Reviewed Preprint
v1 • November 21, 2025
Not revised

Reviewed Preprint
v2 • January 16, 2026
Revised by authors

Non-visual light modulates behavioral memory and gene expression in *C. elegans*

Zhijian Ji[1,§], Bingying Wang[1,§], Rashmi Chandra[1,§], Junqiang Liu[1,§], Supeng Yang[1,2], Yong Long[3], Michael Egan[1,2], Fujia Han[4], Han Wang[4], Noelle L'Etoile[5], Dengke K Ma[1,6,7]

[1]Cardiovascular Research Institute, University of California, San Francisco, San Francisco, United States • [2]Department of Molecular Cell Biology, University of California, Berkeley, Berkeley, United States • [3]Key Laboratory of Breeding Biotechnology and Sustainable Aquaculture, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China • [4]Department of Integrative Biology, University of Wisconsin-Madison, Madison, United States • [5]Department of Cell and Tissue Biology, University of California, San Francisco, San Francisco, United States • [6]Department of Physiology, University of California, San Francisco, San Francisco, United States • [7]Innovative Genomics Institute, Berkeley, United States

For correspondence: Dengke.Ma@ucsf.edu

[§] Equal contribution

Competing interests: No competing interests declared

Funding: See page 18

Reviewing editor: Sonia Sen, Tata Institute for Genetics and Society, India

eLife Assessment

This **important** study uncovers a previously unrecognized light-responsive pathway in C. elegans that depends on live food bacteria and is mediated by the bZIP factors ZIP-2/CEBP-2 and the cytochrome P450 enzyme, CYP-14A5. The authors show that this bacteria-linked pathway modulates long-term memory and can be harnessed as a low-cost light-inducible expression system, opening new directions for sensory biology and genetic engineering in worms. The exact means by which live bacteria modulate light signal that activates ZIP-2/CEBP-2 in the worm remains to be elucidated. The evidence supporting the pathway's role uses multiple genetic, transcriptional, and behavioural assays, and is **convincing**.

https://doi.org/10.7554/eLife.108507.2.sa4

Abstract

Visible light influences a range of physiological processes, yet how animals respond to it independently of the visual system remains largely unknown. Here, we uncover a previously undescribed light-induced transcriptional pathway that modulates behavioral plasticity in *C. elegans*, a roundworm without eyes. We demonstrate that ambient visible light or controlled-intensity visible-spectrum LED activates an effector gene *cyp-14A5* in non-neuronal tissues through the bZIP transcription factors ZIP-2 and CEBP-2. Light induction of *cyp-14A5* is more prominent at shorter wavelengths but is independent of the known blue light receptors LITE-1 and GUR-3 in *C. elegans*. This bZIP-dependent genetic pathway in non-neuronal tissues enhances behavioral adaptability and olfactory memory, suggesting a body-brain communication axis. Furthermore, we use the light-responsive *cyp-14A5* promoter to drive ectopic gene expression, causing synthetic light-induced sleep and rapid aging phenotypes in *C. elegans*. These findings advance our understanding of light-responsive mechanisms outside the visual system and offer a new genetic tool for visible light-inducible gene expression in non-neuronal tissues.

Introduction

Visible light is crucial for image formation and regulating various physiological processes through the visual system, yet how animals respond to ambient light independently of sight remains understudied and likely through diverse mechanisms. Recent studies have uncovered non-visual photoreception mechanisms that modulate a range of biological processes, from circadian

rhythms to stress responses and metabolic homeostasis(Andrabi et al., 2023; Cronin and Johnsen, 2016; Do and Yau, 2010; Van Gelder, 2008). These mechanisms often involve specialized light-sensitive proteins, such as opsins and cryptochromes, widely expressed in body locations, including the skin, brain, and peripheral organs. For example, mammalian melanopsin-expressing retinal ganglion cells play critical roles in systemic light responses largely independent of image formation(Berson et al., 2002; Hattar et al., 2003; Lucas et al., 2003; Shi et al., 2024). In the nematode roundworm *C. elegans*, blue light photoreception requires the light-activated ion channels LITE-1 and GUR-3 in specific neurons, influencing aversive behaviors and cellular physiology(Bhatla and Horvitz, 2015; Edwards et al., 2008; Gong et al., 2016; Hanson et al., 2023). Visible light irradiation can also generate photo-oxidative reactive oxygen species in diverse living organisms(De Magalhaes Filho et al., 2018; Liebel et al., 2012; Mahmoud et al., 2010). Despite these advances, the molecular pathways and physiological outcomes of non-visual light sensing and responses remain understudied, raising intriguing questions about the mechanistic basis and functional implications of light as an environmental cue beyond vision.

We previously studied how genes encoding cytochrome P450 (CYP) proteins respond to and mediate effects of exposure to environmental stresses in *C. elegans*(Keller et al., 2014; Ma et al., 2013). Among various transcriptional reporters we generated for CYP-encoding genes to monitor environmental regulation, we serendipitously discovered that the *cyp-14A5* promoter-driven GFP expression is particularly sensitive to ambient visible light exposure. Building upon this initial finding, we conduct transcriptome profiling to identify light-inducible genes in addition to *cyp-14A5*, determine key environmental and transcriptional regulators of *cyp-14A5*, and show that the light-inducible CYP-14A5 promotes behavioral plasticity and olfactory memory in *C. elegans*. The findings also provide a genetic tool to use light-inducible *cyp-14A5* promoter to flexibly and ectopically drive gene expression.

Results

Light activates expression of *cyp-14A5* and other genes in *C. elegans*

Cytochrome P450 proteins comprise a highly conserved superfamily of heme-containing monooxygenases critical for metabolizing endogenous and xenobiotic compounds(Denisov et al., 2005). We constructed transcriptional reporters for genes encoding CYPs in *C. elegans* and found that *cyp-14A5p::GFP* was drastically up-regulated by bright-field transmission light from a microscope inadvertently left on overnight. Using controlled light versus dark conditions, we confirmed the finding from an integrated *cyp-14A5p::GFP* reporter and observed its robust widespread GFP expression in many tissues induced by moderate-intensity (500-3000 Lux, 16-48 hr duration) LED light exposure (Fig. 1A). The photometric Lux range is approximately 0.1–0.60 mW/cm² in radiometric (total radiant power) metric given the spectrum of the LED light source. The level of GFP expression increased proportionally with both light intensity and duration (Fig. 1B), the condition of which does not impact ambient temperature (Fig. S1A-S1D). Other common stresses, including transient 32 °C heat shock, constant 24-hr hypoxia or starvation did not apparently induce *cyp-14A5p::GFP* (Fig. S1E-S1H). Interestingly, light induction of *cyp-14A5p::GFP* appears to require live bacteria sustaining a non-starved animal physiological state (Fig. S1I-J). We are investigating how bacteria modulate host light responses in a separate study and, in the present work, focus on the regulation and functional consequences of *cyp-14A5* in *C. elegans* upon light exposure in the presence of the OP50 bacteria as food.

To determine CYP-14A5 protein expression pattern, we constructed a translational GFP reporter (*cyp-14A5p::cyp-14A5::GFP*) and observed robust light-induced expression of CYP-14A5::GFP in many of the non-neuronal tissues, including the pharynx, hypoderm and intestine (Fig. 1C). The transcriptional reporter exhibited similar patterns of non-neuronal GFP induction by light (Fig. 1A). The translational reporter displayed a CYP-14A5::GFP pattern characteristic of endoplasmic reticulum morphology (Fig. 1C), consistent with the established localization of most CYP enzymes to ER membranes.

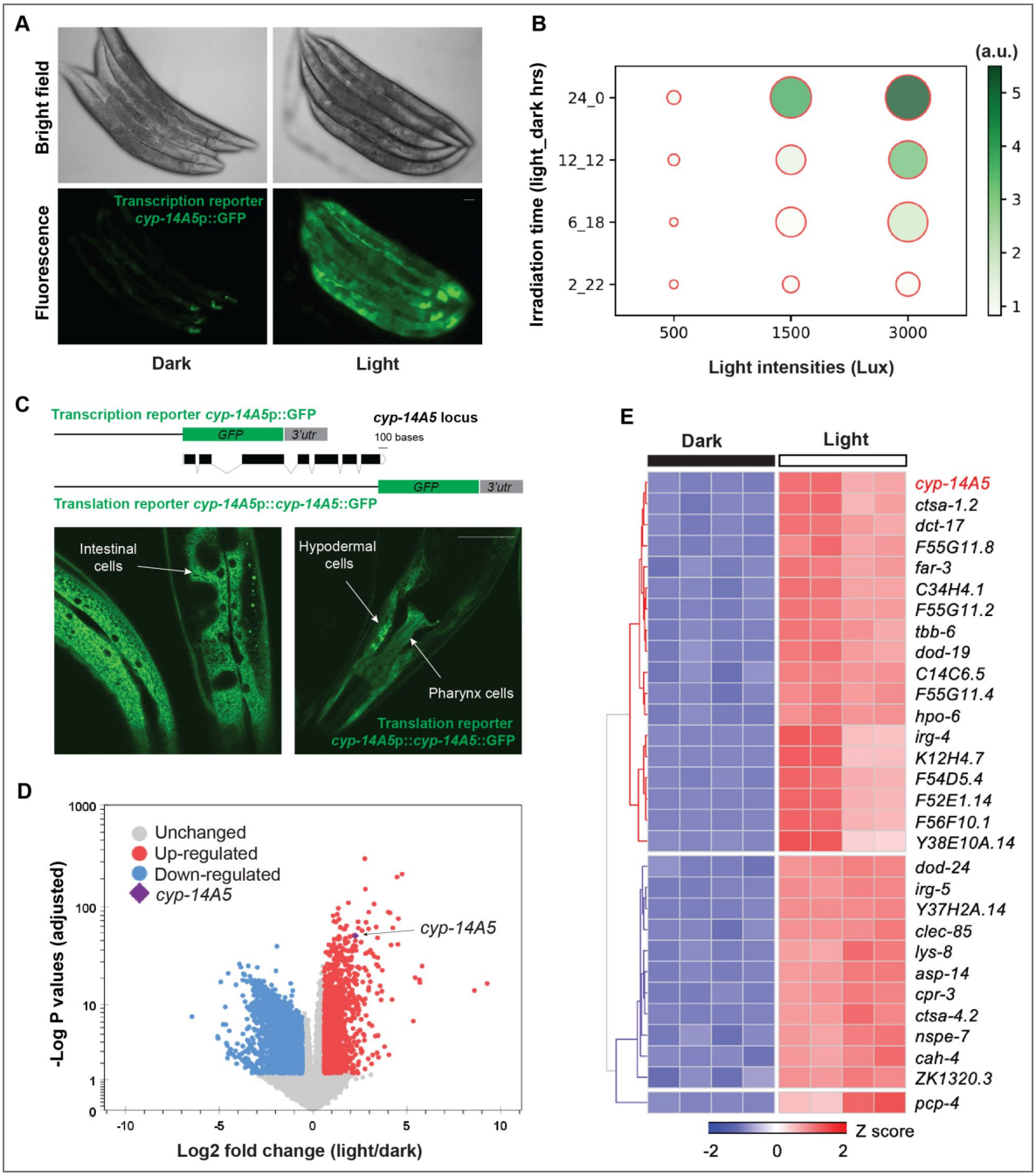

Figure 1. Visible light exposure activates the CYP-encoding gene *cyp-14A5* in a genetic program in *C. elegans*.

A, Representative epifluorescence and brightfield images showing *cyp-14A5*p::GFP induction by visible light exposure (1500 Lux, 24 hrs), in synchronized young adults (24 hrs post L4). Scale bar: 50 µm. B, Bubble plot showing fold induction of *cyp-14A5p::GFP* as a function of light intensity (Lux) and duration (hours of light_dark indicated in Y axis). C, Schematic of *cyp-14A5* transcriptional and translational GFP reporters. The translational reporter shows non-neuronal (indicated by arrows) induction of CYP-14A5::GFP by light (1500 Lux, 24 hrs). Scale bar: 50 µm. D, Volcano plot showing genes differentially regulated by light (1500 Lux, 24 hrs), with *cyp-14A5* highlighted, in synchronized young adults (24 hrs post L4). E, Heat map of top-ranking visible light-regulated genes (top 30 including *cyp-14A5*).

To explore how eyeless *C. elegans* responds to ambient visible light independently of a visual system, we conducted transcriptomic profiling by RNA-seq in *C. elegans* exposed to controlled light or dark conditions. We found that defined visible light exposure (1500 Lux, 24 hr duration) to a synchronized population of young adults (24 hrs post L4) triggered a robust genome-wide transcriptional response, including thousands of genes differentially regulated (adjusted *P* value < 0.05, Fig. 1D and Table S1). Among these, *cyp-14A5* was one of the most strongly upregulated genes (Fig. 1E). Previous work identified *gst-4* as a gene responsive to light exposure(De Magalhaes Filho et al., 2018). In our RNA-seq analysis, *gst-4* also was differentially regulated, and we confirmed this using a *gst-4p::GFP* reporter, although the response was relatively modest compared to the robust induction of *cyp-14A5p::GFP* by the same light condition (Fig. S1J). Gene ontology (GO) analysis of the light-induced genes reveals their significant enrichment in several pathways, including transmembrane signaling, pathogen and stress responses, protein phosphorylation, cellular homeostasis and metabolisms (Fig. S2).

