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The *Drosophila apterous*^{56f} mutation impairs circadian locomotor activity

Kohiyama Mayumi,

Biology Department, Skidmore College, Saratoga Springs, NY USA, mfkohiyama@gmail.com, 917-715-7270.

Bonser Danielle,

Biology Department, Skidmore College, Saratoga Springs, NY USA, danielle.bonser@utah.edu, 518-223-2790.

Leung Lisa,

Biology Department, Skidmore College, Saratoga Springs, NY USA, lleung@skidmore.edu, 617-910-7669.

Fall Alexandra,

Biology Department, Skidmore College, Saratoga Springs, NY USA, afall@skidmore.edu, 845-803-3591.

Canada Nathan,

Biology Department, Skidmore College, Saratoga Springs, NY USA, ncanada@skidmore.edu, 770-359-7123.

Qu Boyang,

Biology Department, Colby College, Waterville, ME USA, boyangqu@colby.edu, 207-616-6117.

S. Tariq Ahmad, and

Biology Department, Colby College, Waterville, ME USA, stahmad@colby.edu, 207-859-5722.

Possidente Bernard

Biology Department, Skidmore College, Saratoga Springs, NY USA, bposside@skidmore.edu, 518-580-5082.

Abstract

We investigated effects of *apterous* mutation *ap*^{56f} on circadian locomotor activity, eclosion rhythms, and transcript levels of *period* and *timeless* in *Drosophila*. We investigated circadian locomotor activity and eclosion rhythms in *ap*^{56f} and wild-type flies, their F1 and F2 offspring, and wingless *vestigial* mutants and show that *ap*^{56f} disrupts circadian locomotor rhythms in a genetically recessive manner, that is not caused by the absence of wings. The *ap*^{blt} strain also showed impaired circadian activity rhythms, providing independent evidence for a significant role of *apterous* in circadian locomotor rhythm expression. The *ap*^{56f} mutation did not disrupt a circadian eclosion rhythm or rhythmic expression of the *period* and *timeless* clock genes,

indicating that *apterous* is not essential for circadian clock function, but is necessary for coupling locomotor activity to a circadian clock. *Timeless* transcription was reduced in *ap^{56f}* flies in 12:12 LD, suggesting that *apterous* may modulate core clock gene expression.

Keywords

Drosophila; circadian; *apterous*; locomotor activity; eclosion; gene expression

Introduction

The light-entrainable circadian pacemaker in the brain of *Drosophila melanogaster* is a network of approximately 150 neurons (Helfrich-Forster et al. 2007) responsible for generating circadian rhythms in diverse behaviours such as locomotor activity, pupal eclosion, courtship behaviour, and olfactory responses (Kuhlman et al. 2007). The central *Drosophila* circadian clock oscillation is driven by intracellular transcription-translation feedback loops that depends on a small number of core “clock genes” (Hardin 2005; Kuhlman et al. 2007) essential for its circadian function.

We investigated the potential role of the *Drosophila ap^{56f}* mutation in circadian rhythm expression. The *apterous* locus was originally isolated as a wingless mutant (Metz 1914; Butterworth & King 1965) and was later shown to be a LIM family transcription factor (Cohen et al. 1992) with a homeodomain. The LHX2 human ortholog to *apterous* rescues *Drosophila apterous* mutant function, and both *apterous* and its mouse ortholog mLHX2 are expressed in the peripheral and central nervous system and the visual system (Rincon-Limas et al. 1999; Morante and Desplan 2008). LHX1 regulates the terminal differentiation of SCN neurons and expression of multiple neuropeptides in the SCN of mice, and its absence results in disorganized, low amplitude activity rhythms in constant darkness (DD) (Bedont et al. 2014). These observations are consistent with a potential role for the *apterous* gene in the development of the neural circuitry mediating circadian biological clock function in *Drosophila*.

