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Investigation

Vitamin A deficiency affects gene expression in the *Drosophila melanogaster* head

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Abstract

Insufficient dietary intake of vitamin A causes various human diseases. For instance, chronic vitamin A deprivation causes blindness, slow growth, impaired immunity, and an increased risk of mortality in children. In contrast to these diverse effects of vitamin A deficiency (VAD) in mammals, chronic VAD in flies neither causes obvious developmental defects nor lethality. As in mammals, VAD in flies severely affects the visual system: it impairs the synthesis of the retinal chromophore, disrupts the formation of the visual pigments (Rhodopsins), and damages the photoreceptors. However, the molecular mechanisms that respond to VAD remain poorly understood. To identify genes and signaling pathways that are affected by VAD, we performed RNA-sequencing and differential gene expression analysis in *Drosophila melanogaster*. We found an upregulation of genes that are essential for the synthesis of the retinal chromophore, specific aminoacyl-tRNA synthetases, and major nutrient reservoir proteins. We also discovered that VAD affects several genes that are required for the termination of the light response: for instance, we found a downregulation of both *arrestin* genes that are essential for the inactivation of Rhodopsin. A comparison of the VAD-responsive genes with previously identified blue light stress-responsive genes revealed that the two types of environmental stress trigger largely nonoverlapping transcriptome responses. Yet, both stresses increase the expression of seven genes with poorly understood functions. Taken together, our transcriptome analysis offers insights into the molecular mechanisms that respond to environmental stresses.

Keywords: vision; *Drosophila*; photoreceptor; vitamin A; rhodopsin; chromophore; carotene; retinoic acid; visual pigment; rhabdomere; phototransduction; transcriptome

Introduction

Animals cannot synthesize vitamin A *de novo* and therefore need to generate it from dietary precursors such as β-carotene. These precursors are essential for the synthesis of the retinal chromophore, which binds to an opsin protein to form the visual pigment Rhodopsin (von Lintig 2012; Saari 2016; Dewett *et al.* 2021). Chronic vitamin A deficiency (VAD) in mammals causes a lack of Rhodopsin, damage of the rod outer segments, and rod photoreceptor death (Dowling and Wald 1958, 1960; Cornwall and Fain 1994; Melia *et al.* 1997; Fain 2006). Since vitamin A is also required for retinoic acid signaling in mammals, VAD also affects development and immunity (Sommer 2008). These essential functions of vitamin A make it difficult to study the molecular consequences of chronic VAD in mammalian models.

In contrast to mammals, *Drosophila melanogaster* does not use vitamin A for canonical retinoic acid signaling (Oro *et al.* 1990; Bonneton *et al.* 2003; Kam *et al.* 2012) and therefore does not require vitamin A for survival or essential developmental processes. Yet, VAD causes defects in the fly eye that resemble the ones in the mammalian eye: a lack of mature Rhodopsin 1 (Harris *et al.* 1977; Nichols and Pak 1985; Ozaki *et al.* 1993; Huber *et al.* 1994), dramatically reduced visual sensitivity (Chen and Stark 1992), and rhabdomere damage that is equivalent to mammalian outer segment defects (Lee *et al.* 1996). This predominant use of vitamin A for vision makes *D. melanogaster* an ideal model system for studying the poorly understood molecular response to chronic VAD.

In this study, we took advantage of the *Drosophila* model to ask whether VAD affects the expression of vision-related genes that are required for the synthesis of the retinal chromophore or encode components of the phototransduction machinery (Figure 1A). For instance, the evolutionarily conserved β-carotene 15,15'-dioxygenase NinaB, a homolog of mammalian BCO1 (Kiefer *et al.* 2001; von Lintig and Wyss 2001; Lindqvist and Andersson 2002; Hessel *et al.* 2007), generates retinal from β-carotene (von Lintig and Vogt 2000; von Lintig *et al.* 2001; Oberhauser *et al.* 2008; Voolstra *et al.* 2010) (Figure 1A). Because VAD impairs the synthesis of retinal, we asked in the current study whether VAD changes the expression of genes that are required for vitamin A metabolism (see Results and Discussion).

The vitamin A-derived retinal chromophore covalently binds to a specific opsin protein (Figure 1A) to form one of seven *Drosophila* Rhodopsin pigments (Rister *et al.* 2013; Senthilan and Helfrich-Forster 2016). The VAD-induced lack of retinal causes

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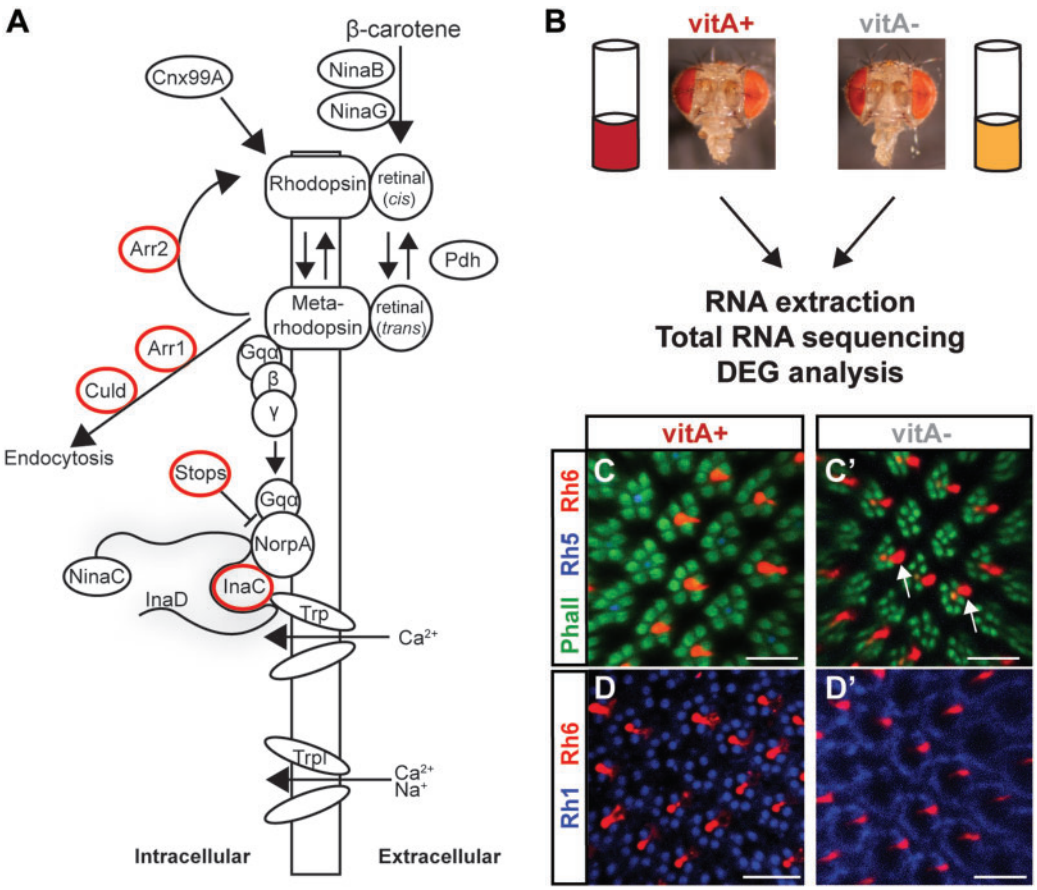

Figure 1 Vitamin A deprivation affects *Drosophila* photoreceptor structure and Rhodopsin expression. (A) The schematic depicts the key steps of phototransduction. Dietary β-carotene is converted by NinaB and NinaG to the retinal chromophore that binds to opsin to form the Rhodopsin pigment. Activation of Rhodopsin triggers the phototransduction cascade and results in the opening of two types of cation channels, Trp and Trpl. The termination of the light response is mediated by two Arrestins (Arr1 and Arr2), which inactivate Rhodopsin, and several downstream factors (Stops, InaC, and Culd). The factors that terminate phototransduction are highlighted by a red outline. For details, see text. (B) Flies were raised on minimal medium with β-carotene (vitA+, left) or without β-carotene (vitA−, right). The images show that vitamin A deprivation had no obvious effect on the external morphology of the head or the eye. Total RNA was extracted from heads of adult flies for sequencing and differential gene expression (DEG) analysis. (C) Vitamin A replete (vitA+) wild-type adult eye. The rhabdomeres (green) have a round shape and the inner photoreceptors express Rh5 (blue) or Rh6 (red). (C') Chronic vitamin A deprivation (vitA−) causes small rhabdomeres (green, compare to C) and affects Rhodopsin expression in the adult eye: Rh6 (red) is abnormally accumulated (arrows) outside of the rhabdomeres (green) and Rh5 is not detectable. (D) The vitamin A replete (vitA+) wild-type retina expresses mature Rh1 (blue). (D') Vitamin A deprivation (vitA−) impairs Rh1 (blue) maturation and results in an abnormal localization (compare to D). Scale bars, 10 μm.

the accumulation of immature opsin in the endoplasmic reticulum (Ozaki *et al.* 1993; Huber *et al.* 1994). This results in a lack of mature Rhodopsin that is required for the initiation of phototransduction (Hardie and Juusola 2015). In contrast, in vitamin A replete flies, light-activated Rhodopsin isomerizes to Metarhodopsin and causes the release of the Gqα subunit that activates the phospholipase C NorpA (Figure 1A). This ultimately opens two types of Ca^{2+} channels, Trp (Montell and Rubin 1989) and Trp-like (Trpl) (Phillips *et al.* 1992), and the Ca^{2+} influx depolarizes the photoreceptor.

