Current Biology 23, 2215–2223, November 18, 2013 ©2013 Elsevier Ltd All rights reserved http://dx.doi.org/10.1016/j.cub.2013.09.028

Article

An AP2 Transcription Factor Is Required for a Sleep-Active Neuron to Induce Sleep-like Quiescence in *C. elegans*

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Summary

Background: Sleep is an essential behavior that is found in all animals that have a nervous system. Neural activity is thought to control sleep, but little is known about the identity and the function of neural circuits underlying sleep. Lethargus is a developmentally regulated period of behavioral quiescence in *C. elegans* larvae that has sleep-like properties.
Results: We studied sleep-like behavior in *C. elegans* larvae and found that it requires a highly conserved AP2 transcription factor, *aptf-1*, which was expressed strongly in only five interneurons in the head. Expression of *aptf-1* in one of these neurons, the GABAergic neuron RIS, was required for quiescence. RIS was strongly and acutely activated at the transition from wake-like to sleep-like behavior. Optogenetic activation of *aptf-1*-expressing neurons ectopically induced acute behavioral quiescence in an *aptf-1*-dependent manner. RIS ablation caused a dramatic reduction of quiescence. RIS-dependent quiescence, however, does not require GABA but requires neuropeptide signaling.
Conclusions: We conclude that RIS acts as a sleep-active, sleep-promoting neuron that requires *aptf-1* to induce sleep-like behavior through neuropeptide signaling. Sleep-promoting GABAergic-peptidergic neurons have also been identified in vertebrate brains, suggesting that common circuit principles exist between sleep in vertebrates and sleep-like behavior in invertebrates.

Introduction

Sleep or sleep-like behavior is found in all animals that have a nervous system and have been studied carefully [1]. It is characterized by periods of quiescence. Sleep thus appears essential for animals that have a nervous system, and it may be evolutionarily conserved [2, 3]. Sleep and wake are controlled by the activity of neural circuits, but little is known about how neuronal activity controls sleep and wake, and it is unclear whether neural circuitry principles underlying sleep and wake are conserved. It has been proposed that sleep and wake are controlled by wake-active, wake-promoting neurons and sleep-active, sleep-promoting neurons, both of which are found in vertebrate brains [4, 5].

Sleep- and wake-controlling neurons have also been found in *Drosophila*, indicating some similarity between circuitry principles in vertebrates and invertebrates, but sleep-active neurons have not yet been described in invertebrates [6–9]. Thus, it is unclear whether, at least in some systems, sleep is induced by the absence of wake-promoting neural activity or whether sleep generally is actively induced by the neural activity of sleep-active neurons.

Quiescence behavior is also found in *C. elegans* larvae, where it is coupled to development: prior to each of the four molts, larvae go through a phase of behavioral quiescence called lethargus that has long been known but has recently been shown to have properties that define sleep in higher organisms, such as an absence of voluntary movement, reversibility, reduced responsiveness to stimulation, homeostatic regulation, a relaxed body posture, and reduced neuronal activity. Because quiescence in *C. elegans* larvae fulfills all behavioral criteria that define sleep in higher organisms, we call it sleep-like behavior [10–15]. In other systems, sleep is typically controlled by a circadian rhythm. Sleep-like behavior in *C. elegans* is controlled by a molting rhythm that is faster than the circadian rhythm. Interestingly, genes that control the circadian rhythm in other animals have homologs in *C. elegans* that control the molting rhythm [16, 17]. Thus, the biological context in which sleep-like behavior occurs differs in *C. elegans*, and it is unclear whether sleep-like behavior in *C. elegans* and sleep in higher organisms have a monophyletic origin and share common molecular mechanisms and neural circuit principles.

Studying sleep-like behavior in *C. elegans* is attractive because it allows the combination of behavioral analysis with genetics and functional neural imaging in a small nervous system containing only about 300 neurons. Using a genetic screen in *C. elegans* larvae, we have found that the AP2 transcription factor *aptf-1* is required for sleep-like behavioral quiescence. *aptf-1* is expressed in only a few interneurons, and expression in RIS is required for quiescence. Using calcium imaging, we found that RIS is active specifically at the transition from wake-like to sleep-like behavior. Using optogenetics, we show that *aptf-1*-expressing neurons can induce sleep-like quiescence. The ability to induce quiescence is conferred by *aptf-1*. Ablation of RIS caused a dramatic reduction in quiescence. RIS-dependent quiescence requires not GABA but neuropeptide signaling.

Thus, sleep-promoting neurons are an important circuit principle that governs sleep-like quiescence of *C. elegans*. Sleep-like behavior in *C. elegans* and sleep in higher organisms thus both use common circuit principles to achieve quiescence.

