Drosophila TRPA1 isoforms detect UV light via photochemical production of H_2O_2

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The transient receptor potential A1 (TRPA1) channel is an evolutionarily conserved detector of temperature and irritant chemicals. Here, we show that two specific isoforms of TRPA1 in *Drosophila* are H_2O_2 sensitive and that they can detect strong UV light via sensing light-induced production of H_2O_2. We found that ectopic expression of these H_2O_2-sensitive *Drosophila* TRPA1 (dTRPA1) isoforms conferred UV sensitivity to light-insensitive HEK293 cells and *Drosophila* neurons, whereas expressing the H_2O_2-insensitive isoform did not. Curiously, when expressed in one specific group of motor neurons in adult flies, the H_2O_2-sensitive dTRPA1 isoforms were as competent as the blue light-gated channelrhodopsin-2 in triggering motor output in response to light. We found that the corpus cardiacum (CC) cells, a group of neuroendocrine cells that produce the adipokinetic hormone (AKH) in the larval ring gland endogenously express these H_2O_2-sensitive dTRPA1 isoforms and that they are UV sensitive. Sensitivity of CC cells required dTRPA1 and H_2O_2 production but not conventional phototransduction molecules. Our results suggest that specific isoforms of dTRPA1 can sense UV light via photochemical production of H_2O_2. We speculate that UV sensitivity conferred by these isoforms in CC cells may allow young larvae to activate stress response—a function of CC cells—when they encounter strong UV, an aversive stimulus for young larvae.

UV sensing | dTRPA1 isoforms | reactive oxygen species | *Drosophila* | optogenetics

Light is an important sensory cue that has a wide-ranging influence on animal physiology and behavior. In addition to its role in vision, light detection also contributes to circadian rhythm regulation, sleep, phototaxis, and even mood control (1–5). Animals of different species have evolved diverse light sensors and light-detection cells to regulate various light-dependent physiological processes. For example, whereas rods and cones in mammals are critical for detecting and relaying image-forming visual information to the visual cortex, the intrinsically light-sensitive melanopsin-expressing retinal ganglion cells relay nonimage-forming visual information primarily to the suprachiasmatic nucleus to regulate circadian rhythm (6, 7).

Similar to the diversity of cell types that sense light, the molecular mechanisms for light detection also vary. For example, in mammalian rods and cones, light activates a Rhodopsin-dependent phototransduction pathway that hyperpolarizes the cells via closing a cyclic nucleotide-gated channel (5). In the intrinsically light-sensitive retinal ganglion cells, however, light activates a melanopsin-dependent pathway that depolarizes the cells via opening TRP channels (6, 8). Further, in *Drosophila* PDF neurons, light has been shown to activate cryptochrome signaling to depolarize the cells (9). And in the ASJ sensory neuron in *Caenorhabditis elegans*, UV and blue light have been shown to activate the cell via signaling a specific GPCR known as LITE-1 (high energy light unresponsive protein 1) (10, 11).

It has been shown that short wavelength UV and blue light can trigger reactive oxygen species (ROS) and H_2O_2 production in cultured cells (12), and H_2O_2 can modulate ion channel activities directly or indirectly (13–15). We therefore reasoned that sensing H_2O_2 might constitute another light-sensing mechanism. Indeed, a recent finding has suggested that two specific GPCRs—GUR-3 and LITE-1—both of which play important roles in light sensing in *C. elegans*, may also be H_2O_2 sensitive (16). Moreover, TRPA1, an evolutionarily conserved TRP channel known for its role in sensing many chemical irritants, has been shown to sense H_2O_2 (17–24), thus TRPA1 may be activated by light via sensing light-induced H_2O_2 production. Some recent evidence has demonstrated a role for TRPA1 in light sensing. First, in human melanocyte, TRPA1 has been shown to act to increase melanin production in response to UV (25). Moreover, in *Drosophila*, TRPA1 has been shown to increase neuronal activities of a specific group of larval somatosensory neurons (also known as the C4da neurons) in response to UV (26). However, in both cases, GPCRs—rhodopsin in the case of human melanocyte and Gr28b in the case of C4da neurons—are thought to mediate light-dependent activation of TRPA1 (25, 26); thus, it is not clear whether TRPA1 can be activated by light through H_2O_2 production.

Here, we show that two specific isoforms of *Drosophila* dTRPA1 are H_2O_2 sensitive and that their H_2O_2 sensitivity allows them to detect UV without relying on conventional phototransduction molecules. We found that ectopic expression of these H_2O_2-sensitive dTRPA1 isoforms conferred UV sensitivity to light-insensitive cultured HEK293 cells and a few types of light-insensitive *Drosophila* neurons. The light and H_2O_2 sensitivities are specific to certain dTRPA1 isoforms, consistent with previous findings that different dTRPA1 isoforms exhibit distinct thermal sensitivities (20, 21). Strikingly, the H_2O_2-sensitive dTRPA1 was as effective as channelrhodopsin-2 (ChR2) in triggering light-produced motor responses when expressed in a specific group of motor neurons that control proboscis extension

Significance

Discovering new light-sensing mechanisms and cell types are of considerable interest to researchers across disciplines. Understanding how cells sense light is one of the most fundamental problems in biology. In addition, optogenetics has become a critical research tool and discovery of new light-sensing mechanisms may expand the existing toolbox. In this work, we described our discovery of a dTRPA1-dependent photochemical pathway that is sufficient to confer UV sensitivity to light-insensitive cells. We also discovered a group of neuroendocrine cells that express these isoforms endogenously and can sense UV. These findings demonstrate a new cell type that can sense strong UV and the potential of exploiting dTRPA1 channel as an optogenetic tool.

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in adult *Drosophila*. We further discovered that the corpus cardiacum (CC) cells, a group of adipokinetic hormone (AKH)-producing cells that reside in the *Drosophila* larval ring gland, expressed the H_2O_2-sensitive dTRPA1 isoforms endogenously, and that these cells were UV and H_2O_2 sensitive. Their sensitivity required dTRPA1 and H_2O_2 production: Reducing *dual oxidase* (*DUOX*) or increasing *catalase* (*cat*) expression in them reduced their UV sensitivity significantly. In contrast, reducing *PLC* or *GR28b* in CC cells had little impact. Our results suggest specific H_2O_2-sensitive TRPA1 isoforms can be activated by UV via a photochemical transduction cascade and that these isoforms may be exploited as a UV-dependent optogenetic tool for controlling neuronal activities.

Results

Two Specific Isoforms of *Drosophila* TRPA1 Are H_2O_2 Sensitive. To begin exploring whether dTRPA1 can detect light via sensing H_2O_2, we first assessed H_2O_2 sensitivity of different dTRPA1 isoforms. The *dTRPA1* locus has two promoters (A and B) that drive expression of at least five transcripts, of which three isoforms have been shown to have distinct thermal sensitivities (20, 21) (Fig. 1*A*). The *dTRPA1(B)10a* isoform is derived from the B promoter and is commonly used as a thermogenetic tool for neuronal activation by heat (27). The *dTRPA1(A)10a* and *dTRPA1(A)10b* isoforms are derived from the A promoter, and both contain a stretch of 97 aa at their N terminus that is absent in *dTRPA1(B)10a*. The exact physiological function of these A promoter-derived isoforms has not been fully characterized in vivo, but ectopic expression experiments in vitro have shown that both isoforms are sensitive to allyl isothiocyanate (AITC, a major chemical component of wasabi) and that *dTRPA1(A)10a*, but not *dTRPA1(A)10b*, can also sense high temperature (>34 °C) (21).

