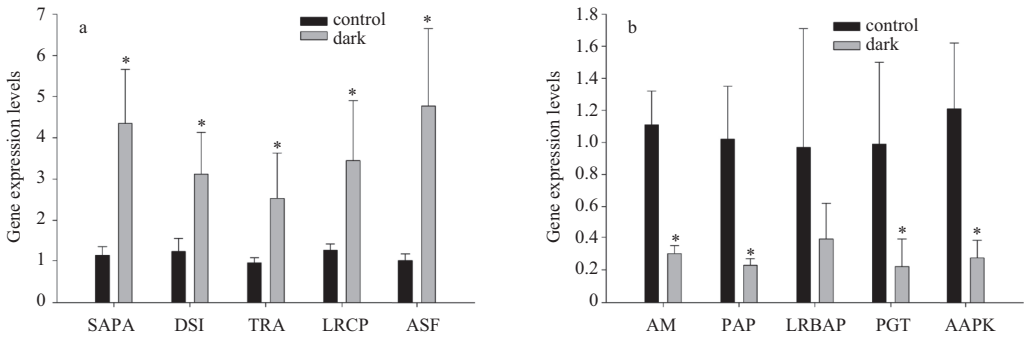

Fig. 3. Real-time PCR analysis for the top five up-regulated and five down-regulated genes in "dark" vs. "control". a. SAPA represents serum amyloid protein A; DSI dynactin subunit 5-like isoform 1; TRA transcobalamin I-like; LRCP Leucine-rich repeat-containing protein; and ASF ATP synthase-coupling factor 6, mitochondrial-like. b. AM represents alpha-2-macroglobulin-like; PAP papilin-like; LRBAP lipopolysaccharide-responsive and beige-like anchor protein-like, partial; PGT procollagen galactosyltransferase 1-like; and AAPK AP2-associated protein kinase 1-like. * indicates significant differences between dark and control groups ($P<0.05$). Values indicate the mean±SD (N=3).

Dynein works associated with another large protein complex called dynactin which is required for virtually all known functions of dynein (Haghnia et al., 2007). After binding to dynactin, dynein transports various cellular cargos, which vary from mRNAs to entire organelles, by "walking" along cytoskeletal microtubules towards the minus-end of the microtubule (Mallik and Gross, 2004). The results of this study revealed the differential expression of numerous movement-associated genes under

Table 6. Key DEGs associated with light influence

Gene	Gene ID	Normal light (RPKM)	Dark light (RPKM)	Strong light (RPKM)
DEGs associated with movement of cell or subcellular component				
Dynein light chain roadblock-type 2	isotig08721	2.01	9.91	5.44
Cytoplasmic dynein 1 heavy chain 1-like isoform 2	isotig17784	35.45	7.28	44.50
Cytoplasmic dynein 1 light intermediate chain 2-like	isotig14932	44.63	13.62	45.16
Dynactin subunit 5-like isoform 1	isotig09020	1.03	12.81	3.92
Dynactin subunit 1-like	isotig13914	52.68	21.35	56.15
Kinesin-like protein KIF1B-like	isotig13747	10.23	1.89	10.42
Kinesin heavy chain	isotig13276	70.32	31.54	83.28
Myosin VI	isotig16090	33.15	6.43	29.10
Myosin phosphatase Rho-interacting protein-like	isotig15515	18.64	4.99	17.80
Dedicator of cytokinesis protein 9	isotig15901	36.91	12.50	45.66
Unconventionnal myosin-X	isotig18746	67.90	23.13	68.92
Unconventional myosin-Id isoform 2	isotig09887	32.04	11.11	32.50
Amoeboid myosin I	isotig17115	73.39	26.92	79.33
Amoeboid myosin I	isotig18961	58.36	23.61	69.64
Myosin-IIIB	isotig13235	58.08	24.39	85.71
Unconventional myosin-XVIIIa	isotig13782	100.30	48.78	101.05
DEGs associated with metabolic process				
Pancreatic alpha-amylase-like	isotig10221	370.71	801.41	365.99
6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase	isotig13300	47.33	19.40	42.36
1-phosphatidylinositol 4, 5-bisphosphate phosphodiesterase gamma-1	isotig20602	77.37	11.79	81.98
Glucosamine-6-phosphate deaminase 1-like	isotig03620	5.00	16.57	7.16
Pancreatic alpha-amylase	isotig10221	370.71	801.41	365.99
Procollagen galactosyltransferase 1	isotig18726	31.59	1.99	28.10
60S ribosomal protein L10a	isotig10422	5.49	10.88	0.01
60S ribosomal protein L23a	isotig11170	136.53	321.65	123.20
Serum amyloid protein A	isotig08093	1.58	32.79	7.52
MICAL-3	isotig01569	5.56	0.24	3.79
ATP synthase-coupling factor 6, mitochondrial-like	isotig03824	3.4	17.29	7.03
ATP synthase subunit gamma, mitochondrial-like	isotig08835	14.37	34.42	17.24
ATP-binding cassette sub-family A member 3	isotig19240	18.77	2.08	20.79
ATP-binding cassette transporter subfamily A	isotig17944	43.16	6.75	58.96
Phospholipid-transporting ATPase ID-like	isotig28066	48.73	10.88	27.27
ATP-binding cassette transporter subfamily A	isotig14200	73.21	19.70	91.71
Potassium-transporting ATPase subunit beta-1-like isoform 1	isotig06693	44.26	14.35	43.90
DEGs associated with stimulus and Immune defense				
C-type lectin	isotig10929	32.86	99.83	45.56
Fucolectin-7	isotig03693	35.74	101.54	16.75
Fibrinogen-like protein A	isotig19241	298.89	823.54	538.43
Fibrinogen C domain-containing protein 1-like	isotig16485	16.98	45.01	2.70
Fibropellin-1	isotig05245	835.64	1 925.45	899.84
Alpha-2-macroglobulin	isotig16383	51.50	2.05	3.17
DEGs associated with signal transduction				
Translationally-controlled tumor protein	isotig09381	21.08	56.66	24.34
Translocon-associated protein subunit gamma	isotig07409	24.06	59.79	29.43
Alpha-1D adrenergic receptor	isotig10079	19.04	44.94	16.74
Thioredoxin	isotig10271	19.01	40.24	12.42
Lipopolysaccharide-responsive and beige-like anchor protein-like, partial	isotig24634	35.58	2.19	49.82
DEGs associated with photoreceptors				
CREB-regulated transcription coactivator 1	isotig21193	28.70	3.32	22.26