ZIP-2 and CEBP-2 are essential for light-induced transcriptional responses

We next investigated the molecular regulators driving *cyp-14A5* activation in response to light. To test if it requires previously identified blue-light receptors LITE-1 or GUR-3, we crossed the *cyp-14A5p::GFP* reporter with *lite-1* and *gur-3* double loss-of-function mutants. Surprisingly, light-induced GFP expression was largely preserved in *lite-1 gur-3* double mutants, indicating that *cyp-14A5* activation operates through an alternative, non-visual light-sensing mechanism (Fig. 2A). Prolonged photonic light exposure may also cause photo-oxidation of DNA and genotoxicity, leading to DNA damage and ATM protein-dependent check points and transcriptional responses(Ciccia and Elledge, 2010; De Magalhaes Filho et al., 2018; Schuch et al., 2017). However, loss of the DNA damage sensor ATM-1 did not apparently affect light-induced *cyp-14A5p::GFP* (Fig. 2A). These findings underscore the existence of a novel light-responsive pathway in *C. elegans*, distinct from previously characterized photoreceptive systems.

To identify transcriptional regulators driving *cyp-14A5* activation in response to light, we adopted an RNAi-based screening strategy, focusing initially on approximately 400 genes encoding transcription factors, including those responding to various types of stresses. Knockdown of the expression of genes encoding TF from a previously assembled RNAi library or selected for mediating various common stress responses (hypoxia, oxidative stress, heat shock etc.) did not appear to affect *cyp-14A5* activation in response to light (Fig. 2B). Interestingly, we identified from such screen two bZIP-type transcription factors, ZIP-2 and CEBP-2, as critical mediators of light-induced *cyp-14A5* transcription (Fig. 2B). Knockdown or genetic ablation of either *zip-2* or *cebp-2* abolished light-induced *cyp-14A5p::GFP* expression (Fig. 2B, **2C**). ZIP-2 and CEBP-2 have been previously identified(Dunbar et al., 2012; Estes et al., 2010; Kniazeva and Ruvkun, 2025; Reddy et al., 2016) to cooperate in a regulatory complex and mediate transcriptional responses to translational inhibition caused by the bacterial pathogen *Pseudomonas aeruginosa* PA14. In these studies, the *irg-1*p::GFP transcriptional reporter was robustly activated by PA14 as a well-established target for ZIP-2 and CEBP-2. Interestingly, we found *irg-1*p::GFP was not activated by the same light condition (1500 Lux, 24 hrs) that reliably induced *cyp-14A5*p::GFP (Fig. 2D). Although ZIP-2 can be activated by pathogen stresses through ribosomal inhibition and subsequent selective ZIP-2 translation(Dunbar et al., 2012; Estes et al., 2010; Kniazeva and Ruvkun, 2025; Reddy et al., 2016), our results suggest the specific roles of ZIP-2 in mediating light-induced *cyp-14A5* but not *irg-1* reporter expression, suggesting the involvement of additional stress context-specific factors in these processes.

We further explored conditions and mechanisms leading to light-induced *cyp-14A5p::GFP*. To test potential effects of ultraviolet (UV) irradiation from our visible light LED, we used a UV-masking shield to block UV irradiation. However, this did not affect visible LED light-induced *cyp-14A5p::GFP* expression (Fig. 2E). In addition, we found that the LED light exposure of equal

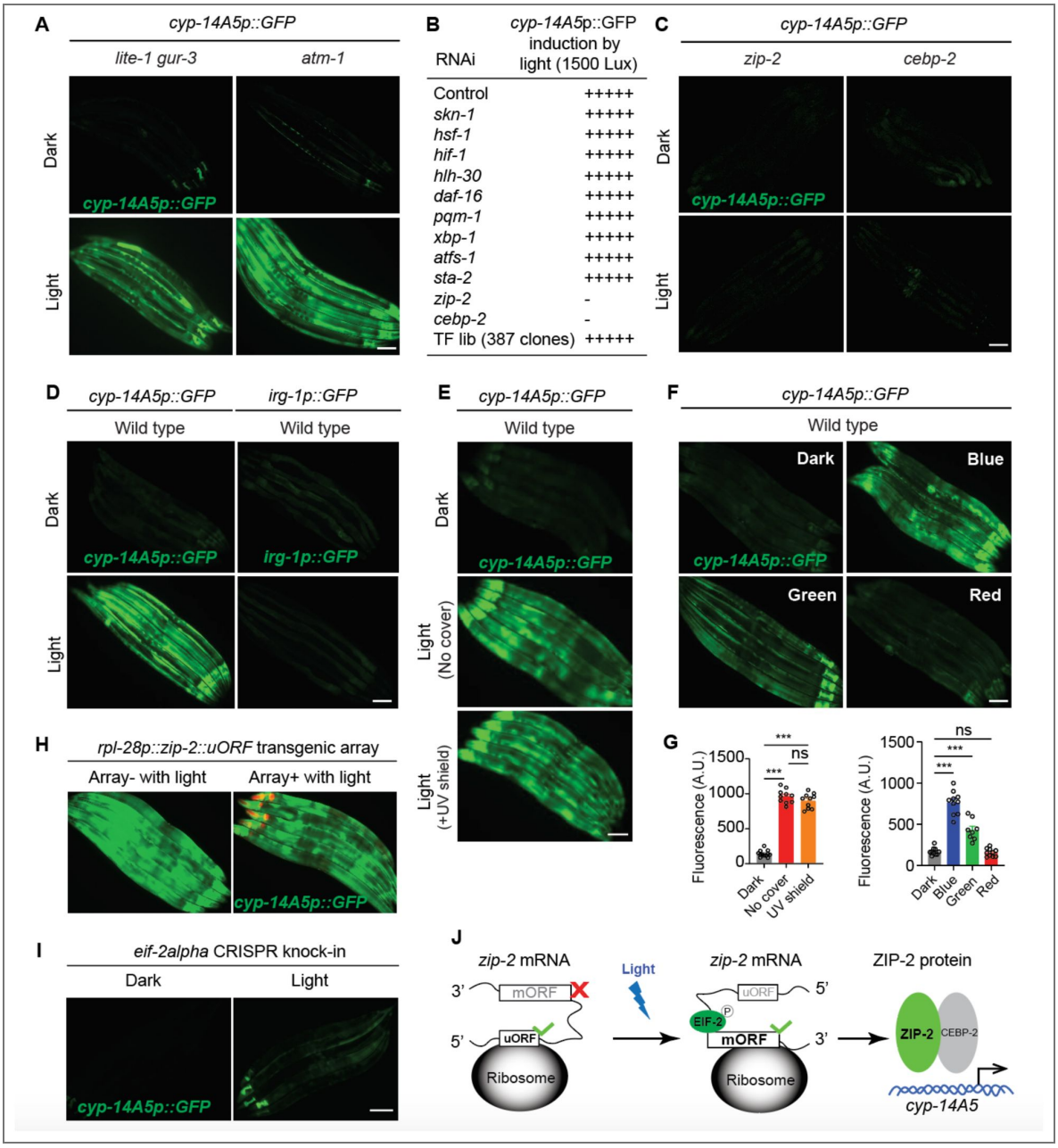

Figure 2. Light induction of *cyp-14A5*p::GFP requires the transcription factors ZIP-2 and CEBP-2.

A, Representative epifluorescence images showing light-induced *cyp-14A5*p::GFP expression in *lite-1 gur-3* double and *atm-1* single loss-of-function mutants. Scale bar: 50 μm. B, Summary of RNAi screens identifying ZIP-2 and CEBP-2 as essential transcriptional regulators of light-induced *cyp-14A5p::GFP* expression. C, Representative epifluorescence images showing light-induced *cyp-14A5p::GFP* expression in wild type, *zip-2* and *cebp-2* loss-of-function mutants. Scale bar: 50 μm. D, Representative epifluorescence images showing light-induced *cyp-14A5p::GFP* expression and no light-induced *irg-1p::GFP* expression in wild type animals. Scale bar: 50 μm. E, Representative epifluorescence images showing light-induced *cyp-14A5p::GFP* expression unaffected by a UV shield. Scale bar: 50 μm. F, Representative epifluorescence images showing light-induced *cyp-14A5p::GFP* expression by red, green, blue LED light sources of equal intensities. Scale bar: 50 μm. G, Quantification of E and F. *** indicates $P < 0.001$, n.s., non-significant. H, Representative epifluorescence images showing light-induced *cyp-14A5p::GFP* expression unaffected in *rpl-28p::zip-2uORF* transgenic animals. Scale bar: 50 μm. I, Representative epifluorescence images showing light-induced *cyp-14A5p::GFP* expression abolished in *eif-2alpha* mutants. Scale bar: 50 μm. J, Schematic model for light-induced transition of *zip-2* mRNA from translating uORF to mORF, leading to increased ZIP-2 and subsequent increased transcriptional *cyp-14A5* expression in cooperation with CEBP-2. Other molecular players are omitted and uORF and mORF are separated for clarity.

intensities (1500 Lux, 24 hrs) but at different wavelengths (red, green, blue) led to differential *cyp-14A5p::GFP* expression (Fig. 2F, **2G**), showing stronger effects of shorter wavelengths in the visible-light spectrum.

A pseudo-open reading frame (uORF) in the 5' untranslated region (UTR) of *zip-2* mRNA inhibits the ribosomal translation of the ZIP-2 main open reading frame (mORF) in the context of PA14 pathogen exposure(Dunbar et al., 2012). However, constitutive expression of *zip-2* uORF by the *rpl-28* promoter did not affect light-induced *cyp-14A5p::GFP* (Fig. 2H). Furthermore, a CRISPR phospho-site knock-in mutation of *eif-2alpha(S49A)* did not affect global translation(Ma et al., 2023), yet abolished light-induced *cyp-14A5p::GFP* (Fig. 2I). As the eukaryotic eIF2alpha complex facilitates translational switch from uORF to mORF upon stress-induced ribosomal stall at uORF(Brito Querido et al., 2024; Costa-Mattioli and Walter, 2020; Mir et al., 2024), these results suggest that it is the *zip-2* uORF translational inhibition, not the uORF protein product function, that mediates visible light-increased ZIP-2 translation and subsequent *cyp-14A5p::GFP* expression (Fig. 2J).

Light-induced CYP-14A5 enhances behavioral memory

Although EIF-2alpha and ZIP-2/CEBP-2 functions appear essential for light-induced up-regulation of *cyp-14A5*, the *zip-2* or *cyp-14A5* loss-of-function null mutants show no apparent body-size, morphological, feeding, defecation or developmental defects under dark or LED light treatment (1500 Lux for 16 or 24 hrs) conditions (Fig. S3). These data suggest that transient visible light exposure or light-induced *cyp-14A5* activation by ZIP-2 does not broadly impact development, aging or physiology, unlike long-term visible light exposure, which has been shown to robustly shorten lifespans in *C. elegans*(De Magalhaes Filho et al., 2018).

The lack of obvious morphological and basal behavioral defects led us to explore whether light exposure influences other aspects of *C. elegans* biology, particularly behavioral plasticity and associative memory formation that might require integration of body physiology. Specifically, we chose a learning paradigm in which animals learn to avoid an innately attractive odor butanone after it is paired with aversive stimuli(Chandra et al., 2023; Kauffman et al., 2010). *C. elegans* can consolidate this learning into a long-lasting memory for up to 16 hours once the repetitive training is followed by sleep and recovery post learning(Chandra et al., 2023). Using this conditioning protocol (Fig. 3A), we observed that animals exposed to ambient light (approximately 500–1000 Lux) during olfactory associative learning and recovery exhibited significantly enhanced memory retention compared to those maintained in darkness (Fig. 3B). To pinpoint the critical period for light exposure, we deprived animals of light in two-hour intervals immediately post learning. Interestingly, light deprivation during the first 2–4 hours post learning resulted in markedly impaired memory retention (Fig. 3C). These results suggest that environmental light exposure enhances aversive cue association post learning and is not required for learning itself but is required post learning concurrent with sleep for consolidation of memory(Chandra et al., 2023).

Does the light-modulated behavioral memory consolidation require light activation of the ZIP-2 pathway? To address this question, we examined the behavioral memory of two independent *zip-2* deletion mutants as compared to wild type (*cebp-2* mutants are pleiotropically sick and thus not included). We found that both the *ok3730* and *tm4246* deletion mutations of *zip-2* caused significantly impaired memory consolidation but not learning (Fig. 3D). Strikingly, the loss-of-function mutation of *cyp-14A5*, but not *F43C1.7* (another ZIP-2 target gene induced by visible light), also impaired memory retention as *zip-2* (Fig. 3E), indicating a crucial role of the ZIP-2/CYP-14A5 regulatory axis in mediating light-modulated memory consolidation. To further delineate the role of CYP-14A5, we performed tissue-specific rescue experiments in the behavioral memory assay. We found that hypoderm-specific expression of *cyp-14A5* restored the behavioral memory in the *cyp-14A5* mutant (Fig. 3F). We observed similar degrees of rescue by two independently derived lines expressing hypoderm-specific *dpy-7p::cyp-14A5* transgenes. These findings strongly suggest that hypodermal induction of CYP-14A5 by ZIP-2 plays a central role in mediating light-modulated behavioral memory.