To test whether any of these isoforms is H_2O_2 sensitive, we first expressed them in cultured HEK293 cells and measured the H_2O_2 sensitivity of these cells by coexpressing in them the genetically encoded calcium indicator GCaMP6 (28). We found that expressing either the *dTRPA1(A)10a* or the *dTRPA1(A)10b* isoform conferred H_2O_2 sensitivity to HEK cells, but expressing *dTRPA1(B)10a* did not (Fig. 1 *B* and *C*); but in keeping with earlier reports, all three isoforms conferred strong AITC sensitivity (Fig. 1*C*). These results show that at least two specific isoforms of *Drosophila* dTRPA1 are H_2O_2 sensitive and that H_2O_2 and AITC may act via distinct mechanisms to activate dTRPA1 because *dTRPA1(B)10a* is sensitive to AITC but not to H_2O_2.

The H_2O_2-Sensitive Isoforms of dTRPA1 Can Confer UV Sensitivity to HEK293 Cells. We next tested whether expressing the H_2O_2-sensitive isoforms of dTRPA1 in HEK cells allows these cells to become UV sensitive, because UV illumination of these cells is expected to lead to H_2O_2 production (12, 16). Indeed, we observed a robust UV-induced Ca^{2+} rise in HEK293 cells that ectopically expressed the H_2O_2-sensitive *(A)10a* or the *(A)10b* isoforms of dTRPA1 but no significant responses in cells that expressed the H_2O_2-insensitive *(B)10b* isoform (Fig. 2 *A* and *B*). These results suggest that expression of H_2O_2-sensitive isoforms of dTRPA1 alone—without coexpressing other known phototransduction molecules—is sufficient to confer UV sensitivity to light-insensitive HEK293 cells.

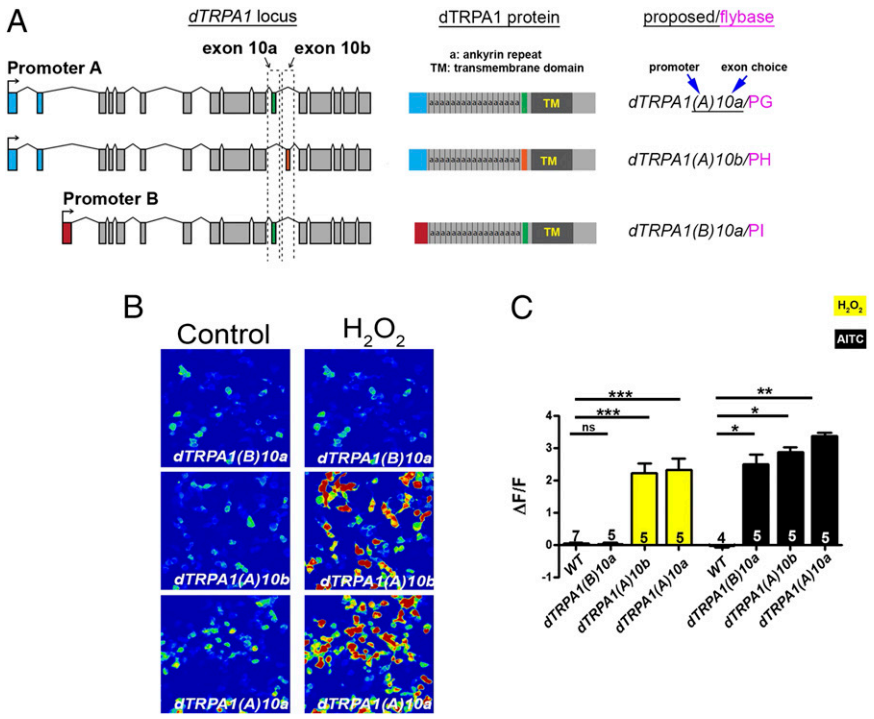

Fig. 1. Some of the dTRPA1 isoforms can confer H_2O_2 sensitivity to HEK cells. (*A*) A schematic of the genomic locus of *dTRPA1*. It is predicted to encode several different isoforms. There are two choices for promoters (A and B) and two choices for exon 10 (10a and 10b). The *dTRPA1(B)10a* isoform is the commonly used thermogenetic tool. The *dTRPA1(A)10a* and *dTRPA1(A)10b* isoforms were referred by Zhong et al. (21) as the D and the C isoforms, respectively. The names of these isoforms annotated in the Flybase are labeled in pink. (*B*) Representative H_2O_2-induced calcium responses of HEK cells that expressed different isoforms of *dTRPA1*. (*Top*) HEK cells that overexpressed the *dTRPA1(B)10a* isoform did not respond to H_2O_2. (*Center* and *Bottom*) HEK cells that overexpressed either the *dTRPA1(A)10a* or the *dTRPA1(A)10b* isoform showed significant response to 100 μM H_2O_2. GCaMP6 was used to report the calcium responses in these cells. Note that all of the calcium responses we described in this work were reported by the genetically encoded calcium indicators GCaMP3 or GCaMP6. (*C*) Quantification of the calcium responses of HEK cells to H_2O_2 (100 μM) and AITC (100 μM) when they ectopically expressed different dTRPA1 isoforms. WT, HEK cells that overexpressed GCaMP6 only. Note that all error bars described in this work indicate SEM. Mann–Whitney *t* test and one-way ANOVA followed with bonferroni test were used in the comparisons described in this work. ns, no significance; ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$.

Ectopic Expression of the H_2O_2-Sensitive Isoforms of dTRPA1 Is Sufficient To Confer H_2O_2 and UV Sensitivity to Larval CNS Neurons. Our data so far have shown that ectopic expression of H_2O_2-sensitive *dTRPA1(A)10a* and *dTRPA1(A)10b* isoforms can endow H_2O_2 and UV sensitivity to cultured mammalian cell lines. However, can they endow H_2O_2 and UV sensitivity to neurons in vivo? To test this idea, we expressed these isoforms in the insulin-like peptide 7 (ILP7)-producing neurons in the ventral nerve cord (the insect equivalent of vertebrate spinal cord) of *Drosophila* larvae (Fig. 3*A*) and monitored the activities of these neurons by calcium imaging. Indeed, ectopically expressing the H_2O_2-sensitive isoforms of dTRPA1 was sufficient to render ILP7 neurons (labeled by *ILP7-GAL4*) sensitive to both H_2O_2 and UV, whereas expressing the H_2O_2-insensitive form was not (Fig. 3 *B*–*E*). To further explore the relationship between H_2O_2 and UV sensitivity, we also ectopically expressed a mouse TRPA1 (mTRPA1) in ILP7 neurons and assessed the consequences. mTRPA1 conferred strong AITC sensitivity to these neurons, but interestingly, it failed to confer significant H_2O_2 or UV sensitivity to ILP7 neurons (Fig. S1). Taken together, these results support the idea that H_2O_2-sensitive isoforms of dTRPA1 can confer UV sensitivity to light-insensitive neurons, and their H_2O_2 sensitivity may be responsible for their ability to confer UV sensitivity.