Several factors terminate the phototransduction cascade (Figure 1A): the visual Arrestins Arr1 and Arr2 inactivate Metarhodopsin (Dolph *et al.* 1993), while the eye-specific protein kinase InaC inhibits NorpA (Smith *et al.* 1991) and Trp (Popescu *et al.* 2006). Moreover, the SOCS box protein Stops promotes the GTPase-activating activity of NorpA, which results in the deactivation of the G protein (Wang *et al.* 2008). In our study, we assessed whether the VAD-induced impairment of light detection affects the expression of these phototransduction-related genes.

In addition to its essential role in vision, β-carotene has been proposed to have anti-inflammatory (Kaulmann and Bohn 2014) and antioxidant properties that protect membranes against oxidative damage (Britton 1995; Gruszecki and Strzałka 2005; Krinsky and Johnson 2005; Edge and Truscott 2018). We, therefore, asked whether VAD altered the expression of genes that have been linked to oxidative stress or inflammation.

Here, we compared the head transcriptomes of vitamin A replete and chronically deprived *D. melanogaster* to characterize the signaling pathways and genes whose expression is affected by VAD (Figure 1B). We identified differentially expressed genes (DEGs) that are essential for the synthesis of the retinal chromophore and the termination of phototransduction. Moreover, we detected significant changes in the expression of genes that encode specific aminoacyl-tRNA synthetases, major nutrient reservoir proteins, calcium buffers, and factors that mediate stress or immune responses. Lastly, we compared these VAD-responsive genes to previously identified blue light stress-responsive genes (Hall *et al.* 2018) and found very little overlap in the transcriptome

response to these two different types of environmental stress. Taken together, our study offers insights into the molecular mechanisms that respond to different environmental stresses.

Materials and methods

Fly stocks and food media

We raised wild-type Canton S flies at 25°C (50% humidity, 12 h light/12 h dark cycle) on minimal baker's yeast-based medium either with (vitA+) or without (vitA−) supplementation of β-carotene as a source of vitamin A. For each food type, we dissolved 0.1 g of stigmasterol (Sigma), a dietary plant sterol that *Drosophila* uses for membrane and hormone production (Knittelfelder *et al.* 2020), in 2 ml of 95% ethanol. For vitA+ food, we additionally dissolved 0.1 g of β-carotene (Sigma) in 2 ml of 95% ethanol. The stigmasterol and β-carotene solutions were vortexed and kept for one hour in a sonicating water bath (Cole-Parmer, set to 37°C) for accelerated dissolution of the solids. For each food type, we then dissolved 10 g of yeast extract (Kerry), 10 g of glucose (Merck), and 1 g of UltraPure Agarose (Invitrogen) in 100 ml of filtered tap water. We microwaved the mixture until it was boiling and then allowed it to cool down to 65–70°C. For both food types, we added the stigmasterol solution (see above) and 1.5 ml of 10% nipagin (Sigma-Aldrich) to the mixture. To obtain vitA+ food, we additionally added the β-carotene solution (see above) to the mixture. After thoroughly mixing for a few minutes, we poured 10–15 ml of vitA+ or vitA− medium into empty *Drosophila* plastic vials (Genesee Scientific) and let the medium solidify at room temperature. We stored the food vials at 4°C for up to 2 weeks until use.

RNA extraction, library preparation, and sequencing

For each biological replicate, we flash froze 100 four-day-old wild-type Canton S female flies in liquid nitrogen and stored them at −80°C. We then separated the frozen fly heads from the bodies using Hogentogler sieves (no. 24 and no. 40). We used TRIzol (Life Technologies) for total RNA extraction, chloroform for purification, and isopropanol for precipitation. We further purified the RNA using the RNeasy Mini Kit (Qiagen).

We constructed the RNA libraries in parallel using the Ovation® *Drosophila* RNA-Seq System 1-16 library preparation kit (Nugen), depleted the ribosomal RNA, and standardized the RNA to a 50 ng input. After quality control using a Bioanalyzer, we performed 2 × 51 paired-end Rapid Run sequencing with a HiSeq 2500 System (Illumina; the runs were performed at the Center for Personalized Cancer Therapy Genomics Core, University of Massachusetts Boston) that yielded ∼100 million reads per lane. All samples were run together in a single pool using on-board cluster generation. For each of the two food conditions (vitA+ or vitA−), we sequenced three biological replicates.

RNA-seq data analysis

We trimmed the sequencing reads using Trim Galore (v0.4.2) and mapped them against the *D. melanogaster* genome (dmel r6.24, www.flybase.org) using the STAR aligner (v2.5.3a) with default parameters (Dobin *et al.* 2013). Next, we generated the raw counts matrix using featureCounts from the Subread package (v1.6.2) (Liao *et al.* 2014) with default parameters. We then performed a differential gene expression (DEG) analysis on genes that showed more than one count per million in at least three samples for the vitA+ and vitA− conditions. To identify DEGs, we used the glmLRT negative binomial generalized linear models in the edgeR package (v3.24.3) (Robinson *et al.* 2010; McCarthy *et al.* 2012) with an FDR <0.05 and abs(logFC) >1.5.

To generate the bar plot and error bars for the RNA-seq data shown in Figure 4, we ran DESeq from the DESeq2 R package (v. 1.22.1) (Love *et al.* 2014) and used the log2FoldChange as well as the lfcSE (the standard error of the log2FoldChange).

Gene ontology term analysis

We performed a gene ontology (GO) term analysis using g:Profiler (Raudvere *et al.* 2019) on the identified DEGs (see above). GO terms with a *P*-value < 0.05 were considered significant.

Eye enrichment analysis

For the identified DEGs, we downloaded eye and brain RNA-seq data as well as enrichment data for adult female *D. melanogaster* from FlyAtlas 2 (http://flyatlas.gla.ac.uk/FlyAtlas2/index.html) (Leader *et al.* 2018). We considered DEGs that had an enrichment score equal or greater than 10 as enriched in a specific tissue.

Immunohistochemistry and confocal microscopy

As previously described (Hsiao *et al.* 2012), we dissected retinas of 3- to 5-day-old female wild-type Canton S flies in cold phosphate-buffered saline (PBS, Sigma). After removing the brain tissue (except the lamina) and most of the cuticle, we fixed the retinas in 3.7% formaldehyde solution for 15 min at room temperature. We then washed the retinas twice with PBS and once with PBST (PBS + 0.3% Triton-X, Sigma). Next, we removed the laminas and incubated the retinas overnight with the following primary antibodies that were diluted with PBST: mouse anti-Rh1 (4C5, 1:10, obtained from Developmental Studies Hybridoma Bank, University of Iowa), mouse anti-Rh5 (1:400, gift from S. Britt, the University of Texas at Austin) and rabbit anti-Rh6 (1:1000, gift from C. Desplan, New York University). The next morning, we performed three PBST washes. Then, we incubated the retinas in Alexa Fluor 488-conjugated Phalloidin (1:100; Invitrogen) and the secondary antibodies diluted in PBST (1:800; Alexa Fluor 555-conjugated or 647-conjugated raised in donkey; Molecular Probes) overnight at room temperature. The next morning, we again performed three washes with PBST. Using SlowFade (Molecular Probes), we mounted the retinas on bridge slides and imaged them with a Zeiss LSM 8 confocal microscope. We converted the confocal images with Fiji (Schindelin *et al.* 2012) and performed further image processing using Adobe Photoshop 2021 and Adobe Illustrator 2021.

RT-qPCR analysis

We performed RT-qPCR analysis using total RNA extracted from the heads of 4-day-old female wild-type Canton S flies that were raised on vitA+ or vitA− food (see above). We used the SuperScript™ IV VILO™ Master Mix with ezDNase™ Enzyme (Thermofisher Scientific) for cDNA synthesis. We designed the primers (Table 1) using NCBI-primer BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) to cover the coding regions and to yield a PCR product of 80–100 base pairs. *rp49* was the housekeeping control gene in all experiments. We used SYBR-green to measure the amount of the qPCR product and the QuantStudio™ 3 Real-Time PCR System (Thermofisher Scientific) for data analysis.