There is evidence for an acute role of *apterous* gene expression in mediating adult *Drosophila* circadian rhythms in locomotor activity and sleep through circadian clock output pathways downstream from clock neurons. The neuropeptides pigment dispersing factor (PDF) and leucokinin (LK), shown to play a role in the downstream output pathway from *Drosophila* circadian clock neurons driving circadian locomotor activity rhythms, are regulated by *apterous* gene expression. PDF is expressed in the small and large lateral ventral *Drosophila* clock neurons (s-LNv and l-LNv, respectively) (Helfrich-Forster et al. 1995; 2007) and PDF mutants display severe abnormalities in their locomotor activity rhythm in DD (Renn et al. 1999). Recently, Shimada et al. (2016) showed that *apterous* expression in the *Drosophila* clock neurons is specific to the s-LNv and the l-LNv, and that *apterous* expression in the l-LNv, but not the s-LNv, mediates light-driven arousal response. Cavey et al. (2016) have identified a role for LK, and the LK receptor in circadian *Drosophila* activity rhythm expression by showing that silencing the LK gene with RNAi significantly degrades circadian locomotor rhythm expression. Four neurons in the

Drosophila brain express LK: two in the lateral horn of the protocerebrum (LHLK neurons), and two subesophageal ganglion (SELK neurons) (Herrero et al. 2003). Only the LHLK neurons express *apterous*, and blocking LHLK *apterous* expression abolishes Leucokinin expression (Herrero et al. 2003).

Although *Drosophila melanogaster vestigial* mutants lacking wings have been shown to display entrained and free-running locomotor rhythms (Subramanian et al. 2009; Sivaperumal et al. 2011) these studies also document multiple quantitative differences from wild-type flies in circadian rhythm characteristics, particularly under monochromatic red and green light photoperiods, and in T-cycles ranging from 20–28 hours. These observations suggest that abnormal circadian phenotypes in wingless mutants may result from either the physical absence of wings, or from pleotropic effects of wingless mutations mediated through other pathways.

Here, our initial experiments were designed to characterize the effect of *apterous* mutation *ap^{56f}* on circadian locomotor rhythms, and to evaluate whether the absence of wings alters circadian locomotor activity. We generated F1 and F2 offspring between *ap^{56f}* and wild-type flies to investigate the pattern of heredity of abnormal circadian activity rhythms in the *ap^{56f}* strain, and to assess circadian activity rhythms in *ap^{56f}* mutants segregating in the F2 against an outbred genetic background. We compared circadian activity rhythms between *ap^{56f}* and *ap^{blt}* flies as an independent assay of the role of the *apterous* gene in circadian activity rhythm expression, and assessed circadian rhythms for eclosion, and clock gene transcription from the *period* and *timeless* genes, to determine whether *ap^{56f}* effects on circadian activity are specific to the activity rhythm. Our results indicate that the *apterous* gene is not essential for circadian clock function, but does mediate circadian locomotor activity expression independently of its effects on wing morphology.

Materials and methods

Experiments one through five were conducted at Skidmore College, and experiment six was conducted at Colby College.

Stocks, rearing conditions, and crosses

All flies in experiments one through five were maintained on Carolina Instant *Drosophila* Medium (Carolina Biological, Burlington North Carolina, USA) and *Drosophila* Instant Mix Bloomington Formulation (Genesee Scientific, El Cajon California, USA) was used for experiment six. Flies were raised at 25°C in a 12:12 light-dark (LD) photoperiod. Mutant strains *ap^{56f}* (stock #4189), *ap^{blt}* (stock #4190) and *vg^l* (stock # 432) were from the Bloomington, Indiana USA *Drosophila* stock center. Canton-S wild-type flies were obtained from Dawson Mohler at the University of Iowa and have been maintained at Skidmore College since 1983. F1 flies were obtained by crossing homozygous *ap^{56f}* males with virgin homozygous wild-type Canton-S (CS+) females. The *apterous* wing phenotype is recessive (Metz, 1914) and all F1 flies (CS+/*ap^{56f}*) exhibited the wild-type phenotype as expected. F1 flies were crossed to obtain F2 flies. This cross is expected to yield 25% homozygous recessive *apterous* mutant offspring, 50% heterozygotes (genetically identical to the F1 parents), and 25% wild-type. F2 flies with wild-type wings (F2W) and F2 flies with the

apterous phenotype (F2A) were assayed for circadian locomotor activity to determine if the *apterous* circadian phenotype segregates with the *apterous* trait.