Ectopic Expression of H_2O_2-Sensitive Isoforms of dTRPA1 Is Sufficient To Confer Behavioral Sensitivity to Light. We next wondered whether the UV sensitivity conferred by dTRPA1 is sufficiently strong to allow optogenetic activation of behavioral responses. To test this possibility, we first expressed the H_2O_2-sensitive dTRPA1 isoforms in the specific motor neurons that innervate the muscles on the internal reproductive tract of adult females (Fig. 4*A*). We have shown that activating these neurons (by expressing in them the ATP-gated P2X2 channel and then exposing them to ATP) can cause a clear contraction of the tract (29, 30); we therefore wondered whether expressing the H_2O_2-sensitive dTRPA1 in these motor neurons (labeled again by the *ILP7-GAL4*) can cause the reproductive tract to contract in response to UV. Indeed, we found that reproductive tract dissected from animals that overexpressed the H_2O_2-sensitive dTRPA1 isoforms showed UV-induced contraction, whereas ones from wild-type animals or animals expressing H_2O_2-insensitive dTRPA1 isoforms showed minimal responses (Fig. 4*B* and Movies S1 and S2).

To test whether H_2O_2-sensitive dTRPA1 can confer UV light-induced behavior in live animals, we next expressed them in the specific motor neurons that control the extension of proboscis (the flies' tongue) in adult flies (31, 32) (Fig. 4*C*). These motor neurons (labeled by the *E49-GAL4*) are of particular interest because a previous study has shown that expressing ChR2 in them caused the flies to readily extend their proboscis in response to blue light illumination (31). Thus, these neurons permit us to test whether light is similarly capable of inducing proboscis extension when we express the H_2O_2-sensitive dTRPA1 in them, and to compare the efficacy of ChR2 vs. dTRPA1 in activating this motor response.

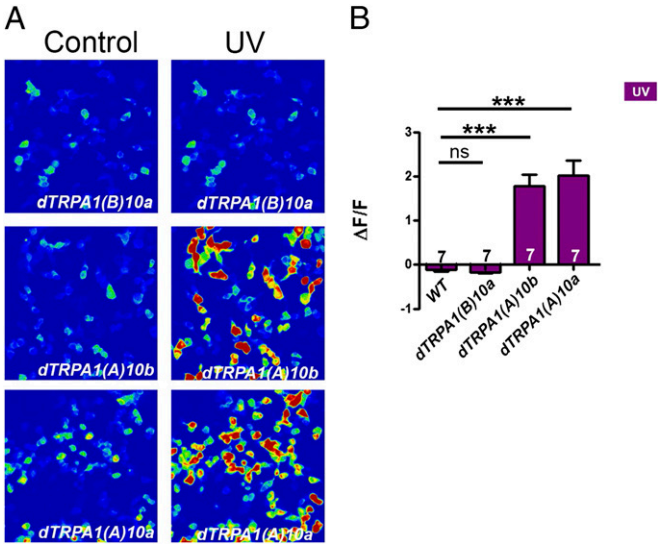

Fig. 2. Ectopic expression of the H_2O_2-sensitive dTRPA1 isoforms conferred UV sensitivity to HEK cells. (*A*) Representative UV responses of HEK cells that overexpressed the H_2O_2-insensitive *dTRPA1(B)10a* (*Top*) and the H_2O_2-sensitive *dTRPA1(A)10a* and *10b* isoforms (*Center* and *Bottom*). (*B*) Quantification of UV responses of HEK cells conferred by different isoforms. WT, HEK cells transfected with GCaMP6 only. The UV intensity used in these experiments was 50 mW/mm^2. ns, no significance; ***$P < 0.001$.

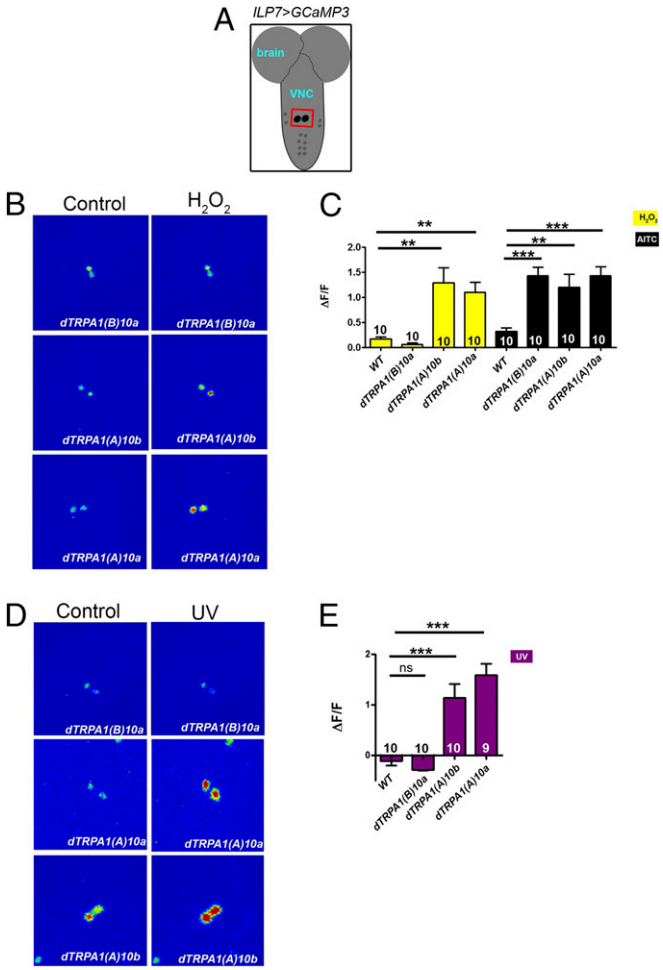

Fig. 3. Ectopic expression of H_2O_2-sensitive dTRPA1 isoforms conferred UV sensitivity to ILP7 neurons in *Drosophila* larvae. (*A*) A picture showing the general arrangement of the ILP7 neurons (labeled by *ILP7-GAL4*) in the larval CNS. The two dorsally located ILP7 neurons (boxed in red) were used to test the efficacy of dTRPA1 in conferring UV and H_2O_2 sensitivity. (*B*) Representative pictures of H_2O_2 response of ILP7 neurons that overexpressed different isoforms of dTRPA1. (*C*) Quantification of responses of ILP7 neurons to H_2O_2 (200 μM) and AITC (500 μM) that overexpressed different dTRPA1 isoforms. WT, ILP7 neurons that overexpressed GCaMP3 only. (*D*) Representative pictures of UV responses of larval ILP7 neurons that overexpressed different isoforms of dTRPA1 isoforms. (*E*) Quantification of UV response of ILP7 neurons conferred by different dTRPA1 isoforms. WT, ILP7 neurons that overexpressed GCaMP3 only. The UV intensity used in *D* and *E* was 6 mW/mm^2. ns, no significance; ***$P < 0.001$; **$P < 0.01$.