environments with different levels of light (Tables S1–S3). It was found that the well-known movement associated genes dynactin subunit 5-like isoform 1 and dynein light chain roadblock-type 2 were over-expressed under dark conditions compared with the normal and strong light environments. It can be inferred that some biomacromolecules involved in movement processes correspond to the active behavior in *A. japonicus* when being placed under dark conditions.

However, cytoplasmic dynein 1 heavy chain 1-like isoform 2, cytoplasmic dynein 1 light intermediate chain 2-like, and dynactin subunit 1-like were up-regulated under normal and strong light conditions when compared with dark environment (Table 6).

In addition, a large variety of myosin genes, such as myosin VI, myosin phosphatase Rho-interacting protein-like, dedicator of cytokinesis protein 9, unconventional myosin-X, unconventional myosin-Id isoform 2, amoeboid myosin I, myosin-IIIB, and unconventional myosin-XVIIIa, were shown to be up-regulated under normal and strong light conditions compared with fully dark environment in this study (Table 6). Myosins are a superfamily of motor proteins that move along actin filaments, while hydrolyzing ATP. They are import for muscular contraction and account for 40%–50% of the total proteins in muscles (Mehl, 1940). Myosin light chain phosphorylation was also found to regulate contraction in the body wall muscles of the sea cucumber *Parastichopus californicus* (Kerrick and Bolles, 1982). Interestingly, the myosin genes that were overexpressed under different light conditions in this study have also been suggested to be involved in muscle differentiation in *A. japonicus* (Sun et al., 2011) and *Holothuria glaberrima* (Ortiz-Pineda et al., 2009). In the previous study, it was found the distribution of *A. japonicus*, being placed in the flume after 1 h, became stable in comparatively dark area (Lin et al., 2013). In this study, the sea cucumbers were sampled after 2 h's stress, and it might bring about a comparative stable status in the latter for sea cucumbers under fully dark environment. The sea cucumbers under strong and normal light conditions might be still searching some area with low illumination level. Maybe it is the reason why the variety of gene homologs associated with movement were up-regulated under normal and strong light environments.

The expression of genes encoding dynein, dynactin and myosin, to which may be attributed the movement of *A. japonicus*, may be coordinated in response to light intensity. This may underlie the molecular mechanism of movement in *A. japonicus* under conditions of different light intensity.