Statistical comparisons for three biological replicates of the vitamin A deficient experimental group (vitA−) and the vitamin A replete normalized control group (vitA+) were performed using a *t*-test. Significance levels are represented as *P*-values and summarized by asterisks: $P > 0.05$ was considered not significant (ns),

Table 1 Primers used for RT-qPCR validation of differentially expressed genes that respond to vitamin A deprivation

Gene	Forward primer	Reverse primer
ninaB	GATTATCCACGCAATGGCAGC	CGTTCCGCTTGCGGATCATT
ninaG	AGAGCTAATCCTCTGCGCTGG	GTTTCTTCAGGGCGGACACG
Cpn	GGAACCATTCCATCGCCTGT	ACTGCCGCAGGACTTACTACT
Arr1	GATCCAGCCTGCAGAAGGTC	TGATATCACCCTCAACGGCG
Arr2	GATCGCCATGGTATCGCCCT	GACTTGCCCTCCTGCACCAT
LeuRS	ATATGGCGGAGCATGTCTGG	CGTTGATGGCTCCCACTTCT
LysRS	CGGCAAAACCAAGAAGGGTG	CAGATGGGGCAGCATGTGTA
CG34138	GCACACCGCTCAACAAACAT	CAACACCCCGAATCCAGACA
CG11426	CCGCAAACGGCTAACTACCA	GGCTCCCCAGTGCTCTCTTA
rp49	GCAAGCCCAAGGGTATCGAC	GCTTGTTCGATCCGTAACCG

a single asterisk indicates significance at $P < 0.05$, two asterisks at $P < 0.01$, and three asterisks at $P < 0.001$. Error bars depict the standard error of the mean (s.e.m.).

Results

Dietary vitamin A deprivation affects photoreceptor morphology and Rhodopsin expression

To identify VAD-responsive genes and pathways, we used two minimal food media (pers. comm., Mukesh Kumar and Andrej Shevchenko, Max Planck Institute of Molecular Cell Biology and Genetics, Dresden) that we hereafter refer to as vitA− and vitA+ medium (Figure 1B). VitA− medium is baker's yeast-based and therefore lacks sources of vitamin A (Isono *et al.* 1988; Randall *et al.* 2015) (see *Materials and Methods*). VitA+ medium is based on vitA− medium but is supplemented with β-carotene as a source of vitamin A (Figure 1B). Four-day-old wild-type female flies that were raised under vitamin A replete conditions (vitA+ medium) had normal rhabdomere morphology and Rhodopsin expression (Figure 1, C and D). In contrast, consistent with previous studies, chronically vitamin A deprived 4-day-old wild-type female flies (vitA− medium) showed abnormally shaped rhabdomeres (Lee *et al.* 1996) and impaired Rhodopsin localization (Nichols and Pak 1985; Ozaki *et al.* 1993) (Figure 1, C'–D'). Since the vitA+ and vitA− food media had the expected effects on the eye, we used the same experimental conditions for our transcriptome analysis.

Identification and annotation of differentially expressed genes that respond to vitamin A deprivation

To identify DEGs that respond to VAD, we profiled the transcriptomes of total RNA from heads of 4-day-old wild-type female flies that had been raised either on vitA+ or on vitA− medium (Figure 1B). We analyzed three biological replicates for each food condition with edgeR [False Discovery Rate, FDR < 0.05; Fold change, abs(logFC) > 1.5] (Robinson *et al.* 2010) and identified 68 genes that were differentially expressed between the vitA+ and vitA− conditions. Of these 68 DEGs, 50 were upregulated (Table 2) and 18 were downregulated (Table 3) in response to VAD (Figure 2, A and B). VAD thus affects the expression of a relatively small set of genes in the adult head and most of these genes were upregulated in response to VAD.

To categorize the 68 DEGs according to their molecular functions, biological processes, or cellular compartments, we used the g: Profiler toolset (https://biit.cs.ut.ee/gprofiler/gost) (Raudvere *et al.* 2019) to perform a GO term analysis (Table 4). Consistent with the Rhodopsin maturation and visual signaling defects that are caused by VAD (Figure 1, C–D'), GO terms such as "response to light stimulus," "phototransduction," "retinoid metabolic process," and "Rhodopsin metabolic process" were highly enriched (Table 4 and Figure 3A). In addition, "aminoacyl-tRNA synthetase multienzyme complex" and "nutrient reservoir activity" were highly enriched GO terms (Table 4 and Figure 3A).

Since we analyzed head transcriptomes, we asked whether some of the 68 DEGs were specifically enriched in the eye or the brain under vitamin A replete conditions. We analyzed the corresponding tissue-specific expression data from FlyAtlas 2 (Leader *et al.* 2018) (see *Materials and Methods*) and identified six phototransduction-related DEGs (*Arr1*, *Pdh*, *Arr2*, *inaC*, *trpl*, and *stops*), whose transcripts were highly expressed in the eye (the FPKM values for the individual genes ranged from 488 to 10,377) and barely detectable in the brain (FPKM values from 1.3 to 30) (Table 5). Other highly eye-enriched genes were *Lsp2*, *CG6656*, and *CG7135*, whose function in this tissue remains to be elucidated. Conversely, we did not find any DEGs that were specifically expressed in the brain but not the eye. In summary, the GO term analysis revealed that VAD affects the expression of genes that are associated with visual signaling, retinoid and Rhodopsin metabolism, tRNA synthesis, and nutrient storage. A fraction of the VAD-responsive DEGs (9 of the 63 for which FlyAtlas data were available) are highly enriched in the eye, which is consistent with the fact that VAD predominantly causes eye defects in *Drosophila*.

Vitamin A deprivation affects genes that are involved in the synthesis of the retinal chromophore

Since vitamin A is essential for the synthesis of the retinal chromophore, we asked whether VAD causes a compensatory response of genes that promote the production of retinal. Indeed, VAD caused a significant change in the expression of three genes that are involved in retinoid metabolism (Figure 3, A and B): *ninaB*, *ninaG*, and *Pdh*. *NinaB* (*neither inactivation nor afterpotential B*) was upregulated by VAD and encodes the key enzyme that produces retinal (von Lintig *et al.* 2001). VAD also caused the upregulation of *ninaG* (*neither inactivation nor afterpotential G*), which encodes an oxidoreductase that has been proposed to mediate a subsequent step of chromophore biogenesis (Figure 3, A and B), the conversion of all-*trans* (3R)-3-hydroxyretinol to all-*trans* (3S)-3-hydroxyretinol (Ahmad *et al.* 2006). The VAD-induced upregulation of *ninaB and ninaG* could thus be a compensatory response to the low levels of retinal to increase the synthesis of the chromophore and to promote Rhodopsin maturation (see Discussion below).

In contrast to the upregulation of *ninaB and ninaG*, *Pdh* (*Photoreceptor dehydrogenase*) was downregulated by VAD (Figure 3A). Pdh is a dehydrogenase that mediates the recycling

Table 2 Differentially expressed genes that are upregulated by vitamin A deprivation

Gene	Adjusted P-value	Abs log2 fold change	Fold change
CG11426	5.40E-164	3.743006981	13.38928467
CG34138	1.20E-10	2.573425186	5.952209005
CG10650	0.016594695	2.347372851	5.088967053
pgant4	0.037672822	2.296827895	4.913761704
Jhl-21	2.40E-45	2.129491276	4.375631595
ninaG	3.63E-27	2.096939029	4.278007551
Lsp1beta	0.000355805	1.985419772	3.95977861
CG31636	0.000163277	1.916812671	3.77587936
CG5999	0.00429766	1.703716444	3.257389962
CG5535	3.47E-08	1.680950013	3.20639022
Cpn	6.19E-31	1.670694288	3.183677691
CG11449	0.004704868	1.531885687	2.891635459
CG5646	2.38E-11	1.528017869	2.883893462
Lsp1alpha	0.003629652	1.455813251	2.743111473
CG14907	0.024641493	1.349628279	2.548464539
LysRS	3.57E-19	1.338418878	2.528740295
CG9760	0.000656052	1.32568354	2.506516168
Cyp309a1	0.003681749	1.307972752	2.475933819
Cnx99A	1.71E-16	1.251012426	2.380083892
LeuRS	1.64E-11	1.205425851	2.306053288
CG16898	0.003582656	1.188064547	2.278468692
Tobi	0.000567102	1.164130325	2.240980857
ninaB	5.41E-11	1.14938166	2.218188024
CG16826	0.001344861	1.133390764	2.193737286
CG9305	1.90E-07	1.121799328	2.176182166
mia	0.006465203	1.115190329	2.166235855
Fie	4.34E-12	1.026500628	2.037077161
per	0.004974918	1.002333885	2.00323807
eys	7.86E-12	0.995791932	1.994174879
Rgk2	2.02E-09	0.979256657	1.971449366
CG7135	9.93E-06	0.932484064	1.908559368
HisCl1	0.018107125	0.917358777	1.88865446
IleRS	2.03E-08	0.899987916	1.866050353
AOX1	9.05E-05	0.821988041	1.767840408
eIF2Bepsilon	0.002826888	0.791094154	1.730386307
Dgp-1	0.000643096	0.757264324	1.690282426
Lsp2	0.000369576	0.753663155	1.686068506
Ack	4.22E-06	0.670488905	1.591612247
stops	0.00035006	0.661031865	1.581213157
Hrs	6.38E-05	0.660812591	1.580972848
Culd	0.009421209	0.642724882	1.561275226
kek4	0.008773579	0.641652346	1.560114966
CG4660	0.044309871	0.608176851	1.524331677
Sodh-1	0.0031575	0.576574957	1.491304598
dnr1	0.000297318	0.573883401	1.488524947
CG8034	0.000349475	0.571992133	1.486574878
cindr	0.000219643	0.569835607	1.484354421
CG9119	0.017844111	0.559528648	1.473787628
GluProRS	0.013104209	0.510704209	1.424745473
inaC	0.02107398	0.506688693	1.420785431