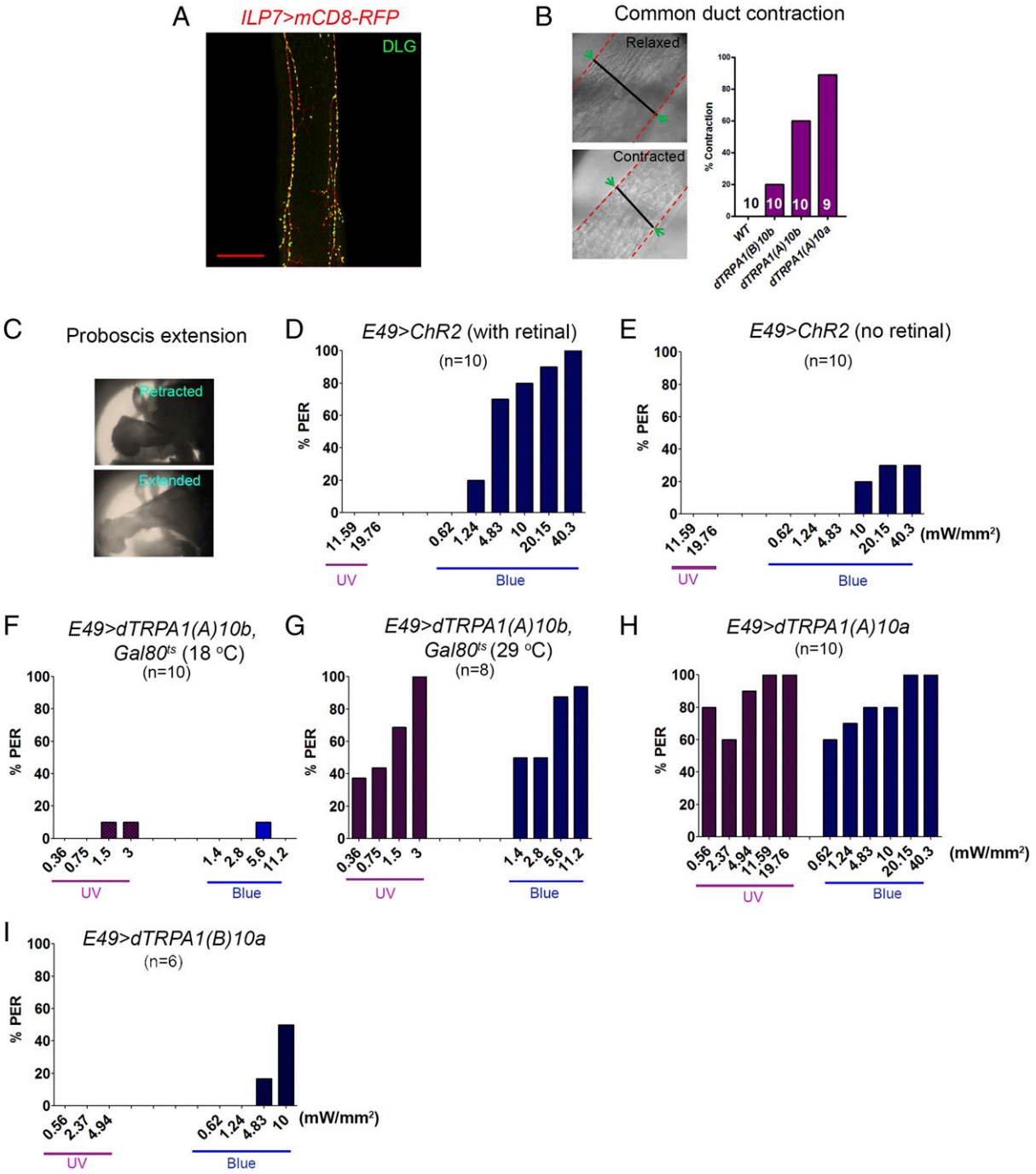

Fig. 4. Ectopic expression of H_2O_2-sensitive isoforms of dTRPA1 in specific motor neurons in adults allowed UV to activate motor responses controlled by these neurons. (*A*) Axons of adult ILP7 neurons (labeled by *ILP7-GAL4*) innervate the common duct of the female internal reproductive tract. Green: DLG, synapse marker. Red: mCD8-RFP, axons. (*B*) (*Top Left*) Common duct in a relaxed state. (*Lower Left*) Common duct in a contracted state. (*Right*) Quantification of the UV-induced common duct contraction from animals that expressed different *dTRPA1* isoforms in their ILP7 neurons. *y* axis, percentage of the animals that showed common duct contraction in response to UV. See also Movies S1 and S2. The UV intensity used in this experiment was 6 mW/mm^2. (*C*) (*Top*) Proboscis in a retracted state. (*Lower*) Proboscis in an extended state. See also Movies S3 and S4. (*D* and *E*) Expressing ChR2 in the proboscis-innervating motor neurons (labeled by *E49-GAL4*) allowed proboscis extension to become blue light inducible. When the animals were not fed with retinal, a critical chromophore for ChR2 activation, they showed little light-induced proboscis extension. Note that ChR2 expression in these neurons did not allow proboscis extension to become UV inducible at the intensities we tested. *y* axis, percentage of the animals that showed proboscis extension in response to light. (*F* and *G*) Expressing the H_2O_2-sensitive isoform of *dTRPA1(A)10b* in E49 neurons allowed proboscis extension to become inducible by both blue and UV light. Because *dTRPA1(A)10b* appeared to cause cell toxicity when chronically overexpressed, we limited its expression duration by introducing the temperature-sensitive *tub-GAL80^{ts}* into the background. When raised and kept at 18 °C (when *dTRPA1* expression was suppressed *GAL80^{ts}*), these animals showed only weak proboscis extension in response to light (*F*). However, once shifted to the restrictive temperature for *GAL80^{ts}* for 3 days, these animals showed strong responses to both blue and UV light (*G*). Note that the behavioral experiments for both sets of animals were conducted at room temperature. (*H*) *dTRPA1(A)10a* did not cause cell toxicity, and its chronic overexpression in *E49* neurons allowed proboscis extension to become inducible by blue and UV light. (*I*) *dTRPA1(B)10a* conferred much reduced blue and UV sensitivities to *E49* neurons.

We found that expressing either of the two H_2O_2-sensitive dTRPA1 isoforms in these motor neurons caused flies to extend their proboscis in response to UV in a dose-dependent manner, whereas expressing the H_2O_2-insensitive one did not (Fig. 4 *F–I* and Movie S3). In contrast, animals bearing ChR2 in these neurons failed to respond at similar UV intensities, probably because ChR2 is tuned specifically to blue light (Fig. 4 *D* and *E*) (33). Next we switched to illuminating the animals with blue light. We found both the ChR2-bearing and the dTRPA1-bearing animals showed blue light-triggered proboscis extension (Fig. 4 *D–H*). However, the timing of the behavior onset in response to light differed between the two groups of animals: ChR2-bearing animals typically extended proboscis with a shorter delay than the dTRPA1-bearing ones upon illumination (Movies S3 and S4), perhaps because ChR2 is directly gated by light whereas dTRPA1 relies on light-induced H_2O_2 production. Supporting this idea, we made a side-by-side comparison of whole-cell recordings of light responses mediated by dTRPA1(A)10a vs. ChR2 in HEK cells and found that dTRPA1 conferred blue light sensitivity also, but its kinetics is slower than that of ChR2 (Fig. S2). Surprisingly, dTRPA1-bearing animals appeared to be more sensitive to blue light than the ChR2-bearing ones. For example, at intensities at or below 1.24 mW/mm^2, few ChR2-expressing animals showed blue light-induced proboscis extension responses but a significant fraction of the dTRPA1-expressing animals did (Fig. 4*D* vs. Fig. 4*H*).

Taken together, our results so far showed that ectopic expression of the H_2O_2-sensitive isoforms of dTRPA1 was sufficient to confer light sensitivity to both cultured mammalian cells and three types of *Drosophila* neurons. In particular, these dTRPA1 isoforms appeared to endow higher UV and blue light sensitivity than the bona fide light-gated ChR2 did in the proboscis extension behavioral assay, further supporting the hypothesis that the H_2O_2-dTRPA1 signaling pathway is capable of converting light detection into neuronal activation.