Measurement of circadian activity rhythms

The circadian rhythms of CS+, *ap^{56f}*, and *vg^l* flies were measured in DAM2 *Drosophila* Activity monitors (TriKinetics Inc, Waltham, Massachusetts). Male and female adult flies less than four days old were collected, anesthetized with FlyNap (Carolina Biological Supply, Burlington, North Carolina USA), sorted by sex, and placed into individual small plastic tubes (5mm outer diameter × 65mm length) containing approximately a centimeter of food (2g of agar, 5g of sucrose per 100 mL of distilled water). The tubes were sealed with a plastic cap on one end and plugged with cotton on the other and inserted into the activity monitors.

Genotype and sex were equally distributed within and among monitors to ensure all genotypes of flies were uniformly exposed to the same environmental conditions. All flies were observed for four days in 12:12 LD to monitor their entrained circadian activity pattern, and for four or five days, without interruption, in constant dark (DD) to examine their free-running circadian period. Activity was recorded in 10-minute intervals, counting the number of times each fly crossed a photobeam in the middle of the tube.

Experiment 1: circadian activity rhythms in CS+, *ap^{56f}*, and *vg^l*

Locomotor activity rhythms were compared among 15 males and 15 females from CS+, *ap^{56f}* and *vg^l* flies. Complete LD data was obtained for 23 CS+ flies, 28 *ap^{56f}* flies, and 18 *vg^l* flies. Complete DD data was obtained for 23 CS+ flies, 20 *ap^{56f}* flies, and 16 *vg^l* flies. Data were combined for males and females because there was no significant sex difference in the numbers of rhythmic flies for any strain (Fisher's exact test, data not shown).

Experiment 2: circadian activity rhythms in F1 and CS+ flies

Virgin CS+ females were mated with *ap^{56f}* males to produce F1 flies, and the parental strains were mated *inter-se*. Thirty F1, *ap^{56f}* and CS+ flies were assayed for circadian locomotor activity. To eliminate sex differences as a variable, only males were used since experiment one showed no significant sex differences in the *ap^{56f}* mutant rhythms. Activity rhythms in 12:12LD and DD were compared among 15 flies of each sex per genotype. Complete LD data was obtained for 27 CS+ flies, 28 *ap^{56f}* flies, and 30 F1 flies. Complete DD data was obtained for 27 CS+ flies, 18 *ap^{56f}* flies, and 30 F1 flies.

Experiment 3: circadian activity rhythms in F2, CS+, and *ap^{56f}* flies

F1 flies from experiment two were mated *inter-se* to produce F2 flies, which were collected and sorted phenotypically into those with the *apterous* phenotype (F2A) and wild-type phenotype (F2W) for the *apterous* trait. The activity patterns of 36 F2A (19 females, 17 males), 39 F2W (21 females, 18 males) were assayed along with 32 CS+ males and 32 *ap^{56f}* males. Both males and females were used to increase the sample size of *apterous* flies, whose viability was reduced approximately 75% compared to the non-*apterous* flies. Complete 12:12 LD data was obtained for 32 CS+ flies, 32 *ap^{56f}* flies, 36 F2A flies, and 39 F2W flies. Complete DD data was obtained for 32 CS+ flies, 27 *ap^{56f}* flies, 26 F2A flies,

and 39 F2W flies. Data were combined for males and females because there was no significant sex difference in the numbers of rhythmic flies for any strain (Fisher's exact test, data not shown).

Experiment 4: circadian activity rhythms in CS+, *ap*^{56f} and *ap*^{blt} flies

Flies from CS+, *ap*^{56f} and *ap*^{blt} strains were compared to provide an independent test for a role of *apterous* in circadian locomotor activity expression. Fifteen males and 15 females of each strain were assayed for circadian activity in 12:12LD and DD. Data were combined for males and females because there was no significant sex difference in the numbers of rhythmic flies for any strain (Fisher's exact test, data not shown). Complete LD data was obtained for 29 CS+ flies, 28 *ap*^{56f} flies, and 29 *ap*^{blt} flies. Complete DD data was obtained for 26 CS+ flies, 22 *ap*^{56f} flies, and 24 *ap*^{blt} flies.