Table 3 Differentially expressed genes that are downregulated by vitamin A deprivation

Gene	Adjusted P-value	Abs log2 fold change	Fold change
Arr1	0.021315545	−0.591023555	1.50631506
CG17108	0.046636279	−0.697624171	1.621831761
CG6656	0.000262771	−0.709349465	1.635066672
mt:ND3	0.008123135	−0.791259233	1.730584316
Fib	0.012092127	−0.793484181	1.733255311
ple	0.010632124	−0.861094229	1.81641547
Arr2	5.97E-06	−0.886093231	1.848164575
Pdh	0.016594695	−0.886538449	1.848735009
CG1690	0.008258544	−1.08700642	2.124327826
trpl	9.79E-12	−1.180072643	2.26588186
TotA	1.12E-08	−1.354423106	2.556948505
Scp1	0.037717653	−1.613353713	3.059622607
CG17005	0.042512739	−1.872521485	3.66172002
TotC	2.31E-07	−1.966422671	3.907978881
CheA7a	0.012122991	−2.185036254	4.547382164
TotM	0.000297318	−2.23390049	4.704040528
Diedel	0.021141153	−2.463750913	5.516491161
Cyp4g1	0.00132429	−4.622322893	24.62962732

of retinal; it converts all-*trans*-3-hydroxyretinal, a product of NinaB's cleavage of vitamin A precursors (see above) or the degradation of activated Rhodopsin, to all-*trans*-3-hydroxyretinol (Wang *et al.* 2010). All-*trans*-3-hydroxyretinol is then further converted to the retinal chromophore. The downregulation of *Pdh* could be mechanistically linked to its circadian downregulation in the dark (Claridge-Chang *et al.* 2001), which resembles the VAD-induced lack of signaling. In summary, VAD affected the expression of three genes (*ninaB*, *ninaG*, and *Pdh*) that are involved in the synthesis of the retinal chromophore.

Vitamin A deprivation affects the expression of phototransduction-related genes

Since VAD causes a lack of mature Rhodopsin and thus impairs the initiation of phototransduction, we asked whether VAD affects the expression of genes that are involved in phototransduction. Indeed, we found seven phototransduction-related DEGs that respond to VAD (Figure 3A); strikingly, most of these DEGs are known for their role in terminating the light response. For instance, VAD significantly decreased the transcription of both *arrestin* genes whose transcripts are highly eye enriched (Table 5) and encode visual Arrestins (Arr1 and Arr2) that turn off activated Rhodopsin (Figure 3B). Their downregulation can be interpreted as a compensatory mechanism to promote Rhodopsin signaling (see a more detailed discussion below).

VAD also caused the upregulation of three eye-enriched genes (*inaC*, *Culd*, and *stops*), whose products mediate the termination of the phototransduction cascade downstream of Rhodopsin (Figure 3, A and B; Table 5). InaC (inactivation no afterpotential C) is an eye-specific protein kinase C (Smith *et al.* 1991) that deactivates phototransduction by inhibiting NorpA (Gu *et al.* 2005) and phosphorylating Trp channels (Popescu *et al.* 2006). Stops (slow termination of phototransduction) is a SOCS box protein that increases NorpA levels and terminates phototransduction by promoting NorpA's GTPase-activating protein activity (Wang *et al.* 2008). Lastly, Culd (CUB and LDLa domain) is a photoreceptor-enriched transmembrane protein that is required for the light-dependent endocytic turnover of Rh1 (downstream of Arr1, Figure 3B) and Trpl (Xu and Wang 2016). It remains to be determined why these three genes, whose products terminate visual signaling downstream of Rhodopsin, are upregulated despite the VAD-induced impairment of visual signaling.

CG11426 was the most upregulated gene (~13-fold, Figure 2A). CG11426 is a lipid phosphate phosphohydrolase (LPP) that is functionally related to the LPP Lazaro (Garcia-Murillas *et al.* 2006), which regulates the termination of phototransduction by mediating the conversion of phosphatidic acid to diacylglycerol (Garcia-Murillas *et al.* 2006). Both *lazaro and CG11426* are expressed in the eye, but only *CG11426* is additionally expressed in the brain (Garcia-Murillas *et al.* 2006). *CG11426*'s functions in the eye and the brain have not been studied in detail.

Trpl is the only phototransduction-related DEG that is not involved in the termination of visual signaling. Trpl is an eye-enriched (Table 5) cation channel that mediates the influx of Ca^{2+} upon light stimulation of the photoreceptor (Phillips *et al.* 1992). *Trpl* was downregulated by VAD (Figure 3, A and B), which contrasts the lack of effect on the expression of *trp*, which

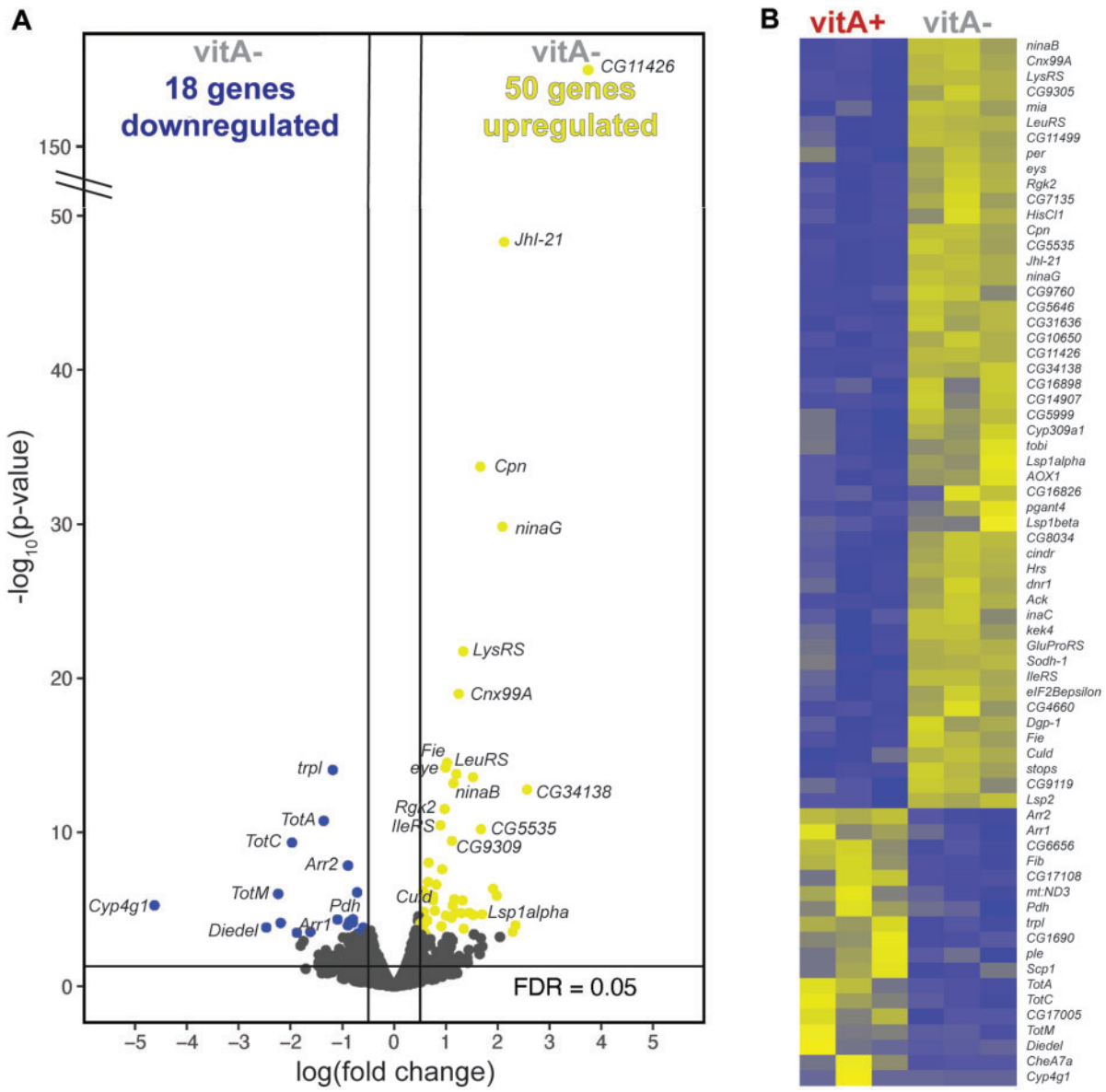

Figure 2 Vitamin A deprivation affects gene expression in the adult *Drosophila* head. (A) The volcano plot shows the profiles of DEGs that respond to vitamin A deprivation (vitA−). Genes with significant differential expression in the adult head are highlighted in blue or yellow color; 18 genes are significantly downregulated upon vitamin A deprivation (blue, left) and 50 genes are upregulated (yellow, right). The fold change is plotted for each gene relative to its *P*-value with a cut-off of abs(logFC) > 1.5-fold and a false discovery rate of FDR < 0.05. (B) Heat map of all DEGs. Three replicates are shown for vitamin A replete (vitA+) and deprived (vitA−) conditions, respectively. Shades of blue represent different levels of downregulation and shades of yellow represent different levels of upregulation.

encodes the major Ca^{2+} channel Trp (Montell and Rubin 1989) (see Discussion below).