CC Cells Express the H_2O_2-Sensitive Isoforms of dTRPA1 and Are UV Sensitive. We next searched for neurons that endogenously express the H_2O_2-sensitive dTRPA1 isoforms and assessed whether they are UV sensitive. The corpus cardiacum (CC) cells of the larval ring gland are good candidates. First, a previous report has shown that CC cells showed a clear signal when stained with an antibody against dTRPA1 (18). Second, a *GAL4* strain that is expected to report expression of H_2O_2-sensitive dTRPA1 isoforms has been shown to be active in CC cells (21). To determine whether the H_2O_2-sensitive dTRPA1 isoforms are expressed in CC cells, we performed RT-PCR experiments. We first extracted RNAs from dissected whole-ring glands and found that they contained the H_2O_2-sensitive isoforms of dTRPA1 (Fig. 5 *A* and *B*). We then repeated the RT-PCR experiment on RNAs extracted from dissociated and hand-sorted CC cells and confirmed that the specific dTRPA1 transcripts we detected in whole-ring glands extract were derived from CC cells (Fig. 5 *C* and *D*).

We next assessed whether CC cells responded to H_2O_2 and UV. In keeping with the observation that they expressed the H_2O_2-sensitive isoforms of dTRPA1, CC cells showed strong calcium responses upon H_2O_2 and AITC stimulation, and both responses are significantly reduced in *dTRPA1* mutant (*dTRPA1^{ko}*) background, indicating that their H_2O_2 as well as AITC responses require dTRPA1 (Fig. 5*E* and Fig. S3). Moreover, CC cells showed clear UV sensitivity and that such sensitivity also critically depended on dTRPA1: *dTRPA1* mutant CC cells showed no UV responses, but the responses were rescued when we restored *dTRPA1(A)10a* expression in them (Fig. 5*F* and Fig. S3). Impressively, CC cells were capable of responding to UV at intensities as low as 80 μW/mm^2, albeit at a reduced level compared with their responses to UV of higher intensities (Fig. S3). Further characterization showed that

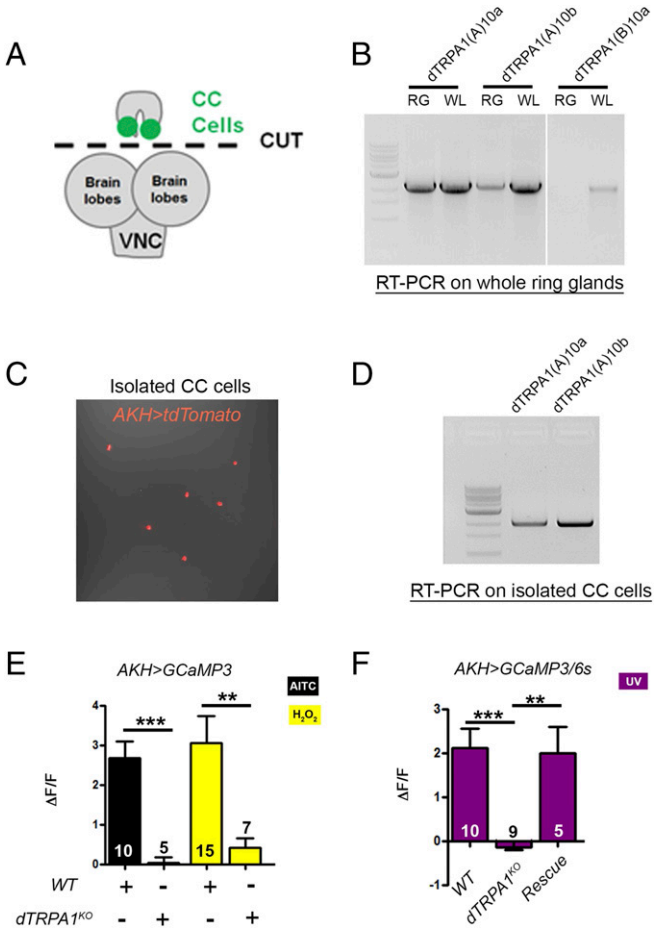

Fig. 5. *Drosophila* larval CC cells (labeled by the *AKH-GAL4*) expressed the H_2O_2-sensitive dTRPA1 isoforms and were sensitive to both H_2O_2 and UV. (*A*) A schematic diagram showing where we severed the ring gland from the larval CNS. (*B*) RT-PCR detected isoforms *dTRPA1(A)10a* and *dTRPA1(A)10b* in RNA extracts prepared from whole ring glands. Isoform *dTRPA1(B)10a* was below detection threshold. RG, ring gland. For positive controls, we performed RT-PCR on RNA extracts prepared from whole larvae (WL). (*C*) A picture showing the dissociated and hand-sorted CC cells. (*D*) RT-PCR on RNA from sorted CC cells detected H_2O_2-sensitivie isoforms *dTRPA1(A)10a* and *dTRPA1(A)10b*. (*E*) Quantification of responses of WT and *dTRPA1* mutant CC cells to AITC (100 μM) and H_2O_2 (200 μM). (*F*) Quantification of UV responses of CC cells from WT, *dTRPA1* mutant (*dTRPA1^{KO}*), and *dTRPA1* mutant with *dTRPA1(A)10a* selectively restored in CC cells (recue). The UV intensities used for WT and rescue experiments were 15 and 6 mW/mm^2, respectively. For *dTRPA1* mutant, we increased the UV intensity to 15 mW/mm^2. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$.

CC cells also exhibited dTRPA1-dependent sensitivity to blue light but were insensitive to strong green light (Fig. S3). Lastly, to confirm that CC cells' UV response can be detected by other means, we also conducted extracellular recording of CC cells and found they significantly increased action potential firing upon UV stimulation (Fig. S3). Taken together, these results showed that CC cells, a group of cells that endogenously expressed the H_2O_2-sensitive dTRPA1, are UV sensitive.

CC Cells' UV Sensitivity Is Sensitive to Manipulations That Affect H_2O_2 Action/Production. We next assessed whether H_2O_2 production mediates UV sensitivity of CC cells. Whereas dTRPA1-conferred H_2O_2 and UV sensitivity seemed to "go hand in hand," it remains possible that dTRPA1 does not detect UV via sensing H_2O_2. To test whether H_2O_2 production plays a critical role in light detection, we conducted three experiments. First, we incubated the

CC cells with the reducing agent DTT and found that their UV light sensitivity reduced significantly (Fig. 6*A*), supporting the idea that an oxidizing agent such as H_2O_2 is responsible for producing the UV responses. Second, we overexpressed in CC cells *catalase (cat)*, an enzyme that degrades H_2O_2 (34), and found that their UV sensitivity also reduced significantly (Fig. 6*B*). Third, we reduced the capacity of CC cells to produce H_2O_2 by reducing their expression of *dual oxidase* (*DUOX*), a flavin-containing oxidase that been shown to produce superoxide—a precursor of H_2O_2—in response to UV light (12), and found that CC cells that overexpressed an RNAi against *DUOX* showed significantly reduced responses to UV (Fig. 6*C*). Taken together, these results support the idea that the ability to sense H_2O_2 (by the H_2O_2-sensitive dTRPA1 isoforms) plays a critical role in CC cells' ability to sense UV.