Results

aptf-1 Is Required for Locomotion Quiescence

As a starting point to understanding sleep-like behavior in *C. elegans* larvae, we wanted to identify genes that control this process. We thus performed a genetic screen to identify mutants that lack sleep-like behavior. Publicly available mutants covered about 4,000 genes, which was about 20% of *C. elegans* open reading frames [18]. We obtained these mutant strains, grew populations of 400–600 animals of mixed developmental stages for each mutant, and visually inspected these populations for the absence of immobile larvae. We found one homozygous viable mutant strain, VC1669, which completely lacked immobile larvae. VC1669 contained a

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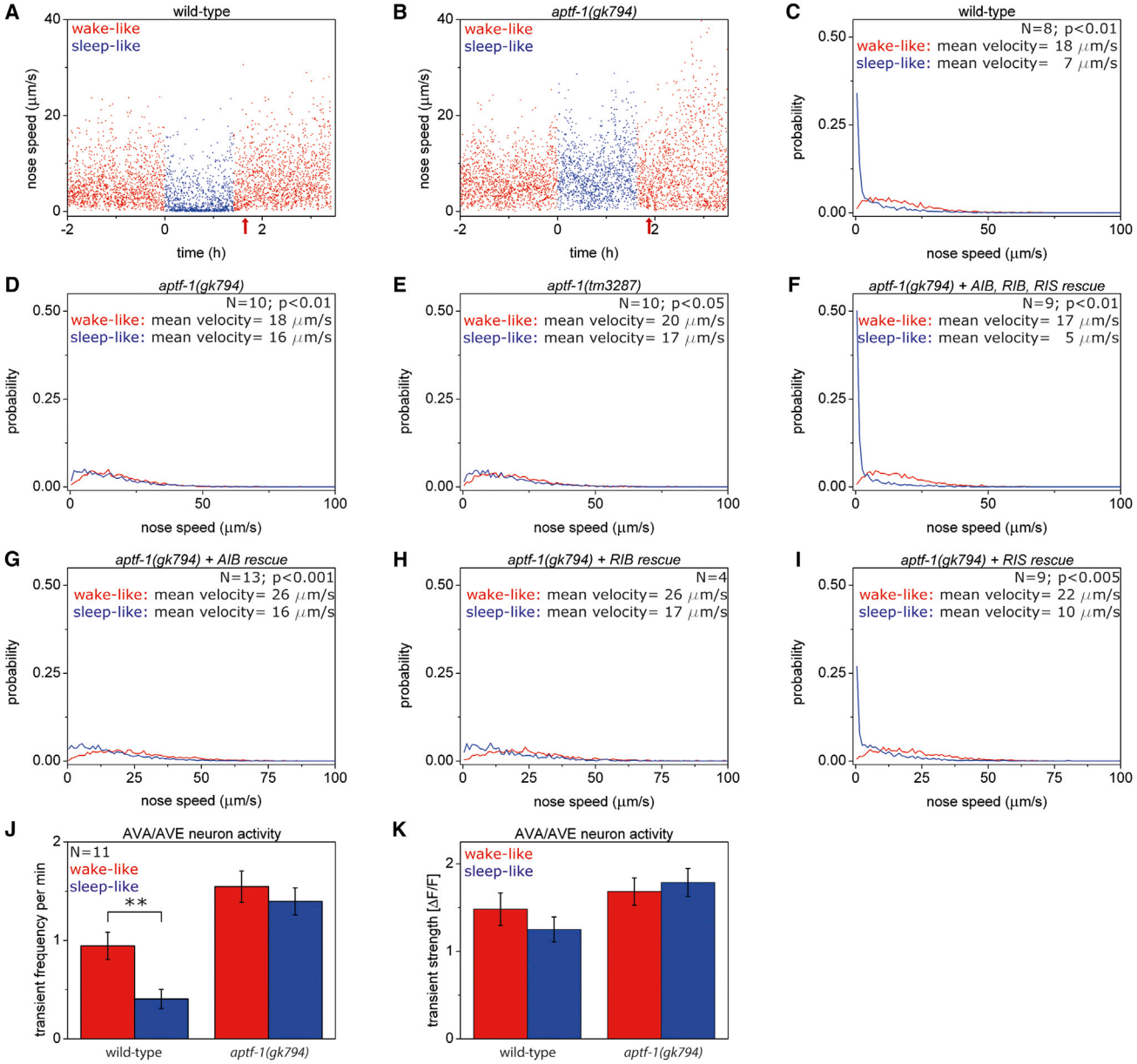

Figure 1. *aptf-1* Is Required for Behavioral Quiescence

Error bars are SEM.

(A and B) Nose speed during sleep-like and wake-like behavior in an individual wild-type (A) and *aptf-1* mutant (B). The red arrow indicates the shedding of the cuticle.

(C–E) Probability distribution of nose speeds during wake-like and sleep-like behavior in wild-type (C), *aptf-1(gk795)* (D), and *aptf-1(tm3287)* (E).

(F–I) *aptf-1* rescue experiments. Probability distribution of nose speeds using endogenous promoter expression (F), AIB expression (G), RIB expression (H), and RIS expression (I) are shown. Nose speed during the wake-like feeding period is displayed in red; nose speed during the nonfeeding sleep-like period is displayed in blue. The wake-like period used was 2 hr before the onset of sleep-like behavior.

(J and K) *aptf-1* mutants lack shutdown of command interneuron activity. Shown are AVA/AVE calcium transient frequency (J) and intensity (K) for wild-type and *aptf-1* mutants. ** denotes statistical significance with $p < 0.01$, Wilcoxon signed-rank test.

deletion in a homolog of AP2 transcription factor genes, *AP2* transcription *f*actor *1*, *aptf-1(gk794)*. We then cultured individual *aptf-1* mutant L1 larvae inside microfluidic microcompartments and observed their behavior across the sleep/wake-like cycle using long-term imaging [19]. *aptf-1(gk794)* mutants developed with normal speed and went normally through the molt. Mutants still stopped feeding for a normal time before each molt, which allowed us to clearly identify the developmental time during which sleep-like behavior should occur. During sleep-like behavior, wild-type larvae showed bouts of complete immobility and an average reduction of nose speed of about 60%. *aptf-1* mutants moved with a normal speed during wake-like behavior. During nonfeeding behavior, however, average nose speed was reduced by only about 10%, and worms were never immobile (Figures 1A–1D; see also Movie S1 available online). We next wanted to confirm that the lack of behavioral quiescence was caused by deletion of *aptf-1*. A second deletion allele, *aptf-1(tm3287)*, showed a