Taken together, VAD affects the expression of a set of eye-enriched and phototransduction-related genes, which is most likely a consequence of the visual signaling defect. Rather than increasing the abundance of phototransduction components that promote visual signaling, VAD predominantly affects the expression of genes that mediate the termination of phototransduction.

Vitamin A deprivation causes the upregulation of genes whose products regulate intracellular Ca^{2+} levels

Phototransduction causes the influx of Ca^{2+} ions through the opening of Trp and Trpl channels (Hardie and Juusola 2015). Although VAD impairs visual signaling and thus Ca^{2+} influx, it unexpectedly caused an upregulation of *Calnexin* (*Cnx99A*) and *Calphotin* (*Cpn*), which encode buffers that protect photoreceptors from Ca^{2+} overload. Calphotin is a photoreceptor-specific and immobile Ca^{2+} buffer that protects against Ca^{2+} overload as well as light-induced degeneration (Ballinger *et al.* 1993; Martin *et al.* 1993; Yang and Ballinger 1994). Similarly, Calnexin serves as a Ca^{2+} buffer that is critical for photoreceptor survival, but additionally acts as an ER chaperone that promotes the maturation of Rh1 (Rosenbaum *et al.* 2006). The increased *Calnexin* expression upon VAD could be a response to the accumulation of high levels of immature Rh1 in the ER (Ozaki *et al.* 1993). However, the expression of *ninaA*, which encodes the main chaperone of Rh1 (Baker *et al.* 1994), was not significantly affected by VAD. *Calnexin*

Table 4 Enriched gene ontology terms, *P*-values, and the corresponding differentially expressed genes that respond to vitamin A deprivation

Gene ontology category	GO term name	GO term ID	Adjusted p-value	DEGs
Biological processes	Response to light stimulus	GO : 0009416	6.90E-07	*Arr1, inaC, per, stops, Arr2, TotC, TotA, ninaB, trpl, CG11426*
Biological processes	Cellular response to light stimulus	GO : 0071482	1.8257E-06	*Arr1, inaC, stops, Arr2, TotC, TotA, ninaB*
Biological processes	Phototransduction, visible light	GO : 0007603	3.61916E-06	*Arr1, inaC, stops, Arr2, ninaB, trpl*
Biological processes	Response to abiotic stimulus	GO : 0009628	4.37429E-06	*Arr1, inaC, HisCl1, ple, per, stops, TotM, Arr2, TotC, TotA, ninaB, trpl, eys, CG11426*
Biological processes	Response to radiation	GO : 0009314	7.95892E-06	*Arr1, inaC, per, stops, Arr2, TotC, TotA, ninaB, trpl, CG11426*
Biological processes	Phototransduction	GO : 0007602	8.63052E-06	*Arr1, inaC, stops, Arr2, ninaB, trpl, CG11426*
Biological processes	Detection of light stimulus	GO : 0009583	1.50047E-05	*Arr1, inaC, stops, Arr2, ninaB, trpl, CG11426*
Biological processes	Cellular response to radiation	GO : 0071478	1.84891E-05	*Arr1, inaC, stops, Arr2, TotC, TotA, ninaB*
Biological processes	Detection of visible light	GO : 0009584	2.8771E-05	*Arr1, inaC, stops, Arr2, ninaB, trpl*
Biological processes	Cellular response to abiotic stimulus	GO : 0071214	7.37673E-05	*Arr1, inaC, stops, Arr2, TotC, TotA, ninaB*
Biological processes	Cellular response to environmental stimulus	GO : 0104004	7.37673E-05	*Arr1, inaC, stops, Arr2, TotC, TotA, ninaB*
Biological processes	Detection of external stimulus	GO : 0009581	8.0115E-05	*Arr1, inaC, stops, Arr2, ninaB, trpl, CG11426*
Biological processes	Detection of abiotic stimulus	GO : 0009582	8.0115E-05	*Arr1, inaC, stops, Arr2, ninaB, trpl, CG11426*
Biological processes	Visual perception	GO : 0007601	0.000329487	*Arr1, inaC, Arr2, trpl, ninaG, Cpn*
Biological processes	Sensory perception of light stimulus	GO : 0050953	0.000434125	*Arr1, inaC, Arr2, trpl, ninaG, Cpn*
Biological processes	Deactivation of rhodopsin mediated signaling	GO : 0016059	0.000732728	*Arr1, inaC, stops, Arr2*
Biological processes	Rhodopsin metabolic process	GO : 0046154	0.000732728	*Culd, ninaB, Cnx99A, ninaG*
Biological processes	Regulation of rhodopsin mediated signaling pathway	GO : 0022400	0.000938974	*Arr1, inaC, stops, Arr2*
Biological processes	Retina homeostasis	GO : 0001895	0.001185447	*Arr1, Culd, Arr2, Cnx99A*
Biological processes	Response to external stimulus	GO : 0009605	0.001496398	*Arr1, Diedel, inaC, HisCl1, ple, per, Lsp2, stops, dnr1, TotM, Arr2, TotC, TotA, ninaB, trpl, JhI-21, CG11426*
Biological processes	Response to temperature stimulus	GO : 0009266	0.002418347	*HisCl1, ple, per, TotM, TotC, TotA, eys*
Biological processes	Adaptation of signaling pathway	GO : 0023058	0.002638889	*Arr1, inaC, Arr2*
Biological processes	Rhodopsin mediated signaling pathway	GO : 0016056	0.00267272	*Arr1, inaC, stops, Arr2*
Biological processes	Receptor-mediated endocytosis	GO : 0006898	0.003895313	*Arr1, inaC, cindr, Hrs, Arr2*
Biological processes	Regulation of G protein-coupled receptor signaling pathway	GO : 0008277	0.005227693	*Arr1, inaC, stops, Arr2*
Biological processes	Import into cell	GO : 0098657	0.006632313	*Arr1, inaC, Culd, cindr, Hrs, Arr2, CG5535, JhI-21*
Biological processes	Negative regulation of binding	GO : 0051100	0.008962269	*Arr1, per, Arr2*
Biological processes	Multicellular organismal homeostasis	GO : 0048871	0.012005025	*Arr1, Culd, per, Arr2, Cnx99A*
Biological processes	Diterpenoid metabolic process	GO : 0016101	0.016327367	*Pdh, ninaB, ninaG*
Biological processes	Retinoid metabolic process	GO : 0001523	0.016327367	*Pdh, ninaB, ninaG*
Biological processes	Retinal metabolic process	GO : 0042574	0.017780302	*Pdh, ninaB*
Biological processes	Desensitization of G protein-coupled receptor signaling pathway by arrestin	GO : 0002032	0.017780302	*Arr1, Arr2*
Biological processes	Receptor internalization	GO : 0031623	0.021158662	*Arr1, Hrs, Arr2*
Biological processes	tRNA aminoacylation for protein translation	GO : 0006418	0.026080388	*GluProRS, IleRS, LeuRS, LysRS*
Biological processes	Pigment metabolic process involved in pigmentation	GO : 0043474	0.026080388	*Culd, ninaB, Cnx99A, ninaG*
Biological processes	Pigment metabolic process involved in developmental pigmentation	GO : 0043324	0.026080388	*Culd, ninaB, Cnx99A, ninaG*
Biological processes	Eye pigment metabolic process	GO : 0042441	0.026080388	*Culd, ninaB, Cnx99A, ninaG*

(continued)

Table 4. (continued)