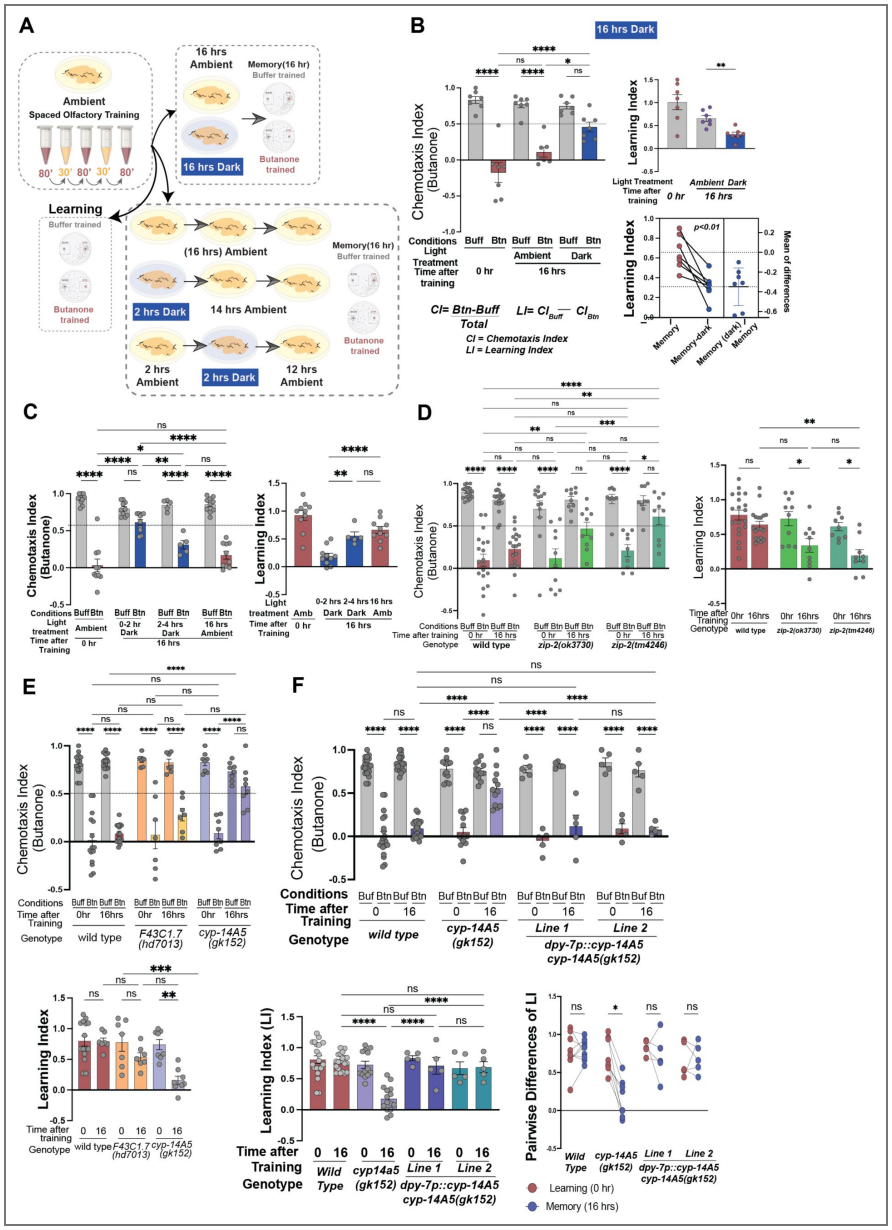

Figure 3. Light promotes behavioral plasticity and memory consolidation via a ZIP-2/CYP axis in hypodermis.

A, Schematic of behavioral setup to test effects of dark/light on olfactory memory. B, Wild-type learning and memory after 16 hours of dark exposure. 7 trials, 50-200 animals per trial/condition. Two-way ANOVA shows significant differences in chemotaxis (CI) under ambient light conditions (approximately 600 Lux) but not when the assay plates were placed in the dark. Learning (LI) indices reflect the differences between buffer- and butanone-treated animals, attenuated under dark (one-way ANOVA). Pairwise t-tests of the amount of memory retention under light and dark recovery reveal the degree of memory loss under dark. C, Dark exposure timeline shows that exposing animals to dark immediately after training (0-2 hr, Dark) hampered memory retention, whereas dark conditions for the 2–4-hour period is less sufficient to induce memory loss (two-way ANOVA). The lack of differences between buffer and butanone-trained animals is reflected in the respective LIs (one-way ANOVA). D, Two different loss-of-function alleles of *zip-2(tm4246)* or *zip-2(ok3730)* both showed impaired memory, but learning remained intact (two-way ANOVA). 5-10 trials, 50-200 animals per trial/condition. E, Memory impairment of *cyp-14A5(gk152)* but not *F43C1.7* null mutant animals (two-way ANOVA for CIs and One-way ANOVA for LIs. 7-14 trials, 50-200 animals per trial/condition. F, Memory defects of *cyp-14A5(gk152)* mutants are rescued by hypodermal expression of wild-type *cyp-14A5*. Two independent transgenic lines show similar results with comparison of pairwise differences in LIs and the amount of memory rescued by hypodermal *cyp-14A5* (Cis: Two-way ANOVA; Lis: One-way ANOVA). * indicates $P < 0.05$, ** indicates $P < 0.01$, *** indicates $P < 0.001$, **** indicates $P < 0.0001$, n.s., non-significant.

The *cyp-14A5* promoter as a versatile tool for light-inducible gene expression

The light-responsive nature of the *cyp-14A5* promoter prompted us to explore its potential as a tool for controlling gene expression. We generated synthetic constructs driving the expression of diverse effectors under the *cyp-14A5* promoter to confer striking organismal phenotypes. We previously found that *zip-10* expression promotes stress-induced organismal death, i.e. phenoptosis(Pandey and Ma, 2022; Wang et al., 2023). Driven by the *cyp-14A5* promoter, light-induced *zip-10* expression indeed caused a robust light-dependent rapid aging or phenoptosis-like phenotype with markedly shortened median and maximal lifespans (Fig. 4A-4D). We confirmed that LED light exposure markedly induced *zip-10* expression, as evidenced by robust ZIP-10-tagging mCherry fluorescence in major non-neuronal tissues of transgenic animals, only after light (1500 Lux, 24 or 48 hrs) exposure (Fig. 4C).

To test organismal behavioral outcomes, we expressed *nlp-22* under the *cyp-14A5* promoter. *nlp-22* was previously identified as a sleep-promoting neuropeptide(Bringmann, 2018; Nelson et al., 2013; Van der Auwera et al., 2020), overexpression of which can cause drastic reduction of pumping and locomotion speed, characteristic of sleep behaviors in *C. elegans*. We found that *cyp-14A5p::nlp-22* can indeed trigger striking behavioral quiescence upon light exposure (1500 Lux, 24 or 48 hrs), as quantified by pumping rates, bending frequencies and locomotion speed (Fig. 4E-4G). Quantitative analysis by WormLab reveals that the behavioral quiescence induced by *nlp-22* corresponded to characteristic bouts of sleep (Fig. S4). These proof-of-concept studies demonstrate that the *cyp-14A5* promoter enables light-dependent ectopic induction of gene expression, offering a flexible tool for probing gene function, studies of organismal biology, behaviors and synthetic physiology applications.

Discussion

Our findings uncover a previously unknown light-induced transcriptional pathway in *C. elegans* that operates independently of known visual light receptors. Our study also establishes a functional link between ambient light and behavioral plasticity through a ZIP-2/CYP regulatory axis. The discoveries of this pathway and its organismal functions open potential avenues for understanding body-brain communication and how environmental cues can shape physiological and behavioral processes.

The specificity of this pathway is particularly intriguing, as *cyp-14A5* is robustly induced by light at wavelength and intensity that do not apparently alter ambient temperature (Fig. S1). Previous studies identified *cyp-14A5* as one of many genes moderately regulated by bacterial pathogens(Sinha et al., 2012; Troemel et al., 2006; Wong et al., 2007), yet the classic pathogen-inducible gene reporter *irg-1p::GFP* was not apparently activated by light conditions that induced *cyp-14A5p::GFP*. We found that heat-killed bacteria or starvation suppressed light induction of *cyp-14A5p::GFP*, suggesting a likely modulatory mechanism from bacteria. These observations raise important questions about how ZIP-2 and *cyp-14A5* are selectively activated by light, directly or indirectly via bacteria. Given the crucial roles of ZIP-2 and eIF2alpha we discovered for light-induced expression of *cyp-14A5* and the established role of the uORF at the 5' untranslated region of *zip-2* RNA in ZIP-2 regulation(Dunbar et al., 2012), it is plausible that light may regulate ZIP-2 translation by *zip-2* RNA photo-oxidation at specific sites, eIF2alpha phosphorylation and specialized ribosomal signaling(D'Orazio and Green, 2021; Genuth and Barna, 2018; Sinha et al., 2024). Of note, bacterial chromophores (Elahi and Baker, 2024), including porphyrins, flavins, or indole metabolites, can generate reactive oxygen or oxidation products upon illumination, thereby possibly producing exogenous oxidative cues that induce *cyp-14A5* in *C. elegans*. Further investigations into the upstream signaling events, the molecular sensors linking light exposure to ZIP-2/CEBP-2 activation, and how live bacteria modulate light-induced ZIP-2/*cyp-14A5* are warranted.

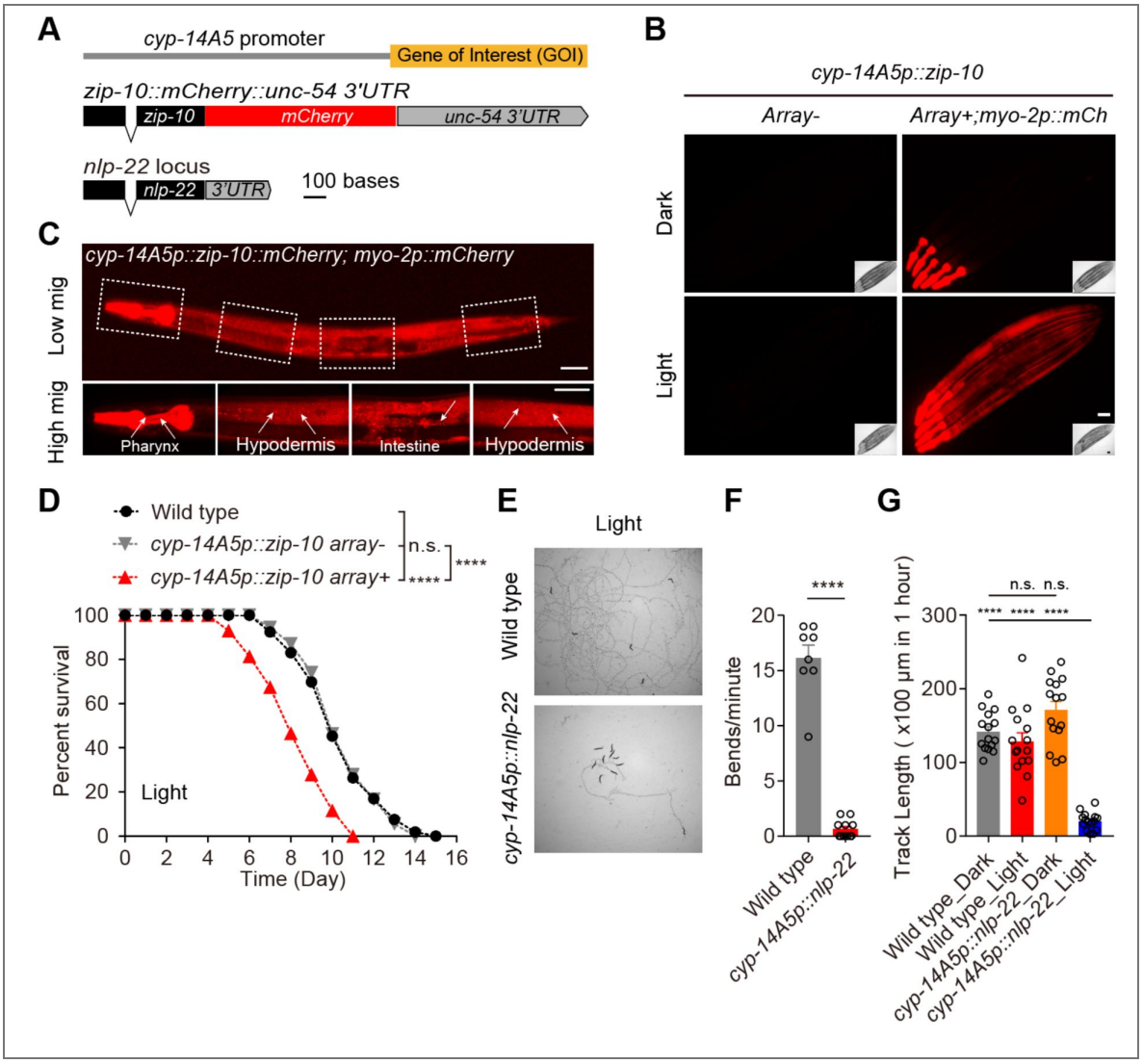

Figure 4. Light-induced gene expression drives organismal phenotypes, including sleep and shortened lifespans.

A, Schematic of synthetic constructs for light-inducible *nlp-22* and *zip-10::mCherry* using the *cyp-14A5* promoter. B, Representative compound epifluorescence images showing light-induced *cyp-14A5p::zip-10::mCherry* activation. Scale bar: 50 μm. Pharyngeal muscle-specific *myo-2p::mCherry* was used as a co-injection marker. C, Representative confocal fluorescence images showing light-induced *cyp-14A5p::zip-10::mCherry* activation (1500 Lux, 24 hrs starting at 24 hrs post L4) in major non-neuronal tissues (hypoderm, intestinal cells indicated by arrows). Scale bar: 50 μm. D, Representative lifespan curves showing that light-induced *zip-10* can markedly shorten lifespan through transient light exposure (1500 Lux, 24 hrs starting at 24 hrs post L4). **** indicates $P < 0.0001$. E, Representative bright field images showing quiescent sleep behaviors by light-induced *cyp-14A5p::nlp-22* expression through transient light exposure (1500 Lux, 24 hrs starting at 24 hrs post L4). F, Quantification of population bending frequencies for transient light-treated (1500 Lux, 24 hrs starting at 24 hrs post L4) control wild type and *cyp-14A5p::nlp-22* animals. G, Quantification of population track lengths for control wild type and *cyp-14A5p::nlp-22* animals with transient light (1500 Lux, 24 hrs starting at 24 hrs post L4) or darkness treatments. *** indicates $P < 0.001$, **** indicates $P < 0.0001$, n.s., non-significant.