CC Cells' UV Sensitivity Does Not Require the Traditional Rhodopsin-PLC Pathway or the Gustatory Receptor Gr28b. Does the H_2O_2-dTRPA1 signaling pathway require contribution from conventional phototransduction molecules to convert UV detection to neuronal activation? Although ectopic expression of H_2O_2-sensitive dTRPA1 isoforms was sufficient to confer UV sensitivity to several cells types, it is conceivable that these cells express some phototransduction molecules endogenously that act together with dTRPA1. To investigate this possibility, we first assessed whether CC cells' UV response required retinal, the chromophore critical for the Rhodopsin-PLC signaling pathway (35). Because flies are unable to synthesize retinal without a dietary supplement of vitamin A (35), flies that were raised on vitamin A-deficient food or that lacked the class B scavenger receptor *santa-maria*, a molecule critical for vitamin A uptake (35), should be unable to sense light via the rhodopsin-PLC pathway. However, CC cells from vitamin A-deprived animals showed UV responses comparably to wild-type

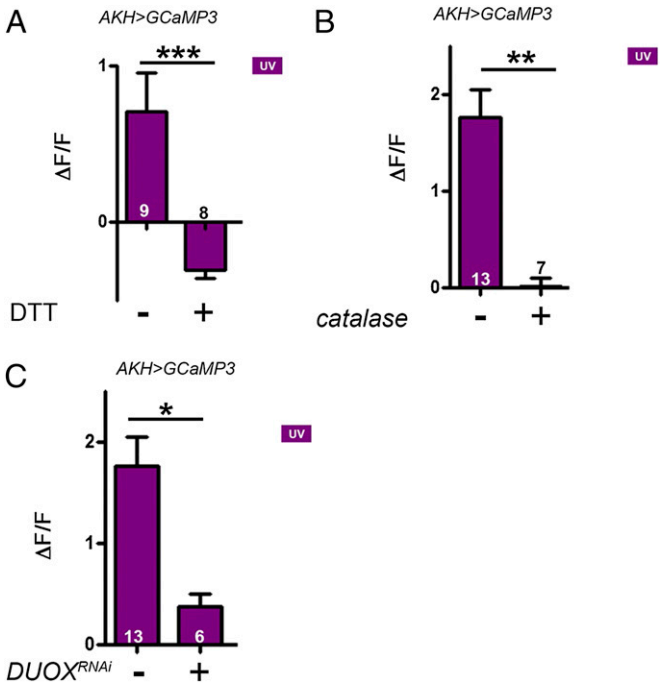

Fig. 6. Manipulating H_2O_2 production and action affected UV responses of CC cells. (*A*) Quantification of UV responses of CC cells when they were incubated with DTT (15 mM). (*B*) Quantification of UV responses of CC cells that overexpressed *catalase*. (*C*) Quantification of UV responses of CC cells that overexpressed RNAi against *DUOX*. The UV intensity used in these experiment was 6 mW/mm^2. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$.

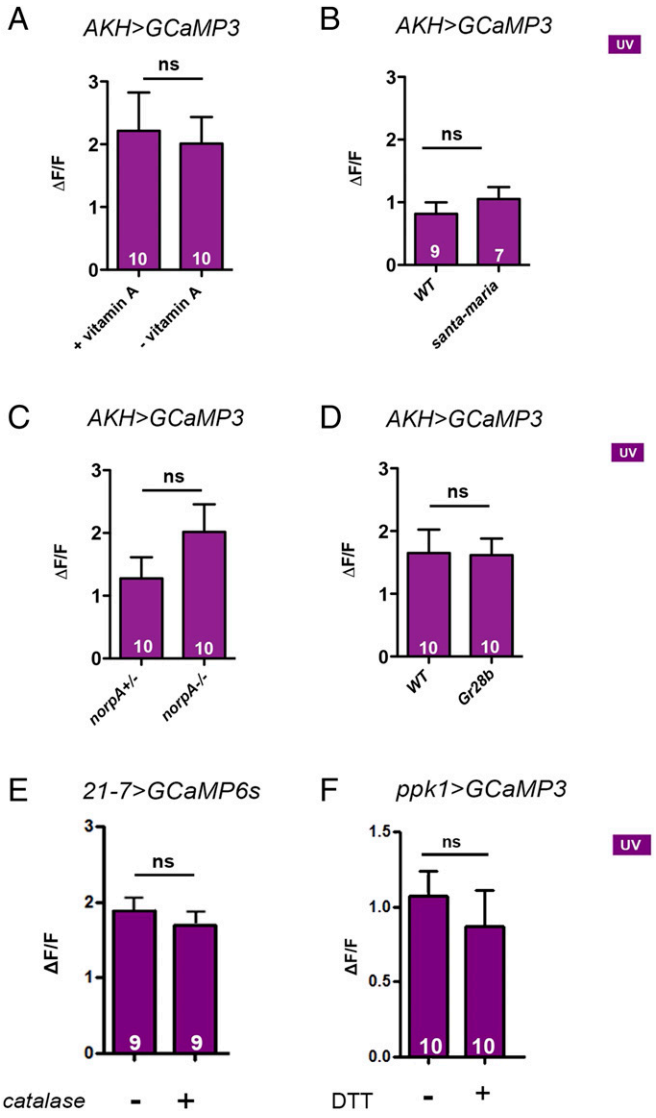

Fig. 7. UV response of CC cells did not require the Rhodopsin-PLC pathway or Gr28b. (*A*) Quantification of UV responses of CC cells from animals raised with or without vitamin A supplement. (*B*) Quantification of UV response of CC cells from control and *santa-maria* mutants. (*C*) Quantification of UV responses of CC cells from control and *norpA* mutants. (*D*) Quantification of UV responses of CC cells from control and *Gr28b* mutants. (*E*) Quantification of UV responses of control and C4da neurons that overexpressed *catalase*. The UV intensity used in these experiment was 15 mW/mm^2. (*F*) Quantification of UV responses of C4da neurons in the presence and absence of DTT (20 mM). ns, no significance.

cells (Fig. 7 *A* and *B*). Consistently, CC cells from *norpA* flies that lacked phospholipase C (PLC) also showed UV response comparably to wild-type cells (Fig. 7*C*). These results suggest that conventional rhodopsin-PLC phototransduction machinery is not required for CC cells to sense UV.

We next tested whether the gustatory receptor Gr28b might act together with H_2O_2-sensitive dTRPA1 in conferring UV sensitivity to CC cells. Although not a component of the conventional phototransduction pathway, Gr28b and its *C. elegans* homolog LITE-1 have been shown to play an important role in sensing blue and UV light (11, 26). In particular, a recent report has provided genetic evidence that LITE-1 is required for H_2O_2 sensing (16), whereas both Gr28b and dTRPA1 have been shown to be required for light sensitivity of *Drosophila* C4da neurons

(26). These results raise the possibility that despite being able to directly sense H_2O_2, dTRPA1s in CC cells may nevertheless receive inputs from an additional H_2O_2 sensor. CC cells from *Gr28b* mutant showed normal UV response (Fig. 7*D*), however. Surprisingly, we found that UV responses of C4da neurons were much less sensitive to *catalase* overexpression or DTT treatment (Fig. 7 *E* and *F*), suggesting that despite the presence of two potential H_2O_2 sensors (i.e., dTRPA1 and Gr28b), C4da neurons do not rely critically on H_2O_2 production for their light sensitivity. Taken together, our results suggest the H_2O_2-dTRPA1 pathway is capable of producing light responses in CC cells without relying on known phototransduction molecules.

Discussion

In this report, we describe our finding that UV can activate cells via signaling an H_2O_2-dTRPA1 photochemical pathway. We found that two specific isoforms of dTRPA1 in *Drosophila* are H_2O_2 sensitive and that ectopically expressing them was sufficient to confer both H_2O_2 and UV light sensitivity to several types of light-insensitive cells. In particular, expressing the H_2O_2-sensitive TRPA1 isoform in a group of proboscis extension-controlling motor neurons was as potent as ChR2 in permitting light to activate the proboscis extension response. We further confirmed the efficacy of the H_2O_2-dTRPA1 pathway in conferring UV sensitivity by demonstrating that CC cells, a group of cells that express these isoforms endogenously, were sensitive to UV. We showed that CC cells' UV response critically depended on dTRPA1 and H_2O_2 but did not require the conventional phototransduction molecules or Gr28b. To our knowledge, our finding is the first to show that specific isoforms of TRPA1 channels can sense UV (and also blue light) via using their H_2O_2 sensitivity, and it raises the intriguing possibility of using these dTRPA1 isoforms as new optogenetics tool.