Gene ontology category	GO term name	GO term ID	Adjusted p-value	DEGs
Biological processes	tRNA aminoacylation	GO : 0043039	0.031732126	*GluProRS*, *IleRS*, *LeuRS*, *LysRS*
Biological processes	Cellular response to UV	GO : 0034644	0.033449645	*TotC*, *TotA*, *ninaB*
Biological processes	Amino acid activation	GO : 0043038	0.034871595	*GluProRS*, *IleRS*, *LeuRS*, *LysRS*
Biological processes	Photoreceptor cell maintenance	GO : 0045494	0.041039061	*Arr1*, *Culd*, *Arr2*
Cellular compartments	aminoacyl-tRNA synthetase multienzyme complex	GO : 0017101	4.25175E-05	*GluProRS*, *IleRS*, *LeuRS*, *LysRS*
Cellular compartments	Larval serum protein complex	GO : 0005616	7.77014E-05	*Lsp1alpha*, *Lsp2*, *Lsp1beta*
Cellular compartments	Rhabdomere	GO : 0016028	0.004645236	*Arr1*, *inaC*, *Arr2*, *trpl*
Molecular function	Nutrient reservoir activity	GO : 0045735	0.000398031	*Lsp1alpha*, *Lsp2*, *Lsp1beta*
Molecular function	Opsin binding	GO : 0002046	0.008377808	*Arr1*, *Arr2*
Molecular function	Aminoacyl-tRNA ligase activity	GO : 0004812	0.013616135	*GluProRS*, *IleRS*, *LeuRS*, *LysRS*
Molecular function	Ligase activity, forming carbon-oxygen bonds	GO : 0016875	0.013616135	*GluProRS*, *IleRS*, *LeuRS*, *LysRS*

has other functions that could explain its response to VAD; for instance, it is also expressed in neurons of the brain and regulates a sodium channel (Xiao *et al.* 2017).

Taken together, VAD affects the expression of two genes whose products regulate intracellular Ca^{2+} levels. Since the main source for an intracellular Ca^{2+} increase is the influx through light-activated Trp and Trpl channels (which is impaired by the defective light response under VAD conditions) it remains to be understood why VAD affects genes that are required when intracellular Ca^{2+} is high rather than low.

Vitamin A deprivation causes the upregulation of genes that are related to tRNA-aminoacylation

One of the most enriched terms in our GO analysis was "aminoacyl-tRNA synthetase multienzyme complex" (Figure 3A and Table 4), which refers to the attachment of a specific amino acid to a specific tRNA. Notably, VAD selectively upregulated four genes, *GluProRS* (*Glutamyl-prolyl-tRNA synthetase*), *IleRS* (*Isoleucyl-tRNA synthetase*), *LeuRS* (*Leucyl-tRNA synthetase*), and *LysRS* (*Lysyl-tRNA synthetase*) (Figure 3A). Since stressed cells can selectively change the abundance of specific tRNAs to increase the translation of specific proteins (Torrent *et al.* 2018), it is possible that the VAD-induced upregulation of genes involved in tRNA-aminoacylation is related to the VAD-induced accumulation of immature Rh1 in the ER (Huber *et al.* 1994; Ozaki *et al.* 1993) and the resulting ER stress (Ryoo 2015). The upregulation of specific tRNA-aminoacylation genes could also promote the translation of the DEGs that we identified in this study and thereby enhance the compensatory response to VAD.

Vitamin A deprivation causes the upregulation of genes that encode major serum and nutrient reservoir proteins

Another highly enriched GO term was "nutrient reservoir activity" (Figure 2A). VAD caused an upregulation of three genes (*Lsp1alpha*, *Lsp1beta*, and *Lsp2*) that encode two major larval serum proteins, which have been proposed to store amino acids and energy for metamorphosis (Roberts *et al.* 1977, 1991). It is conceivable that VAD represents a dietary stress that triggers increased nutrient storage in the larva for the (nonfeeding) pupal stages; however, since we detected the upregulation of *Lsp1alpha*, *Lsp1beta*, and *Lsp2* in the adult head, this suggests that the three DEGs have additional, stage-specific functions. Consistent with this hypothesis, *Lsp2* is differentially regulated in larvae and adults and most of the adult transcript has been detected in adipose tissue of the head (Benes *et al.* 1990; Mousseron-Grall *et al.* 1997).

Vitamin A deprivation causes the upregulation of stress and immune response genes

Several DEGs that were not enriched in our GO term analysis can be classified based on their FlyBase annotation (https://flybase.org/) into the categories "oxidative stress" (*per*, *Cyp309a1*—both upregulated by VAD), "response to stress" (*TotA*, *TotC*, *TotM*—all downregulated by VAD), "immune response" (*dnr1*, *Diedel*), and "transmembrane proteins" (*CG5535*, *CG5646*, *Fie*, *HisCl1*, *CG8034*—all upregulated by VAD). Since most of these DEGs are highly expressed in the head, but not specifically in the eye (Table 5), they are likely a part of molecular mechanisms that are not directly related to vision.

Comparison of genes that respond to vitamin A deprivation and blue light stress

Studies in mammals (Ham *et al.* 1984; Grimm *et al.* 2001) and *Drosophila* (Hall *et al.* 2018) have shown that extended blue light exposure is another important environmental stress that damages the eye. To analyze whether some genes respond to several environmental stresses, we compared our VAD-responsive DEGs with DEGs that respond to blue light phototoxicity in photoreceptors of 6-day-old flies (Hall *et al.* 2018). We identified seven DEGs (Table 6) that were upregulated by both VAD and blue light stress (Hall *et al.* 2018): *CG34138* encodes a transmembrane protein of unknown function and three DEGs encode amino acid transporters: CG5646 is a predicted acyl carnitine and amino acid transmembrane transporter, CG5535 is a predicted L-arginine importer and L-ornithine transmembrane transporter, and JhI-21 (Juvenile hormone Inducible-21) is an L-amino acid transmembrane transporter. Notably, *JhI-21* is the second-most significantly upregulated DEG in our dataset (Figure 2A). Recent studies revealed that JhI-21 is expressed in motor neurons of the larval neuromuscular junction, where it regulates synaptic glutamate signaling as well as locomotor behavior (Ziegler *et al.* 2016); moreover, it is involved in leucine sensing as well as leucine-induced secretion of the insulin-like peptide Dilp2 (Ziegler *et al.* 2018).

Lastly, *GluProRS* (see above), *CG14907* (predicted to encode a protein of the thioredoxin-like family), and *Dgp-1* (encodes a GTP-