Our behavioral assays demonstrate that light exposure enhances olfactory associative memory, providing direct evidence for the functional relevance of light-induced *cyp-14A5* expression. Interestingly, light exposure appears to exert its effects during a specific temporal window hours following learning, suggesting that light-induced transcriptional changes post learning play a key role in memory consolidation. The discovery of *cyp-14A5* as a key effector in this pathway also provides new insights into how non-neuronal tissues contribute to behavioral plasticity. Our findings suggest that light-induced CYP-14A5 and CYP-dependent metabolic or signaling changes in the hypoderm may communicate with the nervous system to influence behavioral memory. Importantly, the moderate light intensities used in our behavioral assays are sufficient to trigger measurable CYP-14A5 induction without eliciting the stress responses observed under higher light levels. This suggests that physiologically tuned, intermediate light exposure engages a systemic metabolic program that supports memory stabilization. This body-brain communication axis highlights the importance of systemic integration in mediating complex physiological and behavioral responses to environmental cues(Aghayeva et al., 2021; Fukuda et al., 2025; Liu et al., 2022; Zhang et al., 2018).

Beyond its biological significance, the light-inducible *cyp-14A5* promoter offers a useful new tool for gene expression studies in *C. elegans*. The ability to drive ectopic gene expression in response to light provides a versatile system for temporally controlled genetic manipulations. Our demonstration of light-induced sleep and mortality phenotypes through ectopic gene expression illustrates the potential applications of this tool in studying diverse biological processes in synthetic biology and physiology.

Previous studies have elegantly used heat shock or drug-inducible promoters for temporally controlled gene expression in *C. elegans*(Monsalve et al., 2019; Stringham et al., 1992; Wei et al., 2012). The light-inducible *cyp-14A5* promoter provides an alternative, simple-to-implement approach and might be particularly useful when the drug-inducible system is cumbersome, or heat shock effects are undesirable. Moreover, the combination of single-copy integrated and validated *cyp-14A5p::cGAL4* (see Method) with modular *UASp::effector* constructs(Wang et al., 2017) further expands the flexibility and generalizability of this system, enabling researchers to readily plug in diverse effectors for broad applications across cell types and pathways.

While our study uncovers a novel light-responding mechanism with functional consequences in *C. elegans*, several limitations exist. First, the precise molecular mechanism by which visible light activates ZIP-2 and/or CEBP-2 remains unclear, as does the upstream signaling cascade linking light exposure to transcriptional activation. Second, although we demonstrate a functional connection between light-induced *cyp-14A5* expression and behavioral outcomes, the exact molecular interplay between peripheral transcriptional changes and neural plasticity requires further exploration. Finally, while the *cyp-14A5* promoter serves as a useful genetic tool, it does not confer tissue specificity, and its ectopic effector expression requires control for light effects. These limitations provide fertile ground for future research to build upon our findings.

Materials and methods

C. elegans strains

C. elegans strains were grown on nematode growth media (NGM) plates seeded with *Escherichia coli* OP50 at 20 °C with laboratory standard procedures unless otherwise specified. The N2 Bristol strain was used as the reference wild type(Brenner, 1974). Mutants and integrated transgenes were back-crossed at least five times. Genotypes of strains used are as follows: *dmaIs156 IV [cyp-14A5p:: cyp-14A5::GFP; unc-54p::mCherry], agIs17 IV [irg-1p::gfp], dmaEx [dpy-7p::cyp-14A5; myo-2p::mCherry], dmaEx [cyp-14A5p::zip-10::mCherry; myo-2p::mCherry], dmaEx [cyp-14A5p::nlp-22; myo-2p::mCherry], cebp-2 (tm5421) I, eif-2alpha(rog3) I, zip-2(tm4248) III, zip-2(ok3730) III, cyp-14A5(gk152) V*. Single-copy integration strains based on the UAS-cGAL4 system (Wang et al., 2017): *jsSi1988 tanSi331 [pHW2186 cyp-14A5p::cGAL-tbb-2 3'UTR; cup-4p::mScarlet::tbb-2 3'UTR] IV, jsTi1453 jsSi1518 [UAS 11X Δpes10 GFP-C1] I; jsSi1988 tanSi331 [pHW2186 cyp-14A5p::cGAL-tbb-2 3'UTR; cup-4p::mScarlet::tbb-2 3'UTR] IV*.

PCR fusion constructs were used to generate transgenes(Hobert, 2002), using primer sequences:

DM1310_*cyp-14A5*Pro c5p TCAACCACATCTTCCGATCA; DM1311_*cyp-14A5*Pro to GFP c3p CGACCTGCAGGCATGCAAGCTgatctttgttggacagaatagtttt; DM2857_*dpy-7*p to *cyp-14A5* codutr Forward TGTCTCTGACGCCTGTGAGT; DM2858_*dpy-7*p to *cyp-14A5* codutr Reverse GATAAAGCAACGATGAAAACGCTCATTTTGTTTTCACAGAGCGGTAGA; DM2944_*zip-2*uORF to *rpl-28*p-GOI-mCherry-5utr fusion F CATCATAAAATAATTTATTTCCAGGTAAAATGTATCACGCAAAGACAACCACCG; DM2945_*zip-2*uORF to *rpl-28*p-GOI-mCherry-5utr fusion catgttatcttcttcacccttgaggagccAAGCTCCCGTGGGAAGCTTGTG; DM2948_*zip-10* to *cyp-14A5*p-GOI-mCherry-5utr fusion F aaaactattctgtccaacaaagatcaaaATGACAACAATGACTAATTCTCTTATTTC; DM2949_*zip-10* to *cyp-14A5*p-GOI-mCherry-5utr fusion R catgttatcttcttcacccttgaggagccGGAATGGTTGATTTGATTATTGAGTTG DM2952_*nlp-22*cod::3utr to *cyp-14A5*p-GOI fusion aaaactattctgtccaacaaagatcaaaATGCGTTCCATAATCGTCTTCATCG; DM2953_*nlp-22*cod::3utr to *cyp-14A5*p-GOI fusion R cggttccactttctcatgagt

Fluorescence microscopy and imaging

SPE confocal (Leica) and epifluorescence microscopes were used to capture fluorescence images. Animals were randomly picked at the same stage and treated with 1 mM levamisole in M9 solution (31742-250MG, Sigma-Aldrich), aligned on a 2% agar pad on a slide for imaging. Identical setting and conditions were used to compare experimental groups with control. For quantification of GFP fluorescence, animals were outlined and quantified by measuring gray values using the ImageJ software. The data were plotted and analyzed by using GraphPad Prism10.

For light-induced reporter imaging, reporter animals (synchronized young adults, 24 hrs post L4) were exposed to white light (1500 Lux for 16 or 24 hrs, by Viribright 12-Watt, 800 Lumen, LED Desk Lamp Dimmable Office Lamp). For blue, green (SPE confocal, Leica), and red light (HQRP 660 nm 14w LED pure red) conditions, animals of the same stage were exposed to the same intensities (1500 Lux, 16 or 24 hours). Control groups from the same batch of animals were maintained in darkness by opaque shields. Light intensities and temperature were quantitatively measured by digital light meters and thermometers.

RNA sequencing

A synchronized population of wild-type young adult (24 hrs post L4) animals were exposed to LED light (1500 lux, 24 hr duration, Viribright 12-Watt, 800 Lumen, LED Desk Lamp Dimmable Office Lamp). Control groups from the same batch of animals were maintained in darkness by opaque light shields. Four independent biological replicates were used for both light-treated and control groups. For sample collection, the animals were washed down from NGM plates using M9 solution and bacteria-cleaned with M9 washing in centrifuge tubes, homogenized by tissue disruptors and subjected to RNA extraction using the RNeasy Mini Kit from Qiagen. 1 mg total RNA from each sample was used for sequencing library construction. The libraries were constructed and sequenced for paired end 150 bp by DNBseq (Innomics). The cleaned RNAseq reads were mapped to the genome sequence of *C. elegans* using hisat2 and the mapped reads were assigned to the genes using featureCounts(Kim et al., 2015; Liao et al., 2014). The abundance of genes was expressed as RPKM (Reads per kilobase per million mapped reads) and identification of differentially expressed genes (Table S1) was performed using the DESeq2 package(Love et al., 2014).

C. elegans behavioral assays

The olfactory behavioral memory assay was as described previously with modification(Chandra et al., 2023; Kauffman et al., 2010). Briefly, one day old adult worms (24 hrs post L4) were washed with S basal buffer (0.1 M NaCI, 0.05 M K3PO4, pH 6.0) off 10 cm NGM plates and into microfuge tubes, where they were washed three times with S basal buffer. The animals were split in two groups; one group was added to a microfuge tube of S basal media and the other group was added to a microfuge tube of 1:10,000 dilution of butanone in S basal. The microfuge tubes were

then rotated for 80 minutes. The odor training includes three 80-minute cycles of training with odor, or a control buffer interspersed with two 30-minute periods of feeding with OP50 *E. coli* bacteria. For chemotaxis assay, 1 µL of (1 M) NaN3 was pipetted onto the odor and diluent spots in 10 cm plastic petri dishes. 1 µL of 200 proof ethanol was added to the diluent spot and 1 µL of 1:1000 butanone was added to the odor spot, while S basal or butanone-trained worms were dropped onto the middle of 10 cm plastic petri dishes. The recovery period was either under darkness or ambient light (approximately 600 Lux) for 16 hrs or were kept under darkness for 2 or 4 hr periods followed by light exposure and chemotaxis to assay behavioral memory.

For sleep analysis induced by *cyp-14A5p::nlp-22*, the bending angles, moving average speed, and track length of *C. elegans* after 48 hours of exposure to either light or dark conditions were analyzed using WormLab. In such experiment, a synchronized population of young adult (24 hrs post L4) animals of indicated genotype or transgene expression were used. After light exposure or control dark treatment, they were transferred to a fresh NGM plate seeded with a small OP50 bacterial lawn and allowed to settle for at least ten minutes to recover at room temperature. After the recovery period, a one-hour recording session was conducted using WormLab. Bending angles were calculated as described in the referenced method as a metric for sleep behaviors(Chandra et al., 2023). Moving average speed was determined by tracking the displacement of the worms over time.

Statistics

Numerical data were analyzed using GraphPad Prism 10 Software (Graphpad, San Diego, CA) and presented as means ± S.D. unless otherwise specified, with *P* values calculated by unpaired two-tailed t-tests (comparisons between two groups), one-way ANOVA (comparisons across more than two groups) and two-way ANOVA (interaction between genotype and treatment), with post-hoc Tukey and Bonferroni's corrections. The lifespan assay was plotted and quantified using Kaplan–Meier lifespan analysis, and *P* values were calculated using the log-rank test.

Supplmentary figures

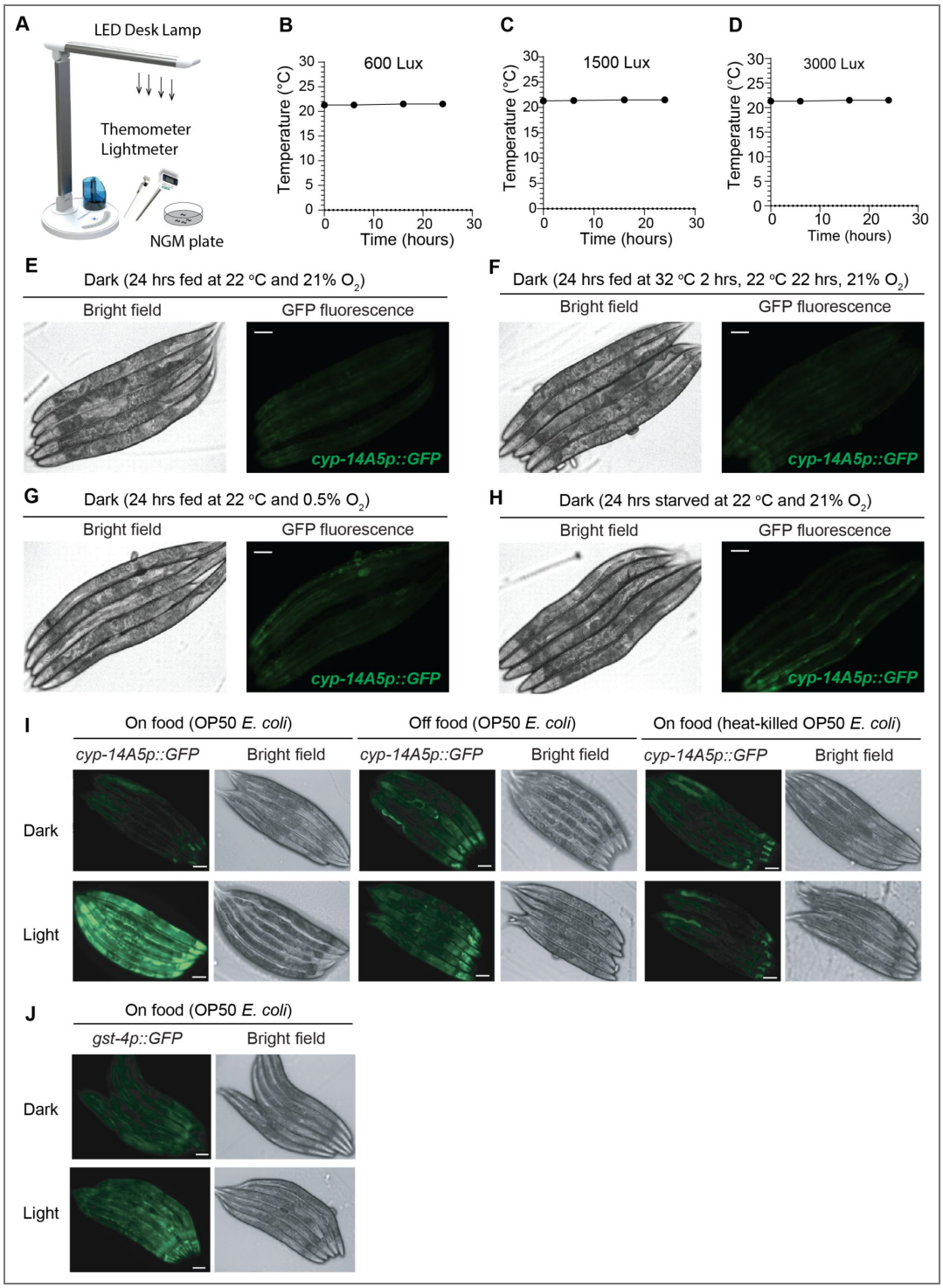

Figure S1. ***cyp-14A5p::GFP*** **induction responds primarily to visible light exposure rather than changes in ambient oxygen, nutrient or temperature.** A, Schematic of the setup for measurements of light intensity and temperature at a plane (in parallel to LED visible light sources) where animals are exposed to LED light in NGM plates. B, Measurements of temperature at the 600 Lux light intensity showing no change of temperature over 24 hrs. C, Measurements of temperature at the 1500 Lux light intensity showing no change of temperature over 24 hrs. D, Measurements of temperature at the 3000 Lux light intensity showing no change of temperature over 24 hrs. E-H, Representative bright-field and epifluorescence images showing no apparent *cyp-14A5p::GFP* induction by transient exposure to heat shock (F), constant exposure to 24 hrs of hypoxia (G) or starvation (H). I,

Representative bright-field and epifluorescence images showing *cyp-14A5p::GFP* induction by light when on-food with OP50 *E. Coli*, but suppressed in starved animals or with heat-killed OP50. Similar results were obtained with the single-copy integrated *UASp::GFP; cyp-14A5p::cGAL4* strains. J, Representative bright-field and epifluorescence images showing relatively mild *gst-4p::GFP* induction by the same light condition (1500 Lux, 24 hrs starting at 24 hrs post L4) that robustly induced *cyp-14A5p::GFP*. Scale bar: 50 μm.