What is the mechanism that allows specific dTRPA1 isoforms to sense H_2O_2 and consequently UV? A comparison of the protein sequences of different dTRPA1 isoforms showed that the two H_2O_2-sensitive isoforms contain a stretch of 97 aa at the N terminus that is absent in the insensitive one, whereas at least one of the H_2O_2-sensitive isoforms shares the same C terminus as the insensitive one. Thus, it seems the critical residue(s) that confers H_2O_2/UV sensitivity might reside at the N terminus of the H_2O_2-sensitive isoforms. The cysteine residue at the N terminus is of particular interest because H_2O_2 is known to oxidize cysteine (22), and covalent modification of cysteine residues has been shown to be able to activate TRPA1 (36). Interestingly, although structure-function analysis of mammalian TRPA1 has implicated that H_2O_2 and AITC may modify the same cysteine residues (23, 24), our results suggest that this rule may not apply to *Drosophila* TRPA1, because at least one of the dTRPA1 isoforms, *dTRPA1(B)10a*, showed robust AITC sensitivity but little, if any, H_2O_2 sensitivity.

One natural question raised by our findings is that why the C4da neurons require Gr28b to sense UV despite the fact that they might also express the H_2O_2-sensitive isoforms—the "A" promoter for dTRPA1 has been shown to be active in these neurons (21). One possibility is that the level of dTRPA1 expression in C4da neurons is too low, because the same dTRPA1 antibody detected a clear signal in CC cells but none in C4da neurons (18, 26). Another possibility is that C4da neurons may express molecules that inhibit dTRPA1 sensitivity to H_2O_2, or molecules that rapidly degrade H_2O_2. Furthermore, it is also conceivable that C4da neurons may not express the H_2O_2-sensitive isoforms of dTRPA1 despite the fact that the promoter for these isoforms appears active in them (21). Regardless of the exact reasons for C4da neurons' lack of dependency on H_2O_2 for light sensing, we speculated their reduced H_2O_2 sensitivity causes them to critically depend on Gr28b to activate dTRPA1 in response to light. We note that although recent reports have suggested that C4da can sense H_2O_2 (37, 38) at certain developmental stages, we have found that H_2O_2 sensitivity of C4da was significantly lower than that of CC cells (e.g., CC cells responded well to 5 μM H_2O_2, whereas C4da neurons showed no significant responses to 50 μM H_2O_2) (Figs. S3 and S4). Identifying the exact isoforms of dTRPA1 expressed in C4da neurons and determining how they interact with Gr28b are important next steps to address the question of why light-induced H_2O_2 production is not sufficient to confer light responses in C4da neurons.

What is the physiological relevance of CC cells' UV response? Because CC cells are known to express and release the adipokenetic hormone (AKH) that can accelerate heart rate and mobilize sugar into the hemolymph (39–41), and that UV is a known aversive stimulus for young *Drosophila* larvae (26, 42), we proposed that CC cells' UV sensitivity may act to promote stress response when young larvae encounter strong UV. It is worth noting that although the sensitivity of CC cells for UV is not high—the lowest we have seen with GCaMP6 reporter is ~80 μW/mm^2, it may nonetheless be sensitive enough to detect sunlight on earth because one report has suggested that UV of sunlight may reach ~75 μW/mm^2 in some regions on earth (10). In addition, although this work primarily focused on CC cells' UV sensitivity, CC cells responded to strong blue light also (Fig. S3). Thus, CC cells likely respond to multiple spectrums of sunlight (blue, violet, UV), and their sensitivity to UV reflects only a fraction of their true light sensitivity. Indeed, our electrophysiological recording showed that CC cells were capable of responding to 1 mW/mm^2 white light emitted from a xenon lamp (Fig. S3), a light source whose spectrum resembles that of sunlight received on the earth surface. While CC cells are residing in the ring gland as opposed to the body surface, given the transparency of the larval cuticle and the anatomical location of the ring gland (they are located directly above the brain lobes), it is conceivable that light can readily reach and activate these cells. We also note that CC cells from at least one other insect species have also been implicated to sense light as EM analysis revealed that they contained rhabdomeres, the bona fide light-sensing organelles (43). Thus, light sensitivity may be a common feature of these cells, especially among insects that have transparent cuticles.

Experimental Procedures

Fly Stocks. Animals were raised in standard molasses/cornmeal/yeast/agar food and maintained in a Darwin Chamber with temperature set at 25 °C and humidity level at 60–65%. The stocks used were as follows: *w^{1118}*, *UAS-GCaMP3 (BL-32236)*, *UAS-GCaMP6* (BL-42746), *UAS-ChR2-YFP*, *E49-GAL4* (31), *AKH-GAL4* (BL-25684), *dTRPA1^{KO}* (27), *Gr28b^{PBac01884}* (BL- 10743), *Gr28b$^{Df(2L)7031}$/CyO* (BL-7804), *norpA^{36}* (BL-9048), *santa-maria* (BL-24520), *UAS-dTRPA1-RNAi* (27), *UAS-DUOX-RNAi^{1}* (BL-32903), *UAS-cat* (BL-24621), *ILP7-GAL4* (44), *UAS-dTRPA1(A)10b* (21), *UAS-dTRPA1(B)10a* (20), *ppk1-GAL4* (45), *UAS-dTRPA1(A)10a* (this work), *UAS-mTRPA1* (a gift from J. Grandl, Department of Neurobiology, Duke University, Durham, NC).

Light and Chemical Responses of dTRPA1 Isoforms in HEK Cells. For the cloning of dTRPA1 isoforms, RT-PCRs were performed on total RNA extracted from third instar larvae followed by ligating the amplified region into pcDNA3.1 vector. HEK cells were grown on cover glasses in 3.5-cm culture dishes with DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin and transfected by 0.5 μg of dTRPA1 plasmids, 0.5 μg of GCaMP6s plasmids and 5 μg of polyethylenimine. The imaging solution (pH 7.2) contained the following ingredients: 130 mM NaCl, 3 mM KCl, 0.6 mM $MgCl_2$, 1.2 mM $NaHCO_3$, 10 mM glucose, 10 mM Hepes, and 1 mM $CaCl_2$. Data were collected by Zeiss LSM700 confocal system with a 20× Plan-Apochromat objective lens under room temperature. UV light (50 mW/mm^2, 5 seconds), 100 μM H_2O_2, or 100 μM AITC were applied on HEK cells. The average peak response was calculated with Zeiss Zen software.

Staging Larvae. We controlled for the age of our larvae when imaging their cells for light response. To do so, eggs were collected for 2–4 h by using standard grape agar plate seeded with yeast paste. The next day, at ~21 h after egg-laying, we cleared the grape plates of any larvae that have already

hatched. We then waited for 1 h and collected the larvae that have just hatched within the last hour. When collected in this way, larvae are more synchronized with respect to their developmental stages. We typically collected and transferred approximately 50 such larvae onto Petri dishes that contained regular molasses/cornmeal food supplemented with yeast paste. Collected larvae were then aged in incubator with temperature and humidity set at 25 °C and 65%, respectively, until the appropriate stage for experiments.