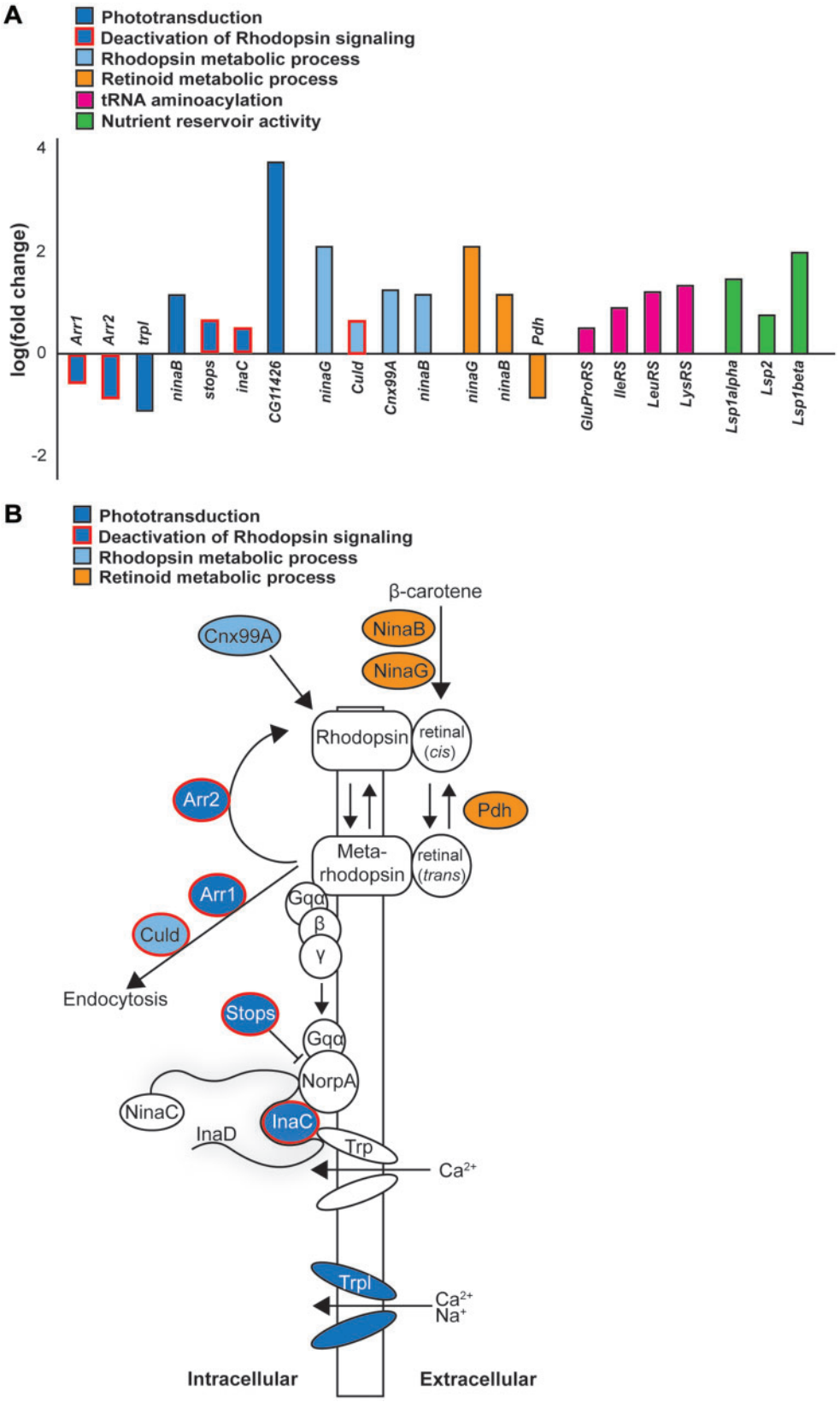

Figure 3 Enriched GO terms for genes that respond to vitamin A deprivation. (A) The bar graph shows the fold change of DEGs that respond to vitamin A deprivation and are associated with the GO terms phototransduction (dark blue), Rhodopsin metabolic process (light blue), retinoid metabolic process (orange), tRNA aminoacylation (magenta), and nutrient reservoir activity (green). Positive values indicate upregulation upon vitamin A deprivation, negative values indicate downregulation. (B) The schematic highlights phototransduction-, Rhodopsin metabolism-, and retinoid metabolism-related genes that respond to vitamin A deprivation. Color code corresponds to (A), white indicates no significant transcriptional response to vitamin A deprivation. Note that the vitamin A deprivation-responsive *Arr1*, *Arr2*, *Culd*, *stops*, and *inaC* all play a role in the deactivation of the light response (emphasized by red outline).

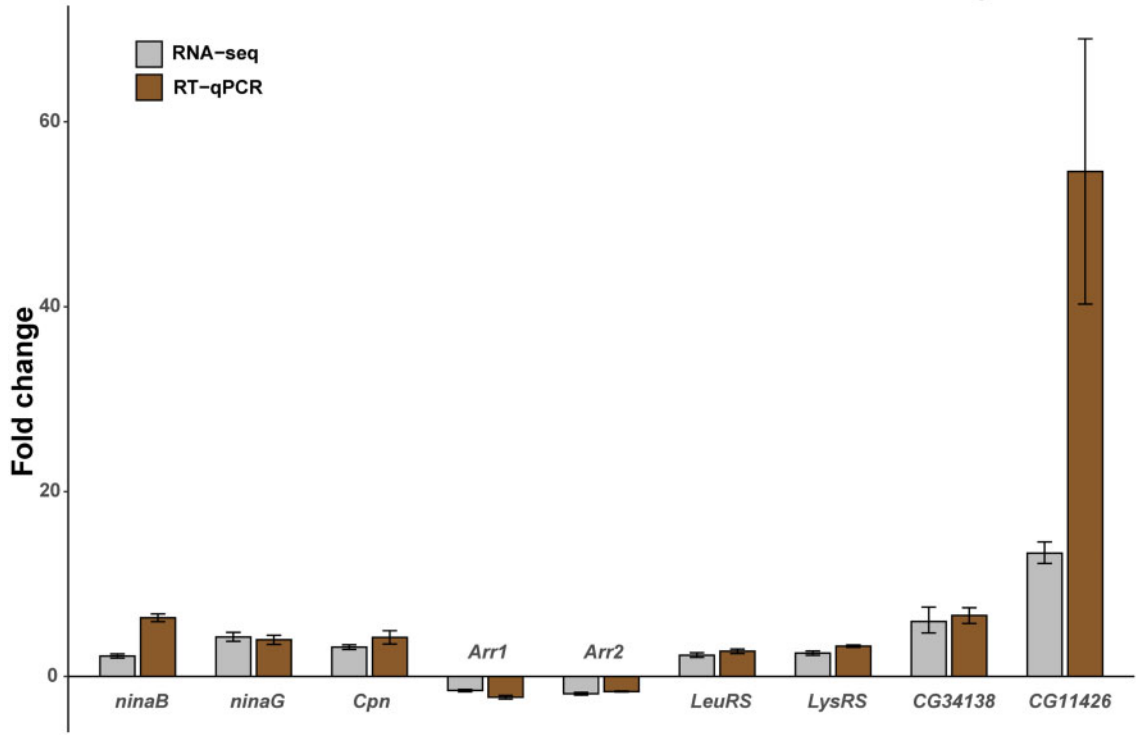

Figure 4 RT-qPCR validates vitamin A deprivation-responsive genes that were identified by total RNA-seq. The bar graph shows the fold change as detected by total RNA-seq (gray) or RT-qPCR (brown) for DEGs that respond to VAD. Three biological replicates were analyzed. Note that the shown genes are associated with different GO term categories such as Rhodopsin metabolic process or retinoid metabolism (*ninaB and ninaG*), phototransduction (*ninaB*, *Arr1*, *Arr2*, and *CG11426*), and tRNA aminoacylation (*LeuRS and LysRS*).

binding protein) were also upregulated by both stresses. Together, the response of these seven DEGs to two different ocular stresses suggests that they play more general roles in responses to environmental stress.

RT-qPCR analysis validates differentially expressed genes that were identified by total RNA sequencing

Next, we sought to validate several VAD-responsive DEGs from different GO term categories by performing RT-qPCR on the heads of 4-day-old wild-type female flies. Consistent with our RNA-seq results, the *Arr1* and *Arr2* transcript levels were also significantly reduced by VAD in the RT-qPCR experiment (Figure 4 and Supplementary Figure S1). Moreover, we confirmed the VAD-induced upregulation of retinoid metabolism-related (*ninaB* and *ninaG*), Ca^{2+} buffer-related (*Cpn*), and tRNA synthetase-related (*LeuRS* and *LysRS*) genes. Lastly, we also validated the most upregulated DEGs *CG11426* and *CG34138* (Figure 4 and Supplementary Figure S1).

Discussion

Genes that respond to vitamin A deficiency

The goal of our study was to gain insights into the molecular mechanisms that respond to VAD. We identified VAD-responsive genes that are associated with the GO term categories retinoid and Rhodopsin metabolism, phototransduction, aminoacyl-tRNA aminoacylation, and nutrient reservoir activity (Figure 5). Although our analysis did not yield a category that fits β-carotene's proposed antioxidant or anti-inflammatory properties (Britton 1995; Gruszecki and Strzałka 2005; Krinsky and Johnson 2005; Kaulmann and Bohn 2014; Edge and Truscott 2018), the DEGs *per* (Krishnan *et al.* 2008)—well-known for its role in circadian rhythms—and *Cyp309a1* (Maitra *et al.* 2019) have been linked to oxidative stress, while *dnr1* is associated with neuroinflammation and negative regulation of innate immune responses (Cao *et al.* 2013).

Transcriptional feedback maintains optimal retinal and Arrestin levels

Our DEG analysis suggests that transcriptional feedback maintains optimal retinal and Arrestin levels. We propose that excessive levels of retinal cause the downregulation of *ninaB*, which encodes the key vitamin A producing enzyme. This negative feedback would ensure that the retinal levels match the opsin production to prevent toxic levels of unbound retinal (Voolstra *et al.* 2010). Conversely, as we observed under VAD conditions, the feedback loop causes the upregulation of *ninaB* in response to the lack of vitamin A/retinal. This homeostasis mechanism is reminiscent of the negative feedback of vitamin A/retinal on the mammalian *ninaB* homolog *Bco1*: an excess of vitamin A/retinal causes a decrease of *Bco1* transcription to prevent toxic levels of retinal (Lobo *et al.* 2013).