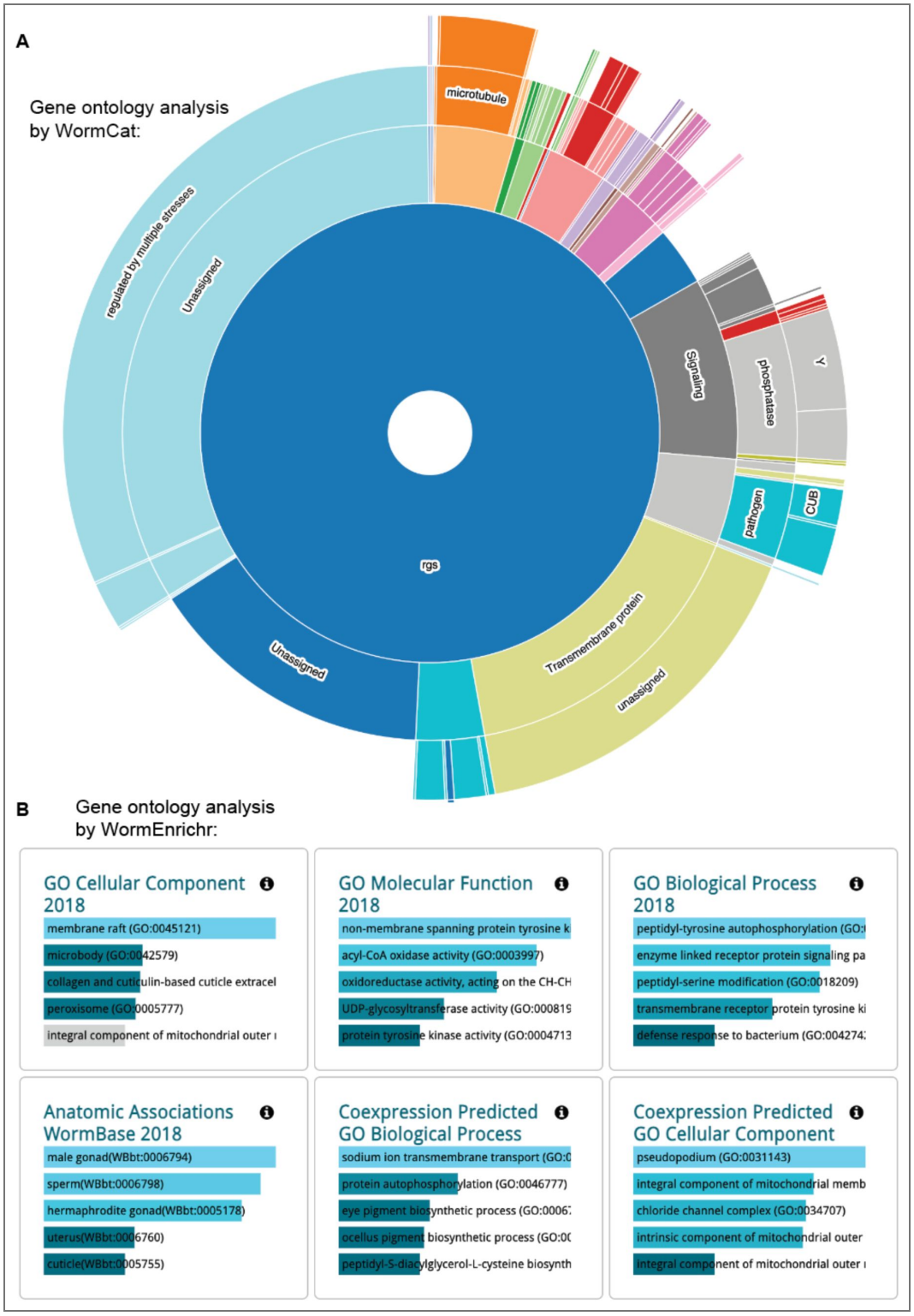

Figure S2. Gene ontology analysis of light-induced transcriptomic changes.

A, Starburst plot of gene ontology analysis of light-induced genes using WormCat (*http://www.wormcat.com/*). B, Table summary of gene ontology analysis of light-induced genes using WormEnrichr (*https://maayanlab.cloud/WormEnrichr/*).

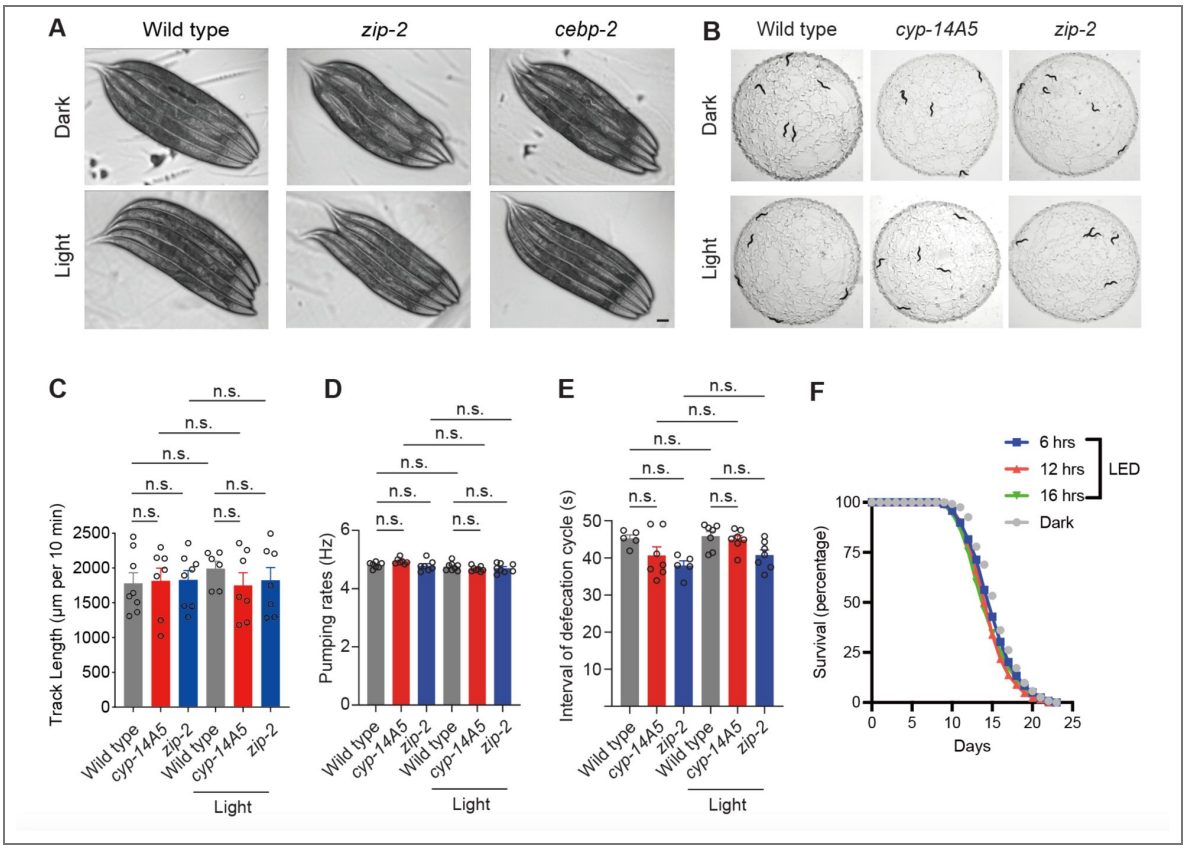

Figure S3. No apparent effects of short-term transient visible LED light exposure on development, morphology, simple behaviors and lifespans.

A, Representative bright-field images showing no apparent effects of visible light exposure (1500 Lux, 24 hrs) on the body lengths and gross morphology of wild type, *cebp-2*, and *zip-2* loss-of-function mutants. Scale bar: 50 μm. B. Representative images showing the movement tracks of wild type, *cyp-14A5*, and *zip-2* loss-of-function mutants under dark and light (1500 Lux, 24 hrs) conditions. C, Quantification of track lengths of wild type, *cyp-14A5*, and *zip-2* loss-of-function mutants under dark and light (1500 Lux, 24 hrs) conditions. D, Quantification of pumping rates of wild type, *cyp-14A5*, and *zip-2* loss-of-function mutants under dark and light (1500 Lux, 24 hrs) conditions. E, Quantification of defecation behaviors of wild type, *cyp-14A5*, and *zip-2* loss-of-function mutants under dark and light (1500 Lux, 24 hrs) conditions. F, Lifespan curves of wild-type animals under conditions of dark or transient LED light exposure for various indicated durations.

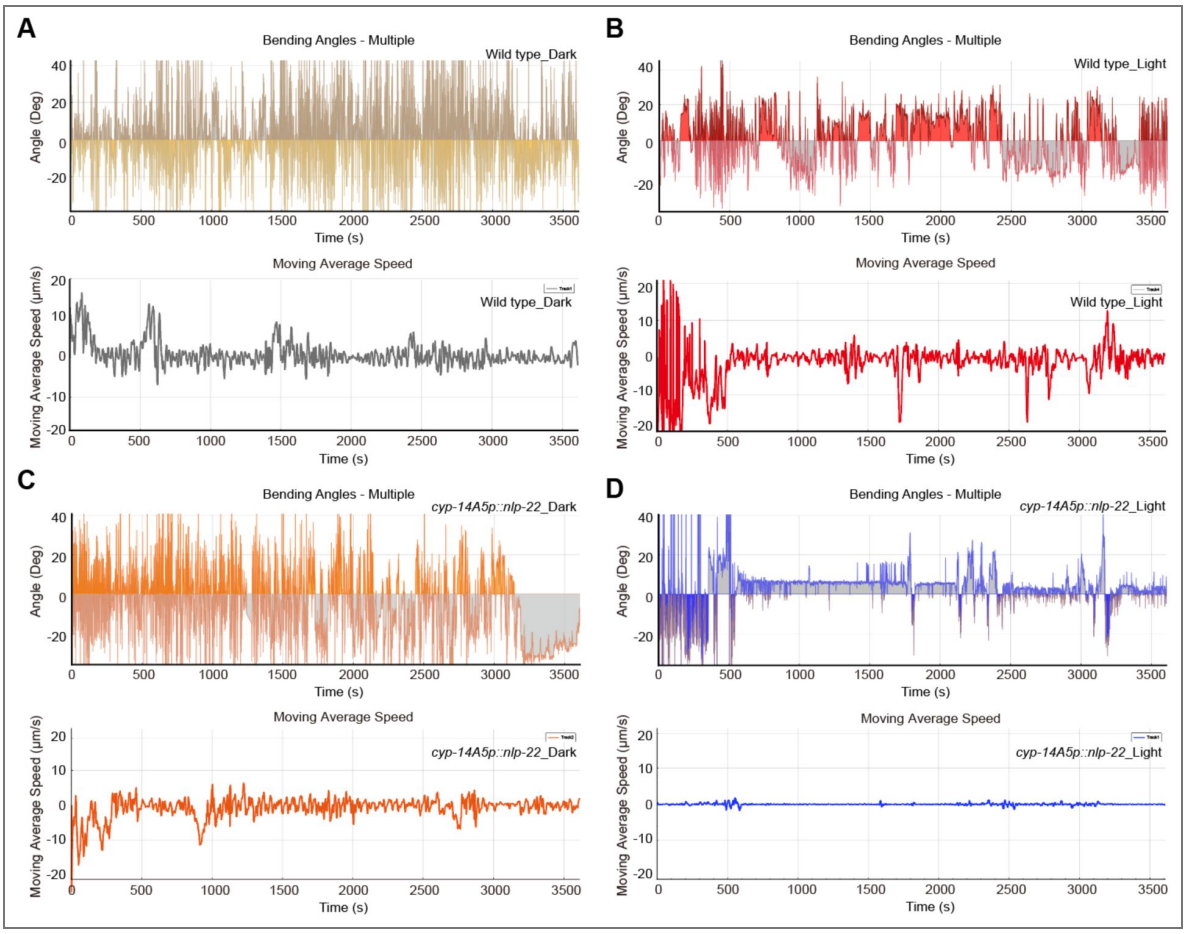

Figure S4. WormLab analysis reveals sleep bouts caused by light-induced *cyp-14A5p::nlp-22* expression.