4.2 *Genes associated with metabolism*

When active behaviors occur, energy will be consumed in *A. japonicus*, as in all other organisms. Biological processes related to metabolism of substances and energy will be activated. Among the annotated unigenes, 60S ribosomal protein L10a, belonging to the ribosomal protein L1P family, which has the function of preventing protein synthesis inhibition, mRNA–rRNA processing and signal transduction (Kim and Jang, 2002; Warner and McIntosh, 2009), was found to be down-regulated under strong light compared with fully dark conditions (Table 6). The protein is a component of the large 60S subunit of ribosome. Ribosomes, which consist of a small 40S subunit and a large 60S subunit, are the organelles functioning in catalyzing protein synthesis. It can be inferred that some protein synthesis function might be restricted in *A. japonicus* under strong light condition, and might be promoted when *A. japonicus* is active under fully dark condition.

Light is a powerful environmental factor for *A. japonicus*. The growth, behavior, and digestive physiology of the sea cucumber may be affected by light intensity and photoperiod (Dong et al., 2010a, b, 2011; Sun et al., 2015). Exposure to different color spectra may also result in different growth performance and energy budgets in *A. japonicus* (Bao et al., 2014). There is a close connection between ATP and energy metabolism (Wang et al., 2012). In this study, some of the mRNA expressions of genes related to ATP synthase, e.g., ATP synthase subunit gamma, mitochondrial-like, ATP synthase subunit gamma, mitochondrial-like, ATP-binding cassette sub-family A member 3, ATP-binding cassette transporter subfamily A were regulated differently under the fully dark and strong light conditions compared with the normal light condition (Table 6), which indicated that the differences of ATP synthesis and energy metabolism may occur when the animals are exposed to different light conditions. The mRNA expression of ATP synthase was down-regulated when *A. japonicus* was in a state of torpor, such as in the aestivation state or when challenged with thermal stress (Zhao et al., 2014a; Shao et al., 2015). It has been suggested that ATP synthase plays an important role in energy metabolism in *A. japonicus* when it responds to light and temperature.

Serum amyloid A (SAA) proteins, found in all mammals, ducks, salmonid fishes, and even echinoderms, comprise a family of highly conserved apolipoproteins (Santiago et al., 2000). They possess enough functional diversity to participate in and regulate metabolic processes. SAAs were up-regulated in the body wall of *A. japonicus* under fully dark conditions and down-regulated under the strong light condition when compared with the normal condition respectively (Table 6). However, in the previous study, SAA was significantly over-expressed in *A. japonicus* undergoing deep aestivation with hypometabolism, compared with non-aestivation (Zhao et al., 2014a, b). The different regulations of SAA at mRNA level, in the less active circumstances of *A. japonicus* under strong light conditions and in the aestivation period, may indirectly indicate the functional diversity of SAA in regulating metabolic processes.

4.3 *Genes associated with stimulus and signal transduction*

Light is a key environmental factor. In the study, when the sea cucumbers were transferred directly from tanks under normal light to tanks under fully dark or strong light environment, the sharp changes of the light condition might be stimuli to *A. japonicus*, and immune defenses could be triggered. Lectins are a group of proteins which bind to cell surface carbohydrates and play critical roles in innate immunity. Fucolectin-7, which recognizes blood group fucosylated oligosaccharides and acts as a defensive agent (Wu et al., 2004), was found to be up-regulated in "dark" compared with "control" in this study (Table 6). Lipopolysaccharide (LPS)-stimulated expression of fucolectins in the Japanese eel *Anguilla japonica* (Honda et al., 2000) suggests that they serve as powerful defense agents. Fucolectin-7 was also found in the pathogen recognition system of *A. japonicus* (Dong et al., 2014). Some genes related to fibrinogen, which is important for the immune system of both vertebrates and invertebrates (Xu and Doolittle, 1990), for example fibrinogen-like protein A and fibrinogen C domain-containing protein 1-like, were also found to be over-expressed under fully dark conditions (Table 6). The changes in immune response genes such as fucolectin-7 and fibrinogen suggest that self-defense mechanisms are activated in response to light density. The different expressions of genes related to immune defense might be the responses of *A. japonicus* to the sharp changes of light condition, which is different from the natural light.

Furthermore, some signal transduction related genes were involved. For example, alpha-1D adrenergic receptor and thioredoxin, were found to be up-regulated under dark condition compared with normal light and strong light conditions (Table 6), when *A. japonicus* is active. The alpha-1D adrenergic receptor mediates its effect through the influx of extracellular calcium, and may play an important role in the phospholipase C-activating G-protein coupled receptor signaling pathway. Wheel-running activity, exploratory rearing behavior in a novel cage environment, and hyperlocomotion are significantly reduced in mice with mutated copies of this gene. The alpha-1D adrenergic receptor signaling gene is required for stimulus-induced locomotor activity (Sadalge et al., 2003). This may be why it is up-reg-

ulated under a fully dark environment, when *A. japonicus* shows active locomotion. Thioredoxins (TRxs), known to be present in all living organisms, are a family of small evolutionarily conserved proteins. They are critical for the maintenance of cellular homeostasis. They act roles in many important biological processes, including positive regulation of protein kinase B signaling, participating in various redox reactions. In the present study, the up-regulation of TRx under dark environment might be because of its participation of some signaling pathway. And further investigation should be conducted to unveil the regulation mechanism.