Moreover, we propose that a second negative feedback loop preserves the sensitivity of Rhodopsin to visual stimuli by maintaining stochiometric Arrestin levels. Previous studies have shown that Arr2 deactivates Rhodopsin by uncoupling it from the G protein (Dolph *et al.* 1993) and that a stochiometric ~1:3 ratio of available Arr2 to activated Rhodopsin keeps the Arr2 levels low enough to maintain Rhodopsin function (Dolph *et al.* 1993; Ranganathan and Stevens 1995; Satoh *et al.* 2010). We propose that the stochiometric Arr2: Rhodopsin ratio is maintained by

Table 5 Differentially expressed genes that respond to vitamin A deprivation sorted by their enrichment in the eye or brain (FPKM values and tissue enrichment data from FlyAtlas 2, see *Materials and Methods*)

Gene	Fold change	Response to vitA-	Enrichment (female eye)	Enrichment (female brain)	FPKM (female eye)	FPKM (female brain)
Arr1	1.50631506	Down	129	0.2	8476	15
Pdh	1.848735009	Down	129	0.3	2963	7
Arr2	1.848164575	Down	126	0.4	10377	30
inaC	1.420785431	Up	81	0.3	488	2
Culd	1.561275226	Up	79	N.A.	158	0.6
trpl	2.26588186	Down	75	0.2	655	1.3
stops	1.581213157	Up	55	1	110	2
ninaB	2.218188024	Up	37	9.6	74	19
CG6656	1.635066672	Down	36	1	127	3.4
Fie	2.037077161	Up	32	23	122	85
Lsp2	1.686068506	Up	31	2	63	4
Cpn	3.183677691	Up	27	N.A.	54	0.2
CG7135	1.908559368	Up	23	2.2	46	4.3
eys	1.994174879	Up	15	6.1	30	12
ninaG	4.278007551	Up	10	N.A.	20	0.6
HisCl1	1.88865446	Up	7.8	N.A.	16	1.7
CG11426	13.38928467	Up	6.3	2.7	17	7.2
kek4	1.560114966	Up	6.1	6.2	12	12
CG1690	2.124327826	Down	6.1	N.A.	12	0.1
TotA	2.556948505	Down	5.7	0.3	443	25
CG4660	1.524331677	Up	4.9	2	9.9	4.1
per	2.00323807	Up	4.6	1.5	9.2	2.9
dnr1	1.488524947	Up	4.5	1.6	20	7
Diedel	5.516491161	Down	4.2	0.2	9	0.5
TotM	4.704040528	Down	4.1	0.1	84	1.8
Cyp309a1	2.475933819	Up	3.4	N.A.	6.8	0.3
Hrs	1.580972848	Up	3.4	0.7	23	5
TotC	3.907978881	Down	3.3	0.2	210	12
CG31636	3.77587936	Up	3.1	N.A.	6.2	0.2
CG5646	2.883893462	Up	3	1.2	11	4.6
Cnx99A	2.380083892	Up	2.2	0.7	56	19
Sodh-1	1.491304598	Up	2.2	0.1	47	3.1
cindr	1.484354421	Up	2.2	1.3	25	14
CG8034	1.486574878	Up	2.1	0.5	13	3
tobi	2.240980857	Up	2	0.2	24	1.8
ple	1.81641547	Down	1.9	1	9	4.6
CG5999	3.257389962	Up	1.7	0.1	4.2	0.3
Ack	1.591612247	Up	1.4	1.3	7.1	6.6
Dgp-1	1.690282426	Up	1.3	1.5	9.2	10
Lsp1beta	3.95977861	Up	1.2	N.A.	2.4	0.2
Rgk2	1.971449366	Up	1.1	3.8	2.2	7.6
eIF2Bepsilon	1.730386307	Up	1.1	0.7	7.1	4.7
JhI-21	4.375631595	Up	1	0.6	21	14
LysRS	2.528740295	Up	0.8	0.4	21	11
CG16898	2.278468692	Up	0.8	0.1	2.5	0.2
IleRS	1.866050353	Up	0.8	0.4	23	10
CG14907	2.548464539	Up	0.7	0.2	3	1
CG16826	2.193737286	Up	0.7	0.2	309	79
CG9119	1.473787628	Up	0.7	0.3	7.7	2.9
CG5535	3.20639022	Up	0.6	0.1	11	2.5
CG9305	2.176182166	Up	0.6	0.6	1.7	1.7
AOX1	1.767840408	Up	0.6	2	13	4
CG17108	1.621831761	Down	0.6	0.1	105	9.9
GluProRS	1.424745473	Up	0.5	0.2	8.7	3.5
mt:ND3	1.730584316	Down	0.5	0.2	1534	775
pgant4	4.913761704	Up	0.4	0.2	0.8	0.5
Fib	1.733255311	Down	0.3	0.1	8.3	2.7
CG10650	5.088967053	Up	0	0.1	0.4	0.9
Scp1	3.059622607	Down	0	0	2.7	2
CheA7a	4.547382164	Down	0	0	0.3	0.1
Cyp4g1	24.62962732	Down	0	0	8.1	1.4

Bold print indicates eye enrichment.

Table 6 Comparison of differentially expressed genes that respond to vitamin A deprivation (vitA−) and prolonged blue-light induced stress (1 or 6 days old adult flies, data from Hall *et al.* 2018)

DEG	Response to vitA-	Blue light (6 days old flies)	Blue light (1 day old flies)
CG34138	Up	Up	NA
Jhl-21	Up	Up	Up
CG5535	Up	Up	NA
CG5646	Up	Up	NA
CG14907	Up	Up	NA
GluProRS	Up	Up	NA
Dgp-1	Up	Up	Up
kek4	Up	Down	NA
dnr1	Up	Down	NA
ple	Down	NA	Up
CG17005	Down	Up	NA

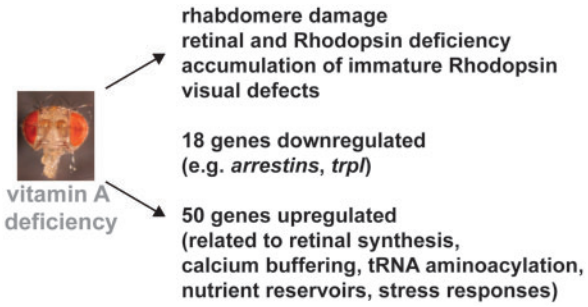

Figure 5 Summary of the effects of vitamin A deprivation in the *Drosophila* head. Vitamin A deprivation causes structural and functional defects in the eye; moreover, it affects gene expression in the adult head (18 genes downregulated, 50 genes upregulated).

transcriptional feedback on *Arr2* transcription. Since VAD impairs Rhodopsin synthesis and causes an excess of Arr2 over the very low residual levels of Rhodopsin, we propose that a compensatory negative transcriptional feedback reduces the transcription of *Arr2* to promote Rhodopsin signaling. Conversely, when Rhodopsin levels increase under vitamin A replete conditions, Arr2 levels would increase accordingly.

Vitamin A deficiency and blue light stress affect different phototransduction genes and have opposite effects on the two genes that encode the major Ca^{2+} channels

We wondered whether different ocular stresses trigger distinct transcriptional responses or whether they share general stress response factors. While VAD impairs visual signaling, prolonged blue light exposure causes excessive visual signaling and phototoxicity. Consistent with these opposing effects of the two environmental stresses on visual signaling, we found that they affect largely nonoverlapping gene sets: for instance, blue light phototoxicity changes the expression of a different set of phototransduction genes (downregulation of *inaF-C*, *rdgA*, *rdgC*, and *trp*) (Hall *et al.* 2018). However, the seven overlapping DEGs that are not related to phototransduction might indeed play a more general role in the response to environmental stresses.

Phototransduction results in the opening of two types of Ca^{2+} channels, Trp and Trpl (Figure 1A). *Trp*, but not *trpl*, is downregulated after extended blue light exposure (Hall *et al.* 2018), which has been proposed to protect the photoreceptor from the excessive Ca^{2+} influx (Hall *et al.* 2018) that is largely mediated by Trp (Hardie and Juusola 2015). Conversely, our study revealed that VAD decreases the transcription of *trpl*, but not *trp*. We propose that this differential expression is related to the circadian modulation of *trpl* transcription: *trpl* expression peaks in the light and decreases in the dark (Claridge-Chang *et al.* 2001). Since VAD impairs visual signaling and thus resembles dark exposure, we suggest that the VAD-induced decrease of *trpl* expression is due to the circadian mechanism that decreases *trpl* expression in darkness. These two examples for a differential regulation of *trp and trpl* complement a previous report of an adaptation mechanism that involves Trpl, but not Trp (Bahner *et al.* 2002): upon light stimulation, Trpl channels translocate from the rhabdomere membranes to intracellular storage compartments (Bahner *et al.* 2002). In darkness, the Trpl channels translocate back to the rhabdomere membranes (Bahner *et al.* 2002). Taken together, differential responses to distinct environmental stresses can help elucidate specializations of structurally and functionally related proteins.

Conclusions

In conclusion, our study offers insights into the transcriptional response to VAD and the resulting impairment of visual signaling (Figure 5). Future studies need to address whether the transcriptomic changes that we identified translate to corresponding changes in the proteome. Moreover, it would be interesting to elucidate whether there are DEGs that specifically respond to the Rhodopsin maturation defect that is caused by VAD. For instance, this could be determined by comparing our VAD dataset to transcriptome data from vitamin A replete *Rh1* (*ninaE*) hypomorphs that have very low levels of Rh1 in their rhabdomeres (Leonard *et al.* 1992). Lastly, an intriguing question is whether insufficient vitamin A uptake makes the eye more vulnerable to other environmental stresses. Together, these studies will further advance our understanding of the molecular mechanisms that respond to environmental stresses and thus have relevance for preventing human eye diseases that result from direct or indirect environmental exposures (Barrett 2005).

Data availability

The raw RNA-seq output files that we generated in this study were deposited under accession number GSE178712 in Gene Expression Omnibus.

Supplementary material is available at *G3* online.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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