Experiment 5: eclosion rhythms in wild-type and *ap*^{56f} flies

Three replicate experiments were performed. Twenty-two half-pint glass bottles were prepared: 11 bottles contained wild-type flies and the other 11 contained *ap*^{56f} mutant flies. Oregon-R was used as the wild-type strain for the first replicate, and CS+ for the second and third replicates. Each bottle was prepared with 15 mL of dry medium and 33 mL of water, and seeded with four female and four male flies. Plastic wrap to collect pupae was placed inside the bottles as well as cotton in the neck to prevent flies from escaping.

Flies were maintained at 25°C in a 12:12 LD. After 10 days, pupae on the plastic wrap were collected. Pupae were sorted by strain, and evenly distributed onto four Petri dishes, two per strain, freshly spread with a thin coating of Elmer's glue. Each Petri dish was inverted over a *Drosophila* Funnel Monitor (TriKinetics, Waltham, Massachusetts USA) that monitored eclosion every hour within each 24-hour period by counting flies falling through the funnel. One Petri dish from each strain was monitored at 25°C in 12:12 LD and in DD for three consecutive days.

Experiment 6: transcription of clock genes in *ap*^{56f} vs. wild-type flies

The relative *period* (*per*) and *timeless* (*tim*) mRNA level in CS+ and *ap*^{56f} flies at Zeitgeber Time (ZT) six and 12 was determined using quantitative real time – Polymerase Chain Reaction (qRT-PCR) as previously described by Ahmad et al., 2013. Briefly, total RNA was isolated from 1–5 day old flies of appropriate genotype and ZT reared in 12:12 LD was isolated using RNeasy miniprep kit (Qiagen, Valencia, California USA). Five independent biological replicates were assayed for each strain and ZT, each consisting of RNA isolated from 25–30 heads. qRT-PCR was performed in duplicates on 50–100 ng total RNA using one step Quantifast SYBR Green RT-PCR kit (Qiagen) on StepONE Real-Time PCR system (Applied Biosystems, Foster City, California USA). Relative normalized transcript level was determined by delta-delta Ct method. *RP49* was used as the normalizing gene. Mean ± SEM *per* transcript was calculated from five independent experiments. *per* primers: 5' – GACCGAATCCCTGCTCAATA– 3' and 5' –GTGTCATTGGCGACTTCTT– 3'. *tim* primers: 5' –CCCTTATACCCGAGGTGGAT– 3' and 5' –TGATCGAGTTGCAGTGCTTC3' *RP49* primers: 5' –CGGTTACGGATCGACAAGC– 3' and 5' –CTTGCGCTTCTTGAGGAGA– 3'.

Experiments 1–4: data analysis

Data was collected using *Drosophila* Activity Monitor Software System 3.0 (TriKinetics). For each experiment, only data from flies that survived the entire LD or DD treatment was used. Actiware software (Minimitter Corporation Bend, OR USA) was used to produce actograms depicting the average activity in both LD and DD cycles for each individual fly (Figure 1). Composite actograms were also made using Actiware J (Schmid et al., 2011) for each treatment group.

Chi-squared periodogram was used to estimate the period of activity rhythms in 12:12LD and DD (Actiware), and statistical significance of estimated circadian periods (Refinetti, 2018). Flies with a significant circadian period (alpha level 0.05) were classified as “rhythmic”. Amplitude of the rhythm was estimated as the QP value of best fit circadian period in the periodogram analysis (Diez-Noguera, 2013)). The Statistical Analysis System’s (Carey, NC USA) GLM procedure was used to compare effects of genotype, sex and genotype-sex interaction. Fisher’s exact test was used to evaluate the frequencies of rhythmic flies.

Each fly was analyzed for mean activity level (XLD and XDD= average beam crossings per 10-minute interval in LD and DD, respectively), circadian period (measured in hours) in LD and DD (TauLD and TauDD, respectively) and circadian amplitude in LD and DD (QPLD and QPDD, respectively). Percent rhythmicity was calculated for each group as the percentage of flies analyzed that showed statistically significant circadian activity rhythms.