(A) Representative tracking of moving angles for body posture and average speed for control animals under dark conditions (N=15). (B) Representative tracking of moving angles for body posture and average speed for control animals after light exposure conditions (1500 Lux, 24 hrs, N=13). (C) Representative tracking of moving angles for body posture and average speed for *cyp-14A5p::nlp-22* transgenic animals under dark conditions (N=12). (B) Representative tracking of moving angles for body posture and average speed for *cyp-14A5p::nlp-22* transgenic animals after light exposure conditions (1500 Lux, 24 hrs, N=13).

Acknowledgements

Some strains were provided by the *Caenorhabditis* Genetics Center (CGC), which is funded by the NIH Office of Research Infrastructure Programs (P40 OD010440), and by Dr. E. Troemel. We also thank the *C. elegans* Reverse Genetics Core Facility (University of British Columbia), National Bioresource Project (S. Mitani, Tokyo Women's Medical University, Tokyo, Japan), *Wormbase.org* (NIH grant #U24 HG002223 to P. Sternberg), *Wormatlas.org* (NIH grant #OD010943 to D.H. Hall.), Aging Atlas (Dr. M. Wang) and CenGen (*cengen.org*) for the resources. The work was supported by NIH grants (R35GM139618 to D.K.M.), BARI Investigator Award (D.K.M.), and UCSF PBBR New Frontier Research Award (D.K.M.)

Additional information

Data availability

All data generated or analyzed during this study are included in the manuscript and supporting files

Author Contributions

Z.J. prepared RNA-seq samples and characterized light effects on the *cyp-14A5* reporter, cellular, behavioral and physiological phenotypes of various mutants with assistance from W.Y. and M.E. Y.L. analyzed the RNA-seq data. B.W. made the initial observation on *cyp-14A5* induction by light and performed RNAi screens and reporter imaging. R.C. designed, performed and analyzed the behavioral memory assays. J.L. designed, performed and analyzed the light-induced sleep and phenoptotic aging assays. N.L., Y.L., B.W., Z.J., R.C., J.L. contributed to research materials, project conceptualization and editing manuscript. D.K.M. designed and analyzed the *C. elegans* experiments, contributed to project conceptualization and editing manuscript.

Funding

Funder	Grant reference number	Author
HHS | NIH | National Institute of General Medical Sciences (NIGMS)	R35GM139618	Dengke K Ma

Author ORCID iDs

Dengke K Ma: https://orcid.org/0000-0002-5619-7485

Additional files

Supplementary Table S1

References

Aghayeva U, Bhattacharya A, Sural S, Jaeger E, Churgin M, Fang-Yen C, Hobert O (2021) DAF-16/FoxO and DAF-12/VDR control cellular plasticity both cell-autonomously and via interorgan signaling. *PLoS Biol* **19**:e3001204 https://doi.org/10.1371/journal.pbio.3001204

Andrabi M, Upton BA, Lang RA, Vemaraju S (2023) An Expanding Role for Nonvisual Opsins in Extraocular Light Sensing Physiology. *Annu Rev Vis Sci* **9**:245-267 https://doi.org/10.1146/annurev-vision-100820-094018

Berson DM, Dunn FA, Takao M (2002) Phototransduction by retinal ganglion cells that set the circadian clock. *Science* **295**:1070-1073 https://doi.org/10.1126/science.1067262

Bhatla N, Horvitz HR (2015) Light and hydrogen peroxide inhibit C. elegans Feeding through gustatory receptor orthologs and pharyngeal neurons. *Neuron* **85**:804-818 https://doi.org/10.1016/j.neuron.2014.12.061

Brenner S (1974) The genetics of Caenorhabditis elegans. *Genetics* **77**:71-94

Bringmann H (2018) Sleep-Active Neurons: Conserved Motors of Sleep. *Genetics* **208**:1279-1289 https://doi.org/10.1534/genetics.117.300521

Brito Querido J, Díaz-López I, Ramakrishnan V (2024) The molecular basis of translation initiation and its regulation in eukaryotes. *Nat Rev Mol Cell Biol* **25**:168-186 https://doi.org/10.1038/s41580-023-00624-9

Chandra R, Farah F, Muñoz-Lobato F, Bokka A, Benedetti KL, Brueggemann C, Saifuddin MFA, Miller JM, Li J, Chang E, *et al.* (2023) Sleep is required to consolidate odor memory and remodel olfactory synapses. *Cell* **186**:2911-2928.e20. https://doi.org/10.1016/j.cell.2023.05.006

Ciccia A, Elledge SJ (2010) The DNA damage response: making it safe to play with knives. *Mol Cell* **40**:179-204 https://doi.org/10.1016/j.molcel.2010.09.019

Costa-Mattioli M, Walter P (2020) The integrated stress response: From mechanism to disease. *Science* **368**:eaat5314 https://doi.org/10.1126/science.aat5314

Cronin TW, Johnsen S (2016) Extraocular, Non-Visual, and Simple Photoreceptors: An Introduction to the Symposium. *Integr Comp Biol* **56**:758-763 https://doi.org/10.1093/icb/icw106

De Magalhaes Filho CD, Henriquez B, Seah NE, Evans RM, Lapierre LR, Dillin A. (2018) Visible light reduces C. elegans longevity. *Nat Commun* **9**:927 https://doi.org/10.1038/s41467-018-02934-5

Denisov IG, Makris TM, Sligar SG, Schlichting I (2005) Structure and chemistry of cytochrome P450. *Chem Rev* **105**:2253-2277 https://doi.org/10.1021/cr0307143

Do MTH, Yau K-W (2010) Intrinsically photosensitive retinal ganglion cells. *Physiol Rev* **90**:1547-1581 https://doi.org/10.1152/physrev.00013.2010

D'Orazio KN, Green R (2021) Ribosome states signal RNA quality control. *Mol Cell* **81**:1372-1383 https://doi.org/10.1016/j.molcel.2021.02.022

Dunbar TL, Yan Z, Balla KM, Smelkinson MG, Troemel ER (2012) C. elegans detects pathogen-induced translational inhibition to activate immune signaling. *Cell Host Microbe* **11**:375-386 https://doi.org/10.1016/j.chom.2012.02.008

Edwards SL, Charlie NK, Milfort MC, Brown BS, Gravlin CN, Knecht JE, Miller KG (2008) A novel molecular solution for ultraviolet light detection in Caenorhabditis elegans. *PLoS Biol* **6**:e198 https://doi.org/10.1371/journal.pbio.0060198

Elahi Y, Baker MAB (2024) Light Control in Microbial Systems. *Int J Mol Sci* **25**:4001 https://doi.org/10.3390/ijms25074001

Estes KA, Dunbar TL, Powell JR, Ausubel FM, Troemel ER (2010) bZIP transcription factor zip-2 mediates an early response to Pseudomonas aeruginosa infection in Caenorhabditis elegans. *Proc Natl Acad Sci U S A* **107**:2153-2158 https://doi.org/10.1073/pnas.0914643107

Fukuda A, Sato K, Fujimori C, Yamashita T, Takeuchi A, Ohuchi H, Umatani C, Kanda S (2025) Direct photoreception by pituitary endocrine cells regulates hormone release and pigmentation. *Science* **387**:43-48 https://doi.org/10.1126/science.adj9687

Genuth NR, Barna M (2018) Heterogeneity and specialized functions of translation machinery: from genes to organisms. *Nat Rev Genet* **19**:431-452 https://doi.org/10.1038/s41576-018-0008-z

Gong J, Yuan Y, Ward A, Kang L, Zhang B, Wu Z, Peng J, Feng Z, Liu J, Xu XZS (2016) The C. elegans Taste Receptor Homolog LITE-1 Is a Photoreceptor. *Cell* **167**:1252-1263.e10. https://doi.org/10.1016/j.cell.2016.10.053

Hanson SM, Scholüke J, Liewald J, Sharma R, Ruse C, Engel M, Schüler C, Klaus A, Arghittu S, Baumbach F, *et al.* (2023) Structure-function analysis suggests that the photoreceptor LITE-1 is a light-activated ion channel. *Curr Biol* **33**:3423-3435.e5. https://doi.org/10.1016/j.cub.2023.07.008

Hattar S, Lucas RJ, Mrosovsky N, Thompson S, Douglas RH, Hankins MW, Lem J, Biel M, Hofmann F, Foster RG, *et al.* (2003) Melanopsin and rod-cone photoreceptive systems account for all major accessory visual functions in mice. *Nature* **424**:76-81 https://doi.org/10.1038/nature01761

Hobert O (2002) PCR fusion-based approach to create reporter gene constructs for expression analysis in transgenic C. elegans. *Biotechniques* **32**:728-730 https://doi.org/10.2144/02324bm01

Kauffman AL, Ashraf JM, Corces-Zimmerman MR, Landis JN, Murphy CT (2010) Insulin signaling and dietary restriction differentially influence the decline of learning and memory with age. *PLoS Biol* **8**:e1000372 https://doi.org/10.1371/journal.pbio.1000372

Keller J, Ellieva A, Ma DK, Ju J, Nehk E, Konkel A, Falck JR, Schunck W-H, Menzel R (2014) CYP-13A12 of the nematode Caenorhabditis elegans is a PUFA-epoxygenase involved in behavioural response to reoxygenation. *Biochem J* **464**:61-71 https://doi.org/10.1042/BJ20140848

Kim D, Langmead B, Salzberg SL (2015) HISAT: a fast spliced aligner with low memory requirements. *Nat Methods* **12**:357-360 https://doi.org/10.1038/nmeth.3317

Kniazeva M, Ruvkun G (2025) Translation elongation defects activate the Caenorhabditis elegans ZIP-2 bZIP transcription factor-mediated toxin defense. *Proc Natl Acad Sci U S A* **122**:e2423578122 https://doi.org/10.1073/pnas.2423578122

Liao Y, Smyth GK, Shi W (2014) featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* **30**:923-930 https://doi.org/10.1093/bioinformatics/btt656

Liebel F, Kaur S, Ruvolo E, Kollias N, Southall MD (2012) Irradiation of skin with visible light induces reactive oxygen species and matrix-degrading enzymes. *J Invest Dermatol* **132**:1901-1907 https://doi.org/10.1038/jid.2011.476

Liu Y, Zhou J, Zhang N, Wu X, Zhang Q, Zhang W, Li X, Tian Y (2022) Two sensory neurons coordinate the systemic mitochondrial stress response via GPCR signaling in C. elegans. *Dev Cell* **57**:2469-2482.e5. https://doi.org/10.1016/j.devcel.2022.10.001

Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* **15**:550 https://doi.org/10.1186/s13059-014-0550-8

Lucas RJ, Hattar S, Takao M, Berson DM, Foster RG, Yau K-W (2003) Diminished pupillary light reflex at high irradiances in melanopsin-knockout mice. *Science* **299**:245-247 https://doi.org/10.1126/science.1077293

Ma DK, Rothe M, Zheng S, Bhatla N, Pender CL, Menzel R, Horvitz HR (2013) Cytochrome P450 drives a HIF-regulated behavioral response to reoxygenation by C. elegans. *Science* **341**:554-558 https://doi.org/10.1126/science.1235753

Ma Z, Horrocks J, Mir DA, Cox M, Ruzga M, Rollins J, Rogers AN (2023) The integrated stress response protects against ER stress but is not required for altered translation and lifespan from dietary restriction in Caenorhabditis elegans. *Front Cell Dev Biol* **11**:1263344 https://doi.org/10.3389/fcell.2023.1263344

Mahmoud BH, Ruvolo E, Hexsel CL, Liu Y, Owen MR, Kollias N, Lim HW, Hamzavi IH (2010) Impact of long-wavelength UVA and visible light on melanocompetent skin. *J Invest Dermatol* **130**:2092-2097 https://doi.org/10.1038/jid.2010.95

Mir DA, Ma Z, Horrocks J, Rogers A (2024) Stress-Induced Eukaryotic Translational Regulatory Mechanisms. *J Clin Med Sci* **8**:1000277

Monsalve GC, Yamamoto KR, Ward JD (2019) A New Tool for Inducible Gene Expression in Caenorhabditis elegans. *Genetics* **211**:419-430 https://doi.org/10.1534/genetics.118.301705

Nelson MD, Trojanowski NF, George-Raizen JB, Smith CJ, Yu C-C, Fang-Yen C, Raizen DM (2013) The neuropeptide NLP-22 regulates a sleep-like state in Caenorhabditis elegans. *Nat Commun* **4**:2846 https://doi.org/10.1038/ncomms3846

Pandey T, Ma DK (2022) Stress-Induced Phenoptosis: Mechanistic Insights and Evolutionary Implications. *Biochemistry (Mosc)* **87**:1504-1511 https://doi.org/10.1134/S0006297922120082

Reddy KC, Dunbar TL, Nargund AM, Haynes CM, Troemel ER (2016) The C. elegans CCAAT-Enhancer-Binding Protein Gamma Is Required for Surveillance Immunity. *Cell Rep* **14**:1581-1589 https://doi.org/10.1016/j.celrep.2016.01.055

Schuch AP, Moreno NC, Schuch NJ, Menck CFM, Garcia CCM (2017) Sunlight damage to cellular DNA: Focus on oxidatively generated lesions. *Free Radic Biol Med* **107**:110-124 https://doi.org/10.1016/j.freeradbiomed.2017.01.029