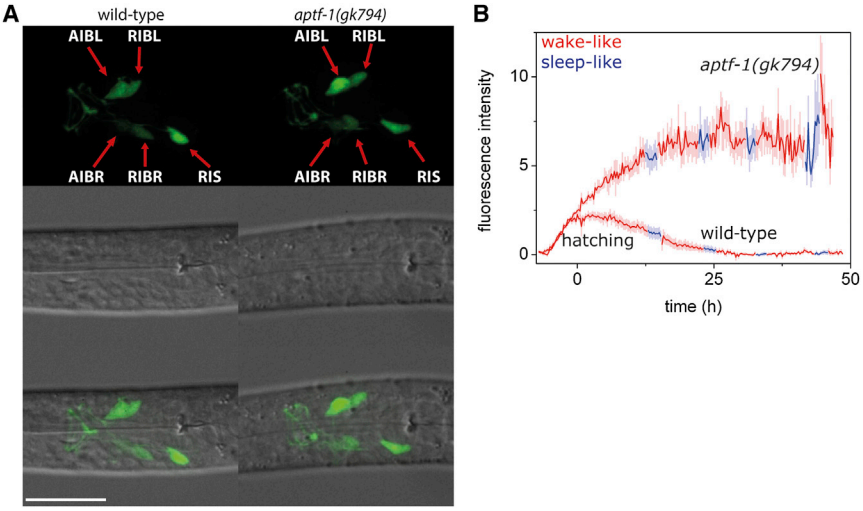

Figure 2. *aptf-1* Expresses Strongly in Only Five Interneurons, AIB, RIB, and RIS

Error bars are SEM.

(A) Spinning-disk and differential interference contrast images of transgenic animals expressing a destabilized version of GFP under the control of the *aptf-1* promoter show that *aptf-1* is expressed in AIB, RIB, and RIS. Scale bar represents 10 μm.

(B) *aptf-1* limits its own expression. Expression quantification from embryo until adult stage showed that *aptf-1* promoter activity was strongest during embryogenesis and early larval development and decreased until the adult stage. In *aptf-1* mutants, promoter activity did not decrease during larval development indicating that *aptf-1* controlled its own expression.

phenotype that was identical to *aptf-1(gk794)* (Figure 1E). Both deletion alleles removed large portions of the *aptf-1* gene and were likely molecular null alleles. A transgene containing wild-type *aptf-1* rescued the *aptf-1(gk794)* phenotype (Figure 1F). Thus, *aptf-1* deletion did not abolish cessation of feeding during sleep-like behavior but severely compromised reduction of locomotion, causing a complete absence of immobility.

The continuous locomotion activity in *aptf-1* mutants suggested that the neural circuits underlying locomotion were not shut down during sleep-like behavior. The command interneurons control coordinated locomotion, and the command interneurons AVA and AVE control backward movement [20]. AVA and AVE activate during reversal from forward to backward locomotion, and their activity is reduced during sleep-like behavior [14, 21]. We measured the activity of AVA and AVE during sleep-like and wake-like behavior with calcium imaging using transgenic animals expressing GCaMP3.35 in this neuron. We cultured individual animals in microcompartments and filmed them every 30 min for 100 s. We then quantified AVA/AVE calcium transients. In wild-type, the frequency of AVA/AVE calcium transients was reduced during sleep-like behavior by about 60%. In *aptf-1* mutants, the frequency of AVA/AVE transients was generally slightly higher than in wild-type and, importantly, was reduced by only 15% during sleep-like behavior (Figures 1J and 1K; Movie S2). Under all conditions, AVA/AVE transients correlated with backward movements in both wild-type and *aptf-1* mutants. Thus, *aptf-1* deletion prevented a normal AVA/AVE shutdown during sleep-like behavior and allowed mutants to move coordinately and continuously during sleep-like behavior.

aptf-1 Is Expressed in AIB, RIB, and RIS

AP2 transcription factors are highly conserved. In other systems, they control developmental processes in neurons and other tissues [22–25]. There are four genes encoding transcription factors that are homologous to vertebrate AP2 in *C. elegans*, *aptf-1* to *aptf-4*, but their functions are unknown. Of those four *aptf* genes, *aptf-1* is most similar to vertebrate AP2 genes (Figure S1A). To find out where and when *aptf-1* acts, we looked at the expression of *aptf-1*. We used an *aptf-1* promoter fusion with a destabilized version of GFP, d1GFP [26, 27], to monitor *aptf-1* promoter activity. *aptf-1* was expressed strongly during late embryogenesis and early larval development in three types of interneurons, the paired neurons AIB and RIB and the unpaired neuron RIS. The expression in RIS was the strongest. Expression after embryonic development decreased until the adult stage (Figure 2). Expression of a GFP-tagged fosmid confirmed the expression in these neurons and showed that APTF-1::GFP was present until the adult stage (Figure S1B). In *aptf-1* mutants, AIB, RIB, and RIS appeared morphologically normal. Expression from the *aptf-1* promoter, however, did not decrease after embryonic development but continued until the adult stage (Figure 2). Thus, *aptf-1* was expressed most highly during early development in only five interneurons where it limited its own expression.

aptf-1 Is Required in RIS for Locomotion Quiescence

aptf-1 expressed in three types of interneurons. In which of these neurons is *aptf-1* required? We used three different promoters that expressed either in AIB, RIB, or RIS, but not in the other *aptf-1*-expressing neurons, to drive expression of an *aptf-1* rescue transgene in each of these types of neurons. *aptf-1* expression in AIB or RIB caused a difference of nose speed during wake- and sleep-like behavior but did not restore quiescence (Figures 1G and 1H). *aptf-1* expression in RIS, however, restored quiescence to almost wild-type levels (Figure 1I). Thus, *aptf-1* appears to play its most important role in quiescence in RIS.