Calcium Imaging of CC Cells. All samples for calcium experiments were prepared in a custom-built imaging chamber. The imaging buffer contains the following ingredients: NaCl (120 mM), KCl (3 mM), $MgCl_2$ (4 mM), $CaCl_2$ (2 mM), $NaHCO_3$ (10 mM), trehalose (10 mM), glucose (10 mM), 2-[(2-Hydroxy-1,1-bis(hydroxymethyl)ethyl)amino]ethanesulfonic acid (TES, 5 mM), sucrose (10 mM), Hepes (10 mM), and we adjust the final pH to 7.25. A Zeiss LSM700 confocal microscope equipped with a 40×/0.8 N.A. water immersion objective and the live-series ZEN image acquisition software were used for acquiring GCaMP3 response. Briefly, baseline GCaMP fluorescence was collected by scanning the cells with a 488-nm laser and acquiring at 128 × 128 pixels at 8-bit dynamic range. We used X-cite LED as the light source for stimulation. The following filter sets were used for obtaining the blue, UV, and green lights used for stimulation. Blue light: filter set 38 from Zeiss, peak 470 nm; UV light: filter set 49 from Zeiss (300-400 nm), peak 365 nm; green light: filter set 43 from Zeiss, peak 545 nm. For stimulation of white light, we used a 300-W xenon lamp from Sutter LB-LS/30. The duration of these lights were controlled by switching the filter cube from confocal mode to the epi-fluorescence mode. Light intensity was measured with a Thorlabs PM100USB optical power meter. Increasing light intensity increased CC cells responses to light (Fig. S1*C*). The GCaMP3/6 signal acquired before light stimulation (baseline) was used as F^0 and the signal after 5 s of light stimulation was used as F'. $\Delta F/F$ was then calculated as $(F' - F^0)/F^0$ to reflect the change in GCaMP signals before and after stimulation. KCl (100 mM) was applied routinely to CC cells that did not show any light response to ensure that they were still capable of producing calcium response.

Electrophysiological Recording of CC Cells. CC cell electrical activity was recorded as described for C4da neurons with several modifications (26). Age-synchronized third instar larvae carrying both *akh*-GAL4 and UAS-GCaMP3 transgenes were dissected by making a dorsal cut along the anterior cuticle and pinning the fillet to expose the ring gland. Fillets were prepared in external saline solution composed of the following ingredients (in micromolars): NaCl 120, KCl 3, $MgCl_2$ 4, $CaCl_2$ 1.5, $NaHCO_3$ 10, trehalose 10, glucose 10, N-tris(hydroxymethyl)methyl-2 aminoethanesulfonic acid (TES) 5, sucrose 10, Hepes 10. The osmolality was 305 $mOsm{\cdot}kg^{-1}$ and the pH was 7.25. GFP-positive (i.e., CC cell) neurons were located under a Zeiss D1 microscope with a 40×/1.0 N.A. water immersion objective lens. Gentle negative pressure to the CC cell trapped the soma in a recording pipette (5 μm tip opening) filled with external saline solution. Recordings were performed with a 700A amplifier (Molecular Devices), and the data were acquired with Digidata 1322A (Molecular Devices) and Clampex 10.4 software (Molecular Devices). Extracellular recordings of action potentials were obtained in voltage clamp mode with a holding potential of 0 mV, a 2 kHz low-pass filter and a sampling frequency of 20 kHz. For light stimulation, a 300-W xenon light source was connected to the microscope with a liquid light guide to allow light stimulation through the lens. Light intensity was measured by a radiometric sensor head (Newport 818P-001-12) coupled with a power meter (Newport 1918-C). The duration (5 s) of light stimulation was controlled by a TTL (transistor-transistor logic)-triggered shutter (Sutter Instruments) in the xenon lamp house triggered by Digidata 1322A (Molecular Devices). Band-pass excitation filters (Semrock) were used to select light wavelength. For each recording trace, average frequency during the 5 s immediately before light exposure was used as control.

Detection of dTRPA1 Isoforms by RT-PCR. RNA was extracted from whole larvae, dissected ring glands, or purified CC cells with TRIzol and RNeasy plus micro kit (Qiagen). SuperScript III RT kit (Invitrogen) with *dTRPA1*-specific primer (5′- CAATCGGAACCACGACAGA-3′, 2 pmol/μL) were used to synthesize the first-strand cDNA. EmeraldAmp Max HS PCR Master Mix (TaKaRa) or GoTaq Green Master Mix (Promega) was used for RT. PCR Program was set as below: 94 °C for 5 min, 94 °C for 20 s, 55 °C for 20 s, 72 °C for 2 min, repeat steps 2–4 for 39 times (40 cycles in total), followed by 72 °C, 7 min. For discriminating different *dTRPA1* isoforms, the primer pairs used were as followed: (*i*) *dTRA1(A)10a*: A-F/10a-R for detection in ring gland. A-F2 /10a-R2 for detection in purified CC cells; (*ii*) *dTRPA1(A)10b*: A-F/10b-R for detection in ring gland. A-F2/10b-R2 for detection in purified CC cells; and (*iii*) *dTrpA1(B)10a*: B-F/10a-R for detection in both ring gland and purified CC cells.

Sequence of the primers described are as follows:

A-F: 5′-GCCGGAACAGCAAGTATT-3′

B-F: 5′-GTGGACTATCTGGAGGCG-3′

10a-R: 5′-CCATGTGTTACCATGGTATTCAAAG-3′

10b-R: 5′-TGTTACCATGGTGTTCACCA-3′

A-F2: 5′-CAATGATAATCGAGCGCAGT-3′

10a-R2: 5′-AATGGGACTGGCGGTCGT-3′

10b-R2: 5′-GCCTCAATCTGTTCGGGTGT-3′

Immunohistochemistry. Tissues were dissected, fixed, and stained according to common protocol (e.g., 4% formaldehyde for 40 min and washed with PBS with 0.3% Triton X-100). For staining for DLG of female reproductive tract, a mouse antibody against Discs-large (DLG, DSHB) was used at dilution 1:100, and secondary donkey anti-mouse antibody (Jackson Laboratory) was used at 1:200. Fixed and immunostained tissue was mounted, and images were then scanned and acquired by using LSM700 confocal microscope and Zen software.

Assay for Contraction of Reproductive Tract. To test whether overexpression of *dTRPA1(A)10a* and *dTRPA1(A)10b* in ILP7 neurons could activate reproductive tract contraction, we dissected the entire reproductive organ from females that are 2–3 d old. The dissected organ were mounted in the same buffer and chamber used for GCaMP3 imaging. A 40× water immersion objective was used to illuminate the tracts with 5–10 inches of UV light (6 mW/mm^2 at 365 nm). Contraction of tract was recorded through the eyepiece. Representative videos (Movies S1 and S2) were taken by using a Canon digital camera mounted on a stand and focused through the eyepiece.

Assay for Proboscis Extension. To test whether overexpression of *dTRPA1(A)10a*, *dTRPA1(A)10b*, and *ChR2* in *E49* neurons would induce proboscis extension, we placed 3- to 5-d-old flies into a 1,000-μL pipette tip and placed the tips on the stage of the Zeiss microscope such that the head of the fly was visible through the eyepiece. We then illuminated lights of different wavelengths and intensities to the flies and assessed whether they would extend proboscis. In the case of *ChR2* flies, we supplemented the food with 1 mM retinal for the experimental group and used regular food for the control group.

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