Shi Y, Zhang J, Li X, Han Y, Guan J, Yilin Li, Shen J, Tzvetanov T, Yang D, Luo X, *et al.* (2024) Non-image-forming photoreceptors improve visual orientation selectivity and image perception. *Neuron* **113**:486-500.e13 https://doi.org/10.1016/j.neuron.2024.11.015

Sinha A, Rae R, Iatsenko I, Sommer RJ (2012) System wide analysis of the evolution of innate immunity in the nematode model species Caenorhabditis elegans and Pristionchus pacificus. *PLoS One* **7**:e44255 https://doi.org/10.1371/journal.pone.0044255

Sinha NK, McKenney C, Yeow ZY, Li JJ, Nam KH, Yaron-Barir TM, Johnson JL, Huntsman EM, Cantley LC, Ordureau A, *et al.* (2024) The ribotoxic stress response drives UV-mediated cell death. *Cell* **187**:3652-3670.e40. https://doi.org/10.1016/j.cell.2024.05.018

Stringham EG, Dixon DK, Jones D, Candido EP (1992) Temporal and spatial expression patterns of the small heat shock (hsp16) genes in transgenic Caenorhabditis elegans. *Mol Biol Cell* **3**:221-233 https://doi.org/10.1091/mbc.3.2.221

Troemel ER, Chu SW, Reinke V, Lee SS, Ausubel FM, Kim DH (2006) p38 MAPK regulates expression of immune response genes and contributes to longevity in C. elegans. *PLoS Genet* **2**:e183 https://doi.org/10.1371/journal.pgen.0020183

Van der Auwera P, Frooninckx L, Buscemi K, Vance RT, Watteyne J, Mirabeau O, Temmerman L, De Haes W, Fancsalszky L, Gottschalk A, *et al.* (2020) RPamide neuropeptides NLP-22 and NLP-2 act through GnRH-like receptors to promote sleep and wakefulness in C. elegans. *Sci Rep* **10**:9929 https://doi.org/10.1038/s41598-020-66536-2

Van Gelder RN. (2008) Non-visual photoreception: sensing light without sight. *Curr Biol* **18**:R38-39 https://doi.org/10.1016/j.cub.2007.11.027

Wang C, Long Y, Wang B, Zhang C, Ma DK (2023) GPCR signaling regulates severe stress-induced organismic death in Caenorhabditis elegans. *Aging Cell* **22**:e13735 https://doi.org/10.1111/acel.13735

Wang H, Liu J, Gharib S, Chai CM, Schwarz EM, Pokala N, Sternberg PW (2017) cGAL, a temperature-robust GAL4-UAS system for Caenorhabditis elegans. *Nat Methods* **14**:145-148 https://doi.org/10.1038/nmeth.4109

Wei X, Potter CJ, Luo L, Shen K (2012) Controlling gene expression with the Q repressible binary expression system in Caenorhabditis elegans. *Nat Methods* **9**:391-395 https://doi.org/10.1038/nmeth.1929

Wong D, Bazopoulou D, Pujol N, Tavernarakis N, Ewbank JJ (2007) Genome-wide investigation reveals pathogen-specific and shared signatures in the response of Caenorhabditis elegans to infection. *Genome Biol* **8**:R194 https://doi.org/10.1186/gb-2007-8-9-r194

Zhang B, Gong J, Zhang W, Xiao R, Liu J, Xu XZS (2018) Brain-gut communications via distinct neuroendocrine signals bidirectionally regulate longevity in C. elegans. *Genes Dev* **32**:258-270 https://doi.org/10.1101/gad.309625.117

Peer reviews

Reviewer #1 (Public review):

Summary:

The authors set out to understand how animals respond to visible light in an animal without eyes. To do so they used the C. elegans model, which lacks eyes, but nonetheless exhibits

robust responses to visible light at several wavelengths. Here, the authors report a promoter that is activated by visible light and independent of known pathways of light resposnes.

Strengths:

The authors convincingly demonstrate that visible light activates the expression of the cyp-14A5 promoter driven gene expression in a variety of contexts and report the finding that this pathway is activated via the ZIP-2 transcriptionally regulated signaling pathway.

Weaknesses:

Because the ZIP-2 pathway has been reported to activated predominantly by changes in the bacterial food source of C. elegans -- or exposure of animals to pathogens -- it remains unclear if visible light activates a pathway in C. elegans (animals) or if visible light potentially is sensed by the bacteria on the plate which also lack eyes. Specifically, it is possible that the the plates are seeded with excess E. coli, that E. coli is altered by light in some way and in this context alters its behavior in such a way that activates a known bacterially responsive pathway in the animals. Consistent with this possibility the authors found that heat-killed bacteria prevented the reporter activation in animals. This weakness would not affect the ability to use this novel discovery as a tool, which would still be useful to the field.

https://doi.org/10.7554/eLife.108507.2.sa3

Reviewer #2 (Public review):

Summary:

Ji, Ma and colleagues report the discovery of a mechanism in C. elegans that mediates transcriptional responses to low intensity light stimuli. They find that light-induced transcription requires a pair of bZIP transcription factors and induces expression of a cytochrome P450 effector. This unexpected light-sensing mechanism is required for physiologically relevant gene expression that controls behavioral plasticity. The authors further show that this mechanism can be co-opted to create light-inducible transgenes.

Strengths:

The authors rigorously demonstrate that ambient light stimuli regulate gene expression via a mechanism that requires the bZIP factors ZIP-2 and CEBP-2. Transcriptional responses to light stimuli are measured using transgenes and using measurements of endogenous transcripts. The study shows proper genetic controls for these effects. The study shows that this light-response does not require known photoreceptors, is tuned to specific wavelengths, and is highly unlikely to be an artifact of temperature-sensing. The study further shows that the function of ZIP-2 and CEBP-2 in light-sensing can be distinguished from their previously reporter role in mediating transcriptional responses to pathogenic bacteria. The study includes experiments that demonstrate that regulatory motifs from a known light-response gene can be used to confer light-regulated gene expression, demonstrating sufficiency and suggesting an application of these discoveries in engineering inducible transgenes. Finally, the study shows that ambient light and the transcription factors that transduce it into gene expression changes are required to stabilize a learned olfactory behavior, suggesting a physiological function for this mechanism.

Weaknesses:

The study implies but does not show that the effects of ambient light on stabilizing a learned olfactory behavior are through the described pathway. To show this clearly, the authors should determine whether ambient light has any further effects on learning in mutants lacking CYP-14A5, ZIP-2, or CEBP-2.

https://doi.org/10.7554/eLife.108507.2.sa2

Reviewer #3 (Public review):

Ji et al. report a novel and interesting light-induced transcriptional response pathway in the eyeless roundworm Caenorhabditis elegans that involves a cytochrome P450 family protein (CYP-14A5) and functions independently from previously established photosensory mechanisms. The authors also demonstrate the potential for this pathway to enable robust light-induced control of gene expression and behavior, albeit with some restrictions. Despite the limitations of this tool, including those presented by the authors, it could prove useful for the community. Overall, the evidence supporting the claims of the authors is convincing, and the authors' work suggests numerous interesting lines of future inquiry.

(1) Although the exact mechanisms underlying photoactivation of this pathway remain unclear, light-dependent induction of CYP-14A5 requires bZIP transcription factors ZIP-2 and CEBP-2 that have been previously implicated in worm responses to pathogens. Notably, this light response requires live food bacteria, suggesting a microbial contribution to this phenomenon. The nature of the microbial contribution to the light response is unknown but very interesting.

(2) The authors suggest that light-induced CYP-14A5 activity in the C. elegans hypoderm can unexpectedly and cell-non-autonomously contribute to retention of an olfactory memory. How retention of the olfactory memory is enhanced by light generally remains unclear. Additional experiments, including verification of light-dependent changes in CYP-14A5 levels in the olfactory memory behavioral setup, appropriate would help further interpret these otherwise interesting results.

https://doi.org/10.7554/eLife.108507.2.sa1

Author response:

The following is the authors' response to the original reviews

> ***Public Reviews:***
>
> ***Reviewer #1 (Public review):***
>
> *Summary:*
>
> *The authors set out to understand how animals respond to visible light in an animal without eyes. To do so, they used the C. elegans model, which lacks eyes, but nonetheless exhibits robust responses to visible light at several wavelengths. Here, the authors report a promoter that is activated by visible light and independent of known pathways of light responses.*
>
> *Strengths:*
>
> *The authors convincingly demonstrate that visible light activates the expression of the cyp-14A5 promoter-driven gene expression in a variety of contexts and report the finding that this pathway is activated via the ZIP-2 transcriptionally regulated signaling pathway.*
>
> *Weaknesses:*
>
> *Because the ZIP-2 pathway has been reported to be activated predominantly by changes in the bacterial food source of C. elegans -- or exposure of animals to pathogens -- it remains unclear if visible light activates a pathway in C. elegans (animals) or if visible light potentially is sensed by the bacteria on the plate, which also lack eyes. Specifically, it*

> *is possible that the plates are seeded with excess E. coli, that E. coli is altered by light in some way, and in this context, alters its behavior in such a way that activates a known bacterially responsive pathway in the animals. This weakness would not affect the ability to use this novel discovery as a tool, which would still be useful to the field, but it does leave some questions about the applicability to the original question of how animals sense light in the absence of eyes.*

Thank you for the insightful questions and suggestions. We have now performed a key experiment requested. Interesting new data (Fig. S1I) show that light induction of *cyp-14A5p::GFP* requires live bacteria that maintain a non-starved physiological state. Neither plates without food nor plates with heat-killed OP50 support robust induction. We now include this interesting new result in the paper and revised discussion on the bacteria-modulated mechanism but note that this bacterial requirement does not alter the central conclusions of the study. Rather, it reveals an intriguing mechanistic layer, namely, that bacterial metabolic activity likely influences the animal's sensitivity to environmental light. We are pursuing this host–microbe interaction in a separate study. In the present work, we focus on the intrinsic regulation and functional significance of *cyp-14A5* under standard laboratory conditions with live OP50. Accordingly, we have revised the Results and Discussion to reflect the appropriate scope.

> ***Reviewer #2 (Public review):***
>
> *Summary:*
>
> *Ji, Ma, and colleagues report the discovery of a mechanism in C. elegans that mediates transcriptional responses to low-intensity light stimuli. They find that light-induced transcription requires a pair of bZIP transcription factors and induces expression of a cytochrome P450 effector. This unexpected light-sensing mechanism is required for physiologically relevant gene expression that controls behavioral plasticity. The authors further show that this mechanism can be co-opted to create light-inducible transgenes.*
>
> *Strengths:*
>
> *The authors rigorously demonstrate that ambient light stimuli regulate gene expression via a mechanism that requires the bZIP factors ZIP-2 and CEBP-2. Transcriptional responses to light stimuli are measured using transgenes and using measurements of endogenous transcripts. The study shows proper genetic controls for these effects. The study shows that this light-response does not require known photoreceptors, is tuned to specific wavelengths, and is highly unlikely to be an artifact of temperature-sensing. The study further shows that the function of ZIP-2 and CEBP-2 in light-sensing can be distinguished from their previously reported role in mediating transcriptional responses to pathogenic bacteria. The study includes experiments that demonstrate that regulatory motifs from a known light-response gene can be used to confer light-regulated gene expression, demonstrating sufficiency and suggesting an application of these discoveries in engineering inducible transgenes. Finally, the study shows that ambient light and the transcription factors that transduce it into gene expression changes are required to stabilize a learned olfactory behavior, suggesting a physiological function for this mechanism.*
>
> *Weaknesses:*
>
> *The study implies but does not show that the effects of ambient light on stabilizing a learned olfactory behavior are through the described pathway. To show this clearly, the authors should determine whether ambient light has any effect on mutants lacking CYP-14A5, ZIP-2, or CEBP-2. Other minor edits to the text and figures are suggested.*

We appreciate the reviewer's comment. Our study indeed implies that ambient light stabilizes learned olfactory behavior through effects on the described pathway. Importantly, the existing data already address this point. Mutants lacking CYP-14A5, ZIP-2, or CEBP-2 display impaired olfactory memory even when exposed to ambient light, indicating that these genes are required for the behavioral effect of light. Consistent with this, ambient light robustly induces *cyp-14A5p::GFP* in wild-type animals but fails to do so in *zip-2* and *cebp-2* mutants, demonstrating that light-dependent transcriptional activation is blocked upstream in these pathway mutants. Together, these results support the conclusion that ambient light acts through the ZIP-2 → CEBP-2 → CYP-14A5 pathway to stabilize memory. Minor textual and figure revisions have been made where helpful to clarify this point.

> ***Reviewer #3 (Public review):***
>
> *Ji et al. report a novel and interesting light-induced transcriptional response pathway in the eyeless roundworm Caenorhabditis elegans that involves a cytochrome P450 family protein (CYP-14A5) and functions independently from previously established photosensory mechanisms. Although the exact mechanisms underlying photoactivation of this pathway remain unclear, light-dependent induction of CYP-14A5 requires bZIP transcription factors ZIP-2 and CEBP-2 that have been previously implicated in worm responses to pathogens. The authors then suggest that light-induced CYP-14A5 activity in the C. elegans hypoderm can unexpectedly and cell-non-autonomously contribute to retention of an olfactory memory. Finally, the authors demonstrate the potential for this pathway to enable robust light-induced control of gene expression and behavior, albeit with some restrictions. Overall, the evidence supporting the claims of the authors is convincing, and the authors' work suggests numerous interesting lines of future inquiry.*
>
> *(1) The authors determine that light, but not several other stressors tested (temperature, hypoxia, and food deprivation), can induce transcription of cyp-15A5. The authors use these experiments to suggest the potential specificity of the induction of CYP-14A5 by light. Given the established relationship between light and oxidative stress and the authors' later identification of ZIP-2, testing the effect of an oxidative stressor or pathogen exposure on transcription of cyp-14A5 would further strengthen the validity of this statement and potentially shed some insight into the underlying mechanisms.*

We appreciate the reviewer's thoughtful suggestion. We would like to clarify that the "specificity" we refer to is the strong and preferential induction of *cyp-14A5* by light among pathogen or detoxification-related genes, rather than an assertion that *cyp-14A5* is exclusively light-responsive. This does not preclude the possibility that *cyp-14A5* can also be activated under other conditions. Indeed, prior work from the Troemel laboratory has identified *cyp-14A5* as one of many pathogen-inducible genes, consistent with its role in stress physiology. Our data show that classical pathogen-responsive genes (e.g., *irg-1*) are not induced by light, whereas *cyp-14A5* is strongly induced, highlighting the selective engagement of this cytochrome P450 by light under the conditions tested. We have revised the text to clarify this point.