RIS Is a Sleep-Active Neuron

How do *aptf-1*-expressing neurons control sleep-like behavior? We measured the activity of *aptf-1*-expressing cells during wake-like and sleep-like behavior using calcium imaging [12, 28, 29]. We imaged individual larvae for short intervals (2 min) every 30 min across the sleep/wake-like cycle and quantified calcium transients. The frequency of spontaneous calcium transients was reduced during sleep-like behavior in AIB and RIB (Figures 3A and 3B; Figures S2A and S2B). RIS showed activity transients only occasionally during wake-like behavior. Around the onset of sleep-like behavior, however, RIS showed a strong increase in calcium activity. Around the time when larvae stopped feeding, average RIS activity rose sharply within 15 min to a maximum (Figure 3C; Figures S2C–S2E). During this time, short bouts of reduced behavioral activity coincided with RIS activity transients (Movie S3). Average RIS activity then decreased during the course of the sleep-like phase. During the sleep-like phase, RIS showed periods during which activation transients occurred that alternated with periods during which RIS did not show activation

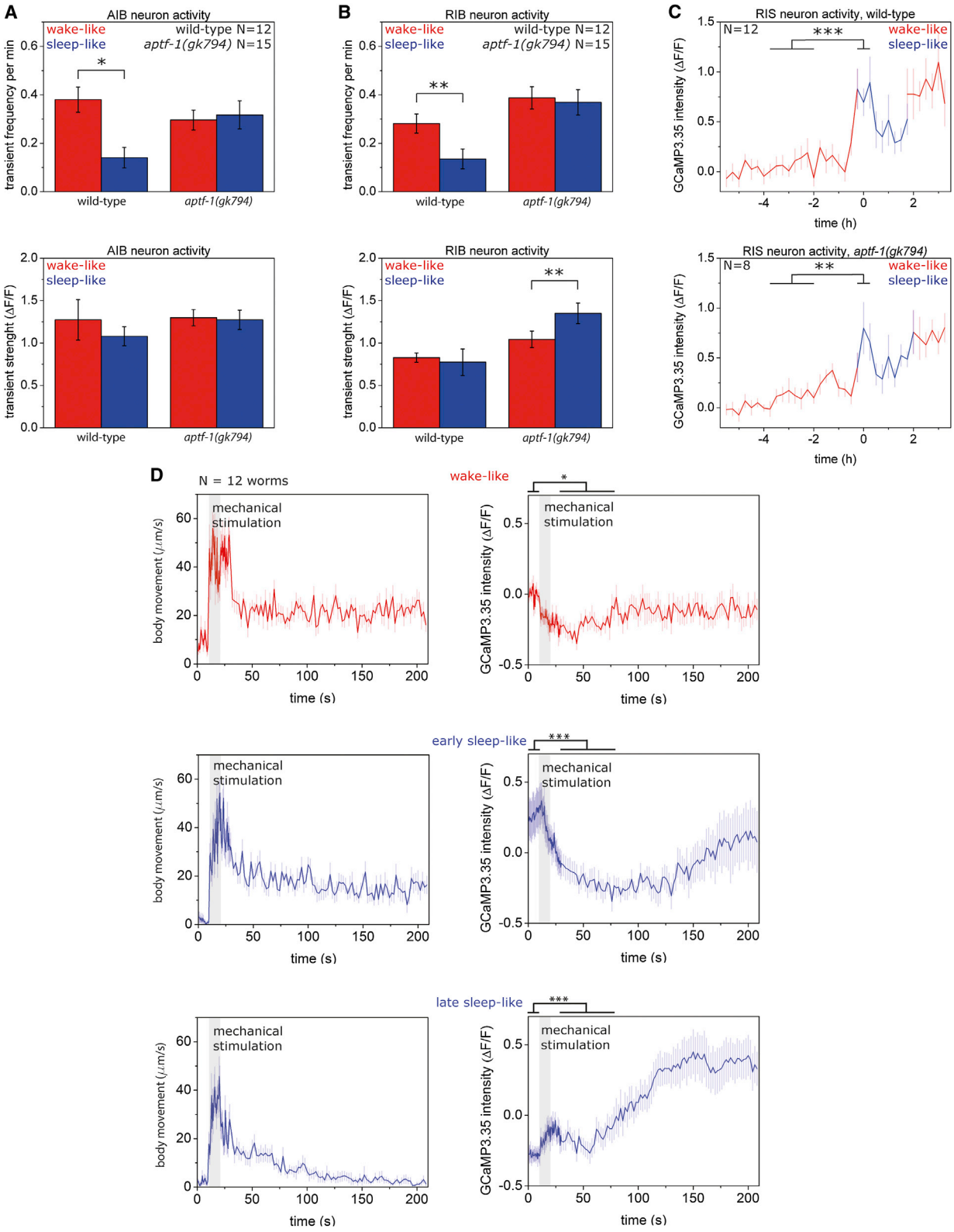

Figure 3. The AIB and RIB Neurons Are Active during Wake-like Behavior, and RIS Is Most Active at the Beginning of Sleep-like Behavior

Error bars are SEM.