Lipopolysaccharide-responsive and beige-like anchor was down-regulated under dark condition compared with strong light and normal light conditions (Table 6). This protein may be involved in coupling signal transduction and vesicle trafficking to enable polarized secretion and/or membrane deposition of immune effector molecules. Compared to dark, the up-regulation lipopolysaccharide-responsive and beige-like anchor protein-like, partial in light group might be an indication that the signal transduction associated with immune system was involved in *A. japonicus* under strong light.

4.4 *Genes associated with photoreceptors*

It is very important for animals to detect light and interact with the environment (Ullrich-Lüter et al., 2011). Optimization in all aspects of an organism's performance according to the daily light–dark cycle is essential for normal physical function (Highland et al., 2014). Photoreceptors can convert light into signals, following which several biological processes may be stimulated. CREB-regulated transcription coactivator 1 (CRTC1) is a transcriptional coactivator for CREB1, which is involved in synchronization of circadian rhythmicity. CRTC1 and salt inducible kinase 1 (SIK1) participate in the CRTC1–SIK1 pathway, which regulates the light-induced entrainment of the circadian clock (Jagannath et al., 2013). In response to a light stimulus, CREB-mediated transcription plays an important role in the photic entrainment of the circadian clock (Sakamoto et al., 2013). Compared to fully dark group, CRTC1 was found to be up-regulated significantly in the body wall of *A. japonicus* under normal and strong light conditions (Table 6). This finding indirectly proves CRTC1 is important in light-induced regulations in *A. japonicus*, and partly reveals the circadian clock of *A. japonicus* might be regulated under conditions of different light intensity (Lin et al., 2013). In addition, CRTC1 may be an important gene for future study of the mechanism underlying the behavioral rhythms of sea cucumbers.

Some species of echinoderm have photosensory organs related to light illumination. Photoreceptor cells have been found in the tube feet of the purple sea urchin, *Trongylocentrotus purpuratus*. The two genes *Sp-opsin4* and *Sp-pax6* are essential for photoreceptor function and development, respectively. Specific reactivity of the Sp-opsin4 antibody with sea star optic cushions, which regulate phototaxis, suggests a similar visual function in sea urchins (Ullrich-Lüter et al., 2011). Opsin protein gene expression has also been found in the brittle star *Amphiura filiformis* and the sea star *Asterias rubens* (Delroisse et al., 2013, 2014). However, in this study, opsin protein genes were not detected, perhaps because of the limitation of the reference genomes currently available.

A large proportion of DEGs were not annotated in this study, and they may include the light-associated genes that encode the photoreceptors, which may play key roles in adaption to different environments. The sea cucumber *A. japonicus*, which has slightly different morphological characteristics from other echinoderms, has undeveloped bones with tiny scattered ossicles inside body wall. Future behavioral and histological studies, and the use of molecular biology methods, are needed to determine whether *A. japonicus* has similar photoreceptors.

5 Conclusions

Large scale gene expression profiling of body wall of *A. japonicus*, comparing animals exposed to natural light ("control"), strong light ("light") and full darkness ("dark") identified a series of candidate genes and GO terms that indicate that proteins involved in these processes are important to regulating the biological responses to different light conditions in echinoderms. Light-specific DEGs identified in this study will be important targets for further investigation to establish the biochemical mechanisms involved in the adaption of *A. japonicus* to changes in the level of environmental light.

Acknowledgements

The authors thank Beijing Genomics Institute (Shenzhen, China) for high throughput sequencing of the mRNA library.

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Supplementary information:

Table S1. The top 20 up- or down-regulated DEGs from "dark" vs. "control".

Table. S2. The top 20 up- or down-regulated DEGs from "light" vs. "control".

Table. S3. The top 20 up- or down-regulated DEGs from "light" vs. "dark".

Fig. S1. Quality assessment of reads (light representing strong light, dark fully dark, and control normal light).

Fig. S2. Sequencing saturation analysis (light representing strong light, dark fully dark, and control normal light).

Fig. S3. Randomness assessment (light representing strong light, dark fully dark, and control normal light).