> *(2) The authors suggest that short-wavelength light more robustly increases transcription of cyp-14A5 compared to equally intense longer wavelengths (Figure 2F and 2G). Here, however, the authors report intensities in lux of wavelengths tested. Measurements of and reporting the specific spectra of the incident lights and their corresponding irradiances (ideally, in some form of mW/mm2 - see Ward et al., 2008, Edwards et al., 2008, Bhatla and Horvitz, 2015, De Magalhaes Filho et al., 2018, Ghosh et al., 2021, among others, for examples) is critical for appropriate comparisons across wavelengths and facilitates cross-checking with previous studies of C. elegans light responses. On a related and more minor note, the authors place an ultraviolet shield in front of a visible light LED to test potential effects of ultraviolet light on transcription of cyp-14A5. A*

> *measurement of the spectrum of the visible light LED would help confirm if such an experiment was required. Regardless, the principal conclusions the authors made from these experiments will likely remain unchanged.*

Thank you. We have revised the text to clarify this point. "Using controlled light versus dark conditions, we confirmed the finding from an integrated *cyp-14A5p::GFP* reporter and observed its robust widespread GFP expression in many tissues induced by moderate-intensity (500-3000 Lux, 16-48 hr duration) LED light exposure (Fig. 1A). The photometric Lux range is approximately 0.1–0.60 mW/cm^2 in radiometric (total radiant power) metric given the spectrum of the LED light source."

> *(3) The authors report an interesting observation that animals exposed to ambient light (~600 lux) exhibit significantly increased memory retention compared to those maintained in darkness (Figure 4). Furthermore, light deprivation within the first 2-4 hours after learning appears to eliminate the effect of light on memory retention. These processes depend on CYP-14A5, loss of which can be rescued by re-expression of cyp-14A5 in mutant animals using a hypoderm-specific- and non-light-inducible- promoter. Taken together, the authors argue convincingly that hypodermal expression of cyp-14A5 can contribute to the retention of the olfactory memory. More broadly, these experiments suggest that cell-non-autonomous signaling can enhance retention of olfactory memory. How retention of the olfactory memory is enhanced by light generally remains unclear. In addition, the authors' experiments in Figure 1B demonstrate - at least by use of the transcriptional reporter - that light-dependent induction of cyp-14A5 transcription at 500 - 1000 lux is minimal and especially so at short duration exposures. Additional experiments, including verification of light-dependent changes in CYP-14A5 levels in the olfactory memory behavioral setup, would help further interpret these otherwise interesting results.*

We thank the reviewer for these thoughtful comments. We agree that understanding how light enhances memory retention at a mechanistic level is an important direction for future work. Regarding the light intensities used in Figure 1B, we would like to clarify that 500–1000 lux does produce a measurable and statistically significant induction of *cyp-14A5p::GFP*, although the magnitude is lower than that observed at higher intensities. We interpret this modest induction as physiologically relevant: intermediate light levels appear sufficient to engage the CYP-14A5–dependent program required for memory stabilization, whereas stronger light intensities are detrimental to learning and reduce behavioral performance. Thus, the behavioral paradigm uses a light regime that activates the pathway without introducing stress-associated confounders.

> *(4) The experiments in Figure 4 nicely validate the usage of the cyp-14A5 promoter as a potential tool for light-dependent induction of gene expression. Despite the limitations of this tool, including those presented by the authors, it could prove useful for the community.*

Thank you and we agree. In addition, we have included in the revised manuscript the single-copy integration strains based on UAS-GAL4 that produced similar results as transgenic strains and will be even more flexible and useful for the community.

> ***Recommendations for the authors:***
>
> ***Reviewing Editor Comments:***
>
> *While appreciating the quality and presentation of this important study, we had two major concerns that the authors need to address.*
>
> *(1) Bacteria-versus-worm origin:*

> *To rule out a bacterially derived stimulus, we suggest testing whether cyp-14A5p::GFP is inducible without bacteria (or killed bacteria). Checking whether the canonical immune reporters irg-5p::GFP and gst-4p::GFP are also light-inducible will further clarify this point.*

We have now performed the key experiment requested by the reviewers. Interesting new data (Fig. S1I) show that light induction of *cyp-14A5p::GFP* requires live bacteria that maintain a non-starved physiological state. Neither plates without food nor plates with heat-killed OP50 support robust induction. Importantly, this requirement does not alter any of the central conclusions of the study. Rather, it reveals an intriguing mechanistic layer, namely, that bacterial metabolic activity influences the animal's sensitivity to environmental light. We are pursuing this host–microbe interaction in a separate study. In the present work, we focus on the regulation and functional significance of *cyp-14A5* under standard laboratory conditions with live OP50.

We included the data (Fig. 2D) to show that the canonical immune reporter *irg-1p::GFP* is not induced by the light condition that robustly induced *cyp-14A5p::GFP*, and *gst-4p::GFP* is only very mildly induced (Fig. S1J).

> *(2) Pathway-behaviour link:*
>
> *The behavioural relevance of the newly described pathway is intriguing, but it needs direct support. Ideally, this would require comparing memory in WT, zip-2-/-, cebp-2-/-, and cyp-14A5-/- under both dark and light conditions. But at the very least, it would require testing if constitutive CYP-14A5 rescue in the dark bypasses the requirement of light.*

We respectfully submit that additional experiments are not required to support the behavioral conclusions. Our model posits that *cyp-14A5* is required but not sufficient for memory stabilization, one component within a broader set of light-induced genes. Thus, constitutive hypodermal expression of *cyp-14A5* would not be expected to bypass the requirement for ambient light. The existing data are fully consistent with this framework and conclusions of the paper.

> ***Reviewer #1 (Recommendations for the authors):***
>
> *Overall, I think this paper is interesting to the field of C. elegans researchers at a minimum, as a light-inducible gene expression system might have a variety of uses throughout the diverse research paradigms that use this model system. With that said, I have a couple of suggestions that I think would substantially impact the ability to interpret these findings, which might be useful for broader implications of the study.*
>
> *(1) Most importantly, the supplemental table of RNA-seq data should likely be updated and discussed further beyond the cyp-14A5 findings. First, the authors report 7,902 genes are differentially expressed in response to light and then break these into upregulated and downregulated genes. But there are only 1,785 upregulated genes and 3,632 downregulated genes. This adds up to 5417 genes, but doesn't match the 7,902 genes reported to change, and I could not find in the text if some other filters were applied that might explain this not adding up.*

Thank you for this helpful comment. We agree that the exact numbers depend on statistical thresholds and are therefore somewhat arbitrary. To avoid implying unwarranted precision, we have revised the text to state that "thousands of genes are differentially regulated by light."

> *(2) Among the upregulated genes in response to light are irg-5, irg-4, irg-6, irg-8, and gst-4. Indeed, all of these well-studied genes (or most) show even more induction by light than cyp-14A5. It is my opinion that this result needs further criticism as there are existing GFP reporters for gst-4 and irg-5 that are similarly well studied to irg-1, which is in the paper (and is not upregulated). In my opinion, the authors should test if they see activation of the irg-4 and gst-4 GFP reporters by light as well. This would not only validate their RNA-seq but might provide more important evidence for the field, as these other reporters are not considered light-inducible previously. If they are, several major studies might be impacted by this.*

Thank you for the comments. We have *irg-1p::GFP* and *gst-4p::GFP* in the lab but did not find other reporters for the genes mentioned from CGC. Neither of the two reporters showed light induction (Figs. 2D and S1J) as strongly as *cyp-14A5p::GFP*. It is possible that *irg-1* and *gst-4* RNA levels are up-regulated but not reflected in our transgenic reporters that used their promoters to drive GFP expression. Stronger light induction of *cyp-14A5p::GFP* is unlikely caused by the multi-copy nature of the transgene since newly generated single-copy integration strains based on the UAS-GAL4 system produced similar robust results for light induction (Fig. S1I and see Method).

> *(3) Along the same lines, if at least 4 (and likely more) well characterized immune response genes are activated by light and these genes are known to mostly respond to differences in C. elegans bacterial food source/diet, then it stands to reason that maybe in this experimental context the light is not acting on "animals" at all, but rather triggering changes in E. coli (i.e. changing E. coli metabolism or pathogenicity like properties). If true, then perhaps the light affects bacteria in such a way that it activates a previously known bacterial pathogen response mechanism. This should be easy to test by seeing if this reporter is still activated by light in the presence of diverse bacterial diets, which are available from the CGC (CeMBio collection, for example). This is likely very important to the conclusions of the manuscript as it relates to animals sensing light, but might not be as important to the use of this system as a tool.*

Thank you for the insightful questions and suggestions. Interesting new data (Fig. S1I) show that light induction of *cyp-14A5p::GFP* requires live bacteria that maintain a non-starved physiological state. Neither plates without food nor plates with heat-killed OP50 support robust induction. Importantly, this requirement does not alter any of the central conclusions of the study. Rather, it reveals an intriguing mechanistic layer, namely, that bacterial metabolic activity influences the animal's sensitivity to environmental light. We are pursuing this host–microbe interaction in a separate study. In the present work, we focus on the regulation and functional significance of *cyp-14A5* under standard laboratory conditions with live OP50. We have revised the Results and Discussion to reflect the appropriate scope of our study and implications of the new findings.

> *(4) Lastly, it seems unlikely that nearly half the C. elegans genome is transcriptionally regulated by light (or nearly half of the detected genes in the RNA-seq results). It seems likely that this list of 7,902 genes contains false positives. I would suggest upping some sort of filter, like moving to padj < 0.01 instead of 0.05, or adding a 4-fold change filter (2-fold and 0.01 still results in near 5000+ genes changing, which might explain the difference in up and down genes just being due to different padj filters. Along these lines, it is worth noting that the padj is generated using DESeq2 it appears and one of the first assumptions of DESeq2 is that the median expressed genes do not change, and there is a normalization. However, if MOST genes do change in expression, then one of the fundamental assumptions of DESeq2 is not valid, and thus would mean it might not be an appropriate analysis tool - perhaps there is some other normalization that could be done before running DESeq2 due to some other noise present in the RNA-seq runs?*

Thank you for this helpful comment. We agree that the exact numbers depend on statistical thresholds and are therefore somewhat arbitrary. To avoid implying unwarranted precision, we have revised the text to state that "thousands of genes are differentially regulated by light."

> *(5) Minor point - I would delete the reference to ER in line 92. While most CYPs do localize to the ER, the images shown are not clearly ER and probably do not have enough resolution to make claims about subcellular localization. To me, it would be easier to just delete this claim as it is not required for the main claims of the manuscript.*

Reference deleted.

> ***Reviewer #2 (Recommendations for the authors):***
>
> *I have one request for clarification that likely requires additional data. Figure 3 shows that ambient light stabilizes learned changes to chemotaxis and further shows that CYP-14A5 has a similar function. The implication is that light promotes CYP-14A5 expression, which somehow promotes memory consolidation. The authors should test whether memory consolidation in cyp-15A5, zip-2, or cebp-2 mutants is no longer affected by ambient light.*

It is also possible to test whether forced expression of CYP14A5 can bypass the effect of 'no light' conditions on memory consolidation.

Thank you for the comments. We respectfully submit that additional experiments are not required to support the behavioral conclusions. Our model posits that *cyp-14A5* is required but not sufficient for memory stabilization, one component within a broader set of light-induced genes. Thus, constitutive hypodermal expression of *cyp-14A5* would not be expected to bypass the requirement for ambient light. The existing data are fully consistent with this framework and conclusions of the paper.

> *I have several minor suggestions relating to the text and figures.*
>
> *(1) In the introduction, the authors assert that little is known about non-visual light sensing and then list many examples of molecular mechanisms of non-visual light-sensing. They should emphasize that non-visual light sensing is important and accomplished by diverse molecular mechanisms.*

Agree and revised accordingly.

> *(2) Check spacing between gene names (line 109).*

Corrected.

> *(3) There should be a new paragraph break when the uORF experiments are described (line 146).*

Corrected.

> *(4) 'Phenoptosis' is an esoteric word. Please define it (line 206).*

Corrected.

> *(5) 'p' in the transgene name cyp-14A5p::nlp-22 is in italics, unlike the rest of the manuscript.*

Corrected.

> *(6) 'Acknowledgment' should be 'Acknowledgments' (line 384).*

Corrected.

> *(7) The color map in panel 1B should have units.*

It was arbitrary unit (now added) to highlight relative not absolute differences.

> *(8) In panel 1E, it is confusing to have 'DARK' denoted by reddish bars and 'LIGHT' denoted by bluish bars. Perhaps 'DARK' is black/dark grey and 'LIGHT' is white?*

Corrected.

> *(9) In panel 1D, it takes a minute to find the purple diamond. Please mark up the volcano plot to make it easier.*

Corrected.

> ***Reviewer #3 (Recommendations for the authors):***
>
> *The authors generally present convincing experiments detailing interesting results in a well-written manuscript.*
>
> *One quick note: the same Bhatla and Horvitz (2015) papers appear to be cited twice [line 52].*

Corrected.

https://doi.org/10.7554/eLife.108507.2.sa0