(A) Calcium transient frequency and intensity in AIB showed reduced activity in this neuron during sleep-like behavior that depended on *aptf-1*.

(B) Calcium transient frequency and intensity in RIB showed reduced activity during sleep-like behavior that depended on *aptf-1*.

(C) Averaged calcium activity of RIS across time showed that RIS was most active during the transition from wake-like (red) to sleep-like (blue) behavior. RIS still activated at the onset of the sleep-like period in *aptf-1* mutant worms.

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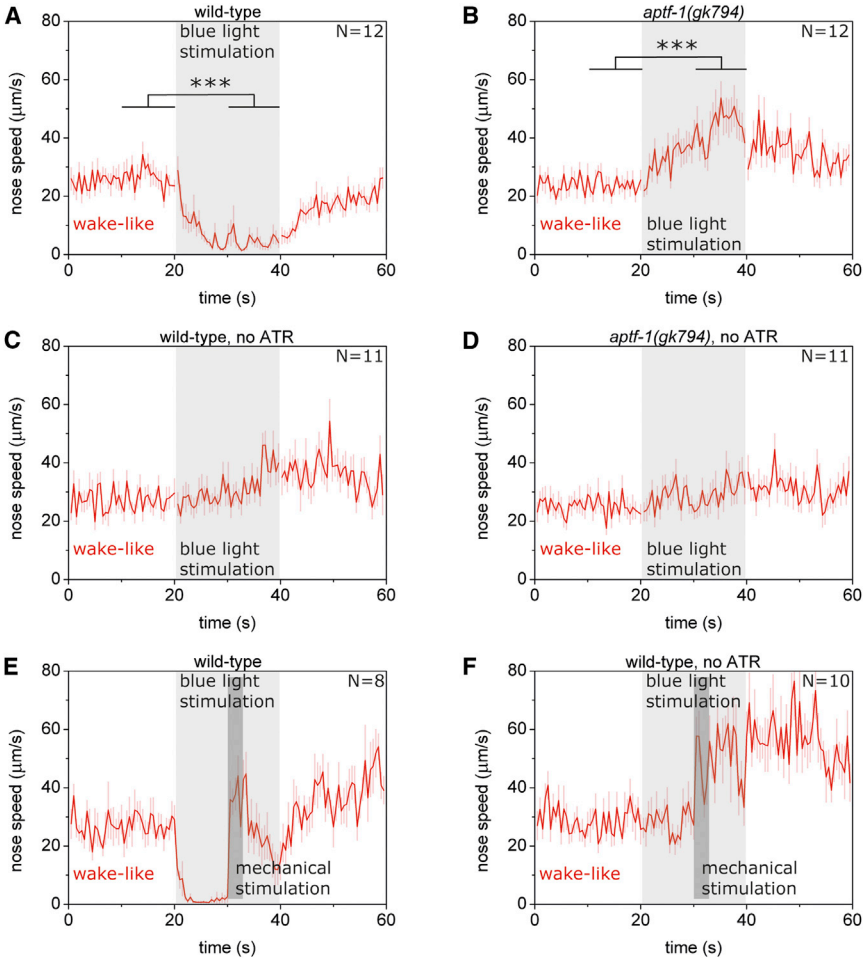

Figure 4. Activating *aptf-1*-Expressing Neurons during Wake-like Behavior Causes Reversible Behavioral Quiescence

Error bars are SEM.

(A) Channelrhodopsin activation of *aptf-1*-expressing neurons during wake-like behavior caused acute immobility.

(B) This immobility depended on *aptf-1*, because blue-light stimulation in *aptf-1* mutants did not cause quiescence but caused an increase in movement instead.

(C and D) Control experiment without all-*trans* retinal (ATR) for wild-type (C) and *aptf-1* mutant worms (D).

(E and F) Quiescence caused by channelrhodopsin-based activation of *aptf-1*-expressing neurons is reversible. A dish-tapping stimulus (dark gray) delivered during ChR2 activation (light gray) causes resumption of movement (E). Control experiment without ATR is shown in (F). *** denotes statistical significance with $p < 0.001$, Wilcoxon signed-rank test.

transients. We then changed the behavioral state of the larvae from sleep-like to wake-like to see whether RIS activity reacted to these changes. Strong mechanical stimulation has been shown to revert sleep-like behavior [11]. Strong mechanical stimulation during wake-like behavior caused an escape response and slightly inhibited RIS activity. Mechanical stimulation during the early phase of sleep-like behavior, when RIS activity was high, reverted the behavioral quiescence, caused locomotion activity, and caused an acute inhibition of RIS activity. Eventually, RIS became active again and worms slowed down again. Mechanical stimulation during the late phase of sleep-like behavior, when RIS activity was low, was less effective in causing a behavioral response compared with the first phase of sleep-like behavior, but it also reverted the behavioral quiescence and caused locomotion activity. After stimulation, RIS was quickly and strongly activated, and worms returned to quiescence (Figure 3D). Thus, the calcium imaging showed that AIB and RIB behaved similarly to AVA and AVE and are wake-active neurons. RIS activity, however, suggested that it might be a quiescence-inducing neuron: RIS activation always correlated with an induction of behavioral quiescence.

aptf-1 deletion caused an absence of locomotion quiescence. Does *aptf-1* act by affecting the calcium activity of *aptf-1*-expressing neurons? To test this hypothesis, we measured calcium activity of *aptf-1*-expressing neurons in *aptf-1* mutants. In *aptf-1* mutants, both AIB and RIB activity was not downregulated during sleep-like behavior (Figures 3A and 3B). AIB and RIB were thus affected by *aptf-1* deletion in a manner similar to AVA and AVE. RIS activity, however, was reduced only by 15% and still strongly increased at the beginning of the non-feeding period in *aptf-1* mutants (Figure 3C; Figure S2C). Thus, *aptf-1* likely does not act by causing RIS calcium transients, and, at least in *aptf-1* mutant worms, RIS activation is not sufficient to trigger quiescence. Thus, *aptf-1* may exert its most prominent effect downstream of RIS calcium transients.

aptf-1-Expressing Neurons Can Induce Quiescence

The activity pattern of *aptf-1*-expressing neurons suggested that RIS actively induces behavioral quiescence. To test this hypothesis, we optogenetically activated *aptf-1*-expressing neurons using channelrhodopsin2 (ChR2) and quantified the behavior of the worms. We generated transgenic worms expressing ChR2 under the control of the *aptf-1* promoter and activated *aptf-1*-expressing neurons using blue light [30–32].

Optogenetic activation of *aptf-1*-expressing neurons in wake-like L1 larvae induced quiescence within seconds of illumination: worms reduced their movement until they were virtually immobile and stopped pumping. After the end of optogenetic activation, worms increased their movement again (Figure 4; Movie S4). Illuminating only RIS also caused quiescence (Figure S3).

RIS is directly and strongly connected via synapses to AVE, which in turn is strongly connected via gap junctions to the command interneuron AVA [33]. RIS may thus directly inhibit command interneurons required for locomotion. To test this

(D) Manipulation of the behavioral state also changed RIS activity. When RIS activity was high and the worms were immobile, strong mechanical stimulation caused a reversal of the sleep-like behavior and an acute inhibition of RIS. When RIS activity was low and the worms were immobile, stimulation caused a reversal of sleep-like behavior and an activation of RIS activity that coincided with a restoration of quiescence. The stimulation period is shown in gray. * denotes statistical significance with $p < 0.05$, *** denotes statistical significance with $p < 0.001$, Wilcoxon signed-rank test.

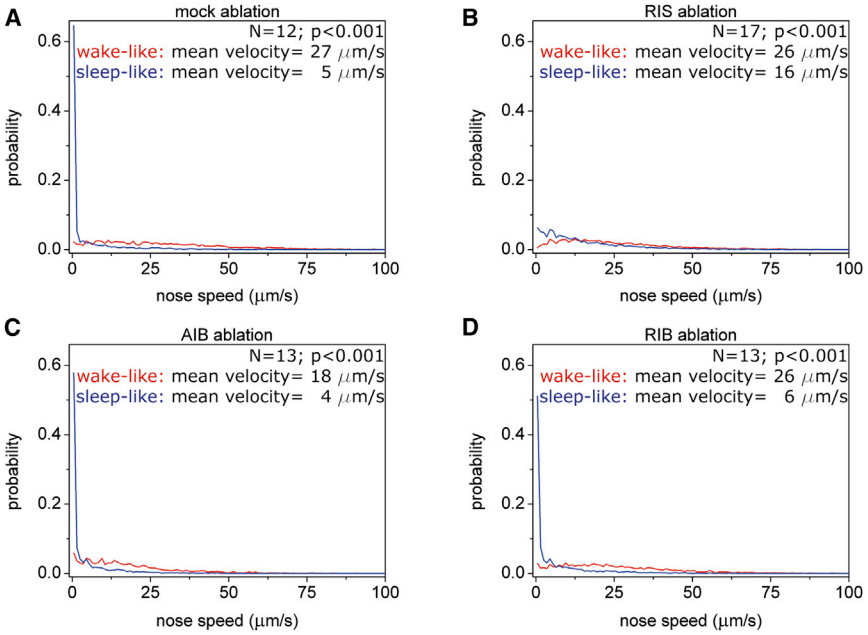

Figure 5. Ablation of a Single Neuron, RIS, Strongly Impairs Behavioral Quiescence

Probability distributions of nose speed during wake-like and sleep-like behavior are shown. Wilcoxon signed-rank test was used.
(A) Mock-ablated worms.
(B) RIS ablation.
(C) AIB ablation.
(D) RIB ablation.

hypothesis, we activated ChR2 in *aptf-1*-expressing neurons and simultaneously imaged neural activity in the command interneurons AVA and AVE using calcium imaging. During ChR2 activation, the frequency of transients in AVA and AVE was reduced by 70% (Figure S4). Thus, ChR2 activation of *aptf-1*-expressing neurons inhibits command interneuron activity.

By definition, sleep is reversible and thus different from paralysis [1]. Is quiescence caused by ChR2 activation of *aptf-1*-expressing neurons also reversible? We immobilized worms using ChR2 expressed in *aptf-1*-expressing neurons again for 20 s using blue light. Ten seconds after the onset of blue-light illumination, when worms were already immobilized, we stimulated them with dish tapping. Immobilized worms quickly responded to dish tapping by increasing their movement. Thus, quiescence induced by ChR2 activation of *aptf-1* expressing neurons is reversible (Figure 4).

Optogenetic activation of *aptf-1*-expressing neurons caused acute, reversible behavioral quiescence and inhibited the activity of the command interneurons AVA and AVE. Together with calcium imaging, which showed an activation of RIS at the sleep/wake-like transition, this suggested that the dominant principle of sleep-like behavior control by *aptf-1*-expressing neurons was active suppression of behavioral activity—probably through RIS activation.

What is the role of *aptf-1* in sleep-controlling neurons? In *aptf-1* mutants, RIS still activated at the onset of nonfeeding behavior but did not cause quiescence. Thus, *aptf-1* may act downstream of RIS activation. We tested this idea by ChR2 activation of *aptf-1*-neurons in *aptf-1* mutants. Unlike in wild-type, in *aptf-1* mutants, activation of *aptf-1*-expressing neurons caused not quiescence but an increase in activity (Figure 4; Movie S4). Thus, *aptf-1* was required for active and acute optogenetic induction of behavioral quiescence, suggesting that *aptf-1* may act downstream of RIS calcium transients.

RIS Is Required for Quiescence

Our results suggested a role for RIS in quiescence induction that may be similar to sleep-active, sleep-promoting neurons in the ventral lateral preoptic area (VLPO) in mammals. Lesion of the VLPO causes a reduction in sleep [4]. We wanted to test whether ablation of RIS causes impairments in quiescence. We ablated RIS with a focused UV laser beam [34] and measured nose speed during wake- and sleep-like behavior as before (Figure 5). After RIS ablation, quiescence was substantially reduced. While RIS-ablated worms slowed down measurably during sleep-like behavior, they were immobile only 5% of the lethargus period, whereas mock-ablated worms were quiescent for 65% of the lethargus period. Ablation of the pair of AIB or the pair of RIB neurons, however, did not cause a detectable quiescence defect. Thus, we could not detect an important role for AIB and RIB in wake- and sleep-like behavior. RIS, however, appears to be crucially required for sleep-like quiescence during lethargus.

Neuropeptide, Not GABA, Signaling Is Important for Quiescence

Sleep-active, sleep-promoting neurons in the VLPO express GABA and neuropeptides as neurotransmitters [4]. RIS also is GABAergic [35]. Is GABA required for quiescence in *C. elegans*? To answer this question we looked at a GABA null mutant and tested the requirement of GABA for optogenetic induction of quiescence. *unc-25* encodes a glutamic acid decarboxylase and null mutants of *unc-25* completely lack GABA [35]. We quantified nose speed during wake- and sleep-like behavior in an *unc-25* null mutant and found that it had normal sleep-like quiescence (Figure 6A). We then activated *aptf-1*-expressing neurons using ChR2 in *unc-25* mutants and found that quiescence induction was normal (Figures 6B and 6C).

Thus, we did not find evidence for a role of GABA in quiescence during lethargus. If GABA is dispensable for quiescence, then neuropeptides may play a role. We tested this idea by looking at a mutant with impaired neuropeptide function. *egl-3* encodes a proprotein convertase that is expressed in most neurons and is required for function of many neuropeptides [36, 37]. *egl-3* mutant worms showed reduced nose speed during sleep-like behavior but showed quiescence during only 5% of the lethargus period. ChR2 activation of *aptf-1*-expressing neurons in *egl-3* mutants did not cause quiescence but caused an increase in activity (Figures 6D–6F). Thus, while we could not detect a role for GABA in quiescence, our results suggest that neuropeptide signaling plays a crucial role in RIS-dependent quiescence.

Discussion

The activity of several neurons was previously analyzed during sleep-like behavior, and in all cases, a reduction of activity was

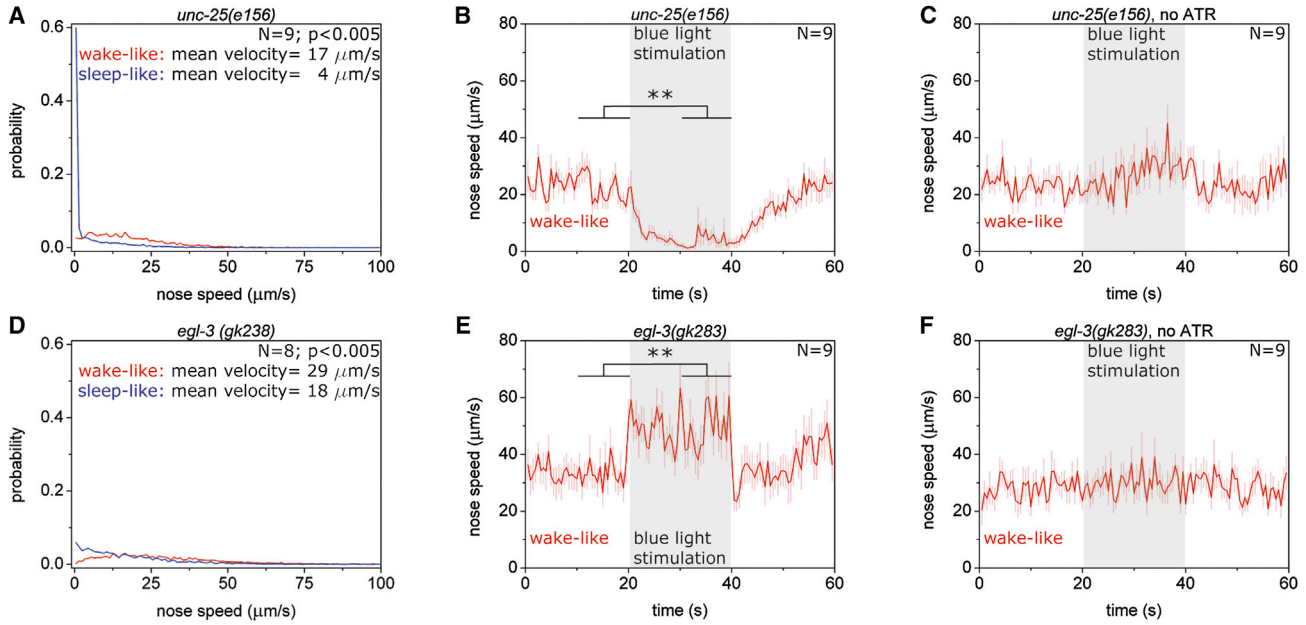

Figure 6. Neuropeptide Signaling, but Not GABA, Is Essential for Sleep-like Quiescence

Error bars are SEM.

(A–C) The GABA null mutant *unc-25(e156)* shows quiescence.

(A) Probability distribution of nose speeds.

(B) Induction of quiescence by channelrhodopsin-based activation of *aptf-1*-expressing neurons.

(C) Control without ATR.

(D–F) The neuropeptide proprotein convertase mutant *egl-3(gk238)* shows quiescence defects. Wilcoxon signed-rank test was used.

(D) Probability distribution of nose speeds shows a strong reduction of quiescence.

(E) No induction of quiescence by channelrhodopsin-based activation of *aptf-1*-expressing neurons

(F) Control without ATR.

found. These neurons include the sensory neuron ALM, the interneuron AVA, and D-type motor neurons [12, 14, 38]. The ALA interneuron has been found to modulate sleep-like behavior through EGF signaling, but it is unclear whether its calcium activity is increased during sleep-like behavior [39]. While AIB, RIB, AVA, and other neurons in *C. elegans* appear to be wake-active, RIS appeared to be a sleep-active and sleep-promoting neuron. RIS is a GABAergic interneuron [35]. We found that RIS-induced quiescence requires peptidergic signaling rather than GABAergic signaling. RIS activity differs from other GABAergic neurons, such as motor neurons, which have reduced activity during sleep-like behavior [38]. RIS has properties that are rather similar to vertebrate sleep-active neurons found in the VLPO that are also GABAergic and peptidergic [4]. Thus, active induction of sleep or sleep-like behavior through activation of sleep-inducing GABAergic peptidergic neurons appears to be a common principle found in vertebrates and invertebrates.

We did not find a role for GABA in controlling sleep-like behavior. GABAergic cell fate, however, appears to be a conserved feature of sleep-active, sleep-promoting neurons. It is thus likely that GABA does play an important role in these neurons. It may be that GABA serves a rather subtle role in sleep regulation in *C. elegans* that we have not detected in our assays.

Neuropeptidergic signaling appears to play an important role in sleep regulation in various organisms, including *C. elegans* [40]. Hypothetically, *aptf-1* may be required for neuropeptidergic cell fate of RIS, and it will be important to identify the neuropeptide that mediates RIS-dependent quiescence.

Disturbing *C. elegans* physically during sleep-like behavior has been shown to be injurious and to cause molting defects and even death [15]. *aptf-1* mutants move continuously during lethargus; however, they do not show obvious defects in viability or molting. This suggests that physical disturbance is different from genetic ablation of locomotor quiescence.

The evolutionary origin of sleep-like lethargus behavior in *C. elegans* and sleep in vertebrates is still unclear. Conserved cyclic nucleotide, TGF-β, and EGF signaling pathways have been found to modulate sleep-like behavior in *C. elegans* and other organisms, showing some similarity in the control of sleep-like behavior in different systems [11, 39, 41]. In some cases, the identification of transcription factors has been crucial in resolving controversies regarding evolutionary relationships. A famous example was the discovery that the transcription factor *pax6* is required for eye development in all vertebrates and invertebrates that have been studied and thus showed that eyes have a common evolutionary origin, which revised the 150-year long view that eyes have evolved multiple times [42]. It would therefore be intriguing to know whether *ap2* mutation actually causes sleep disorders in humans, and whether it controls sleep-promoting circuits.

In humans, mutations in *ap2β* are linked to Char disease, which is characterized by defects in heart, face, and limb development [24]. In two families in which Char disease occurred, sleep disorders were found. Individuals suffering from Char were sleepwalking or were sleeping less than half the normal time [43]. The common circuit principle of sleep induction by GABAergic-peptidergic neurons and the similarities in phenotypes of *ap2* mutations in *C. elegans* and humans

suffering from Char disease support the view that sleep-like behavior in *C. elegans* and sleep in humans share a common evolutionary origin.

Supplemental Information

Supplemental Information includes three figures, Supplemental Experimental Procedures, and four movies and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2013.09.028.

Acknowledgments

Some strains were provided by the Caenorhabditis Genetics Center, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440). *aptf-1(tm3287)* was provided by the Mitani Laboratory at the Tokyo Women's Medical University School of Medicine. M. Sarov provided the *aptf-1* fosmid. J. Borstel and R. Krug assisted with manual data processing.

Received: March 28, 2013
Revised: August 23, 2013
Accepted: September 12, 2013
Published: October 31, 2013

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