Experiment 5: data analysis

Composite graphs of the data were made using Actiware J. Chi-square was used to compare the number of flies eclosing in the 12 hours of light versus the 12 hours of dark for LD, assuming a ratio of 1:1 as the null hypothesis. Numbers of flies eclosing in DD were compared between the first 12 hours of DD that corresponded to the 12 hours of light in the previous photoperiod (subjective day) versus the second 12 hours in DD (subjective night), assuming an approximate 24-hour eclosion rhythm in DD. Chi-square was used to analyze each of the three individual replicates for evidence of rhythmicity, and contingency Chi-square was used to compare day versus night frequencies of eclosion between the wild-type and *ap^{56f}* flies for the combined replicates in LD and DD.

Experiment 6: data analysis:

Student’s t-tests were used to determine differences in *per* and *tim* transcripts between CS+ and *apterous* flies at ZT6 and ZT12.

Results

Experiment 1: circadian activity rhythms in CS+, *ap^{56f}*, and *vg¹* flies

In comparison to wild-type and *vg¹* flies, fewer *ap^{56f}* flies expressed significant circadian activity rhythms in LD and DD ($p < 0.001$; Figure 2; Table 1). Among 28 *ap^{56f}* flies, four had statistically significant circadian rhythms in 12:12 LD and two had significant rhythms in DD. Among the CS+ strain, 20 out of 23 displayed significant circadian rhythms in both LD

and DD. For the *vg^l* strain, 10 out of 18 had significant rhythms in LD and 12 were significant in DD. Since *vg^l* flies displayed rhythms similar to wild-type flies in DD, we conclude that the reduced expression of the activity rhythm in *ap^{56f}* flies is not caused by their wingless phenotype. Both wingless mutants, however, were less rhythmic than wild-type in LD, so it is possible that the absence of wings might reduce daily rhythmicity in a light-dark cycle. The average circadian amplitude of the *ap^{56f}* flies in constant dark (QPDD) was significantly reduced compared to the *vg^l* and wild-type flies ($p < 0.001$; Table 1). Among the flies that had significant free running circadian rhythms in DD, there was no significant difference in the period of the rhythms (Table 1).

Experiment 2: circadian activity rhythms in F1 and CS+ flies

Twenty four of the 30 heterozygous F1 flies, and 21 of 27 CS+ flies had significant circadian rhythms in DD (Figure 3; Table 1). Although the F1 flies carried the *ap^{56f}* mutation, their activity rhythms were similar to wild-type flies, indicating that the *ap^{56f}* mutation is recessive for its effect on circadian locomotor rhythmicity. Of the 28 homozygous *ap^{56f}* controls, only four displayed significant free running circadian rhythms in DD. No significant differences were observed for TauDD (Table 1).

Experiment 3: circadian activity rhythms in F2, CS+, and *ap^{56f}* flies

Among the F2A flies, (homozygous for the recessive *ap^{56f}* gene) only eight of 36 flies had a significant circadian rhythm in DD, compared to 25 of the 39 F2W flies (Fig 4; Table 1, $p < 0.05$). Twenty-seven of 32 CS+ controls also displayed significant circadian rhythms in DD, compared to only five of 27 *ap^{56f}* flies ($p < 0.05$). These results show that the reduced circadian rhythm expression observed in the *ap^{56f}* strain segregates with the wingless *apterous* phenotype in the F2 generation. No significant differences in TauDD were observed (Table 1).

Experiment 4: circadian activity rhythms in CS+, *ap^{56f}* and *ap^{blt}* flies

The *ap^{blt}* strain displayed weak circadian activity rhythms in 12:12 LD and in DD that were similar to *ap^{56f}*, and significantly less rhythmic and CS+ wild-type flies in DD ($p < 0.05$; Table 1, Figure 5). There were no significant differences among strains for TauDD (Table 1).

Experiment 5: eclosion rhythms in CS+ and *ap^{56f}* flies

A total of 329 wild-type flies, and 149 *ap^{56f}* flies eclosed in LD. Each of the three replicate assays in LD showed significantly more flies eclosing during the 12 hours of light than in the 12 hours of dark for both the wild-type flies ($X^2 = 6.0, 13.4, 22.3$ respectively, and the *ap^{56f}* flies ($X^2 = 16.000, 41.832, 5.334$; Figure 6). A total of 415 wild-type flies, and 107 *ap^{56f}* flies eclosed in DD, with significantly more flies eclosing in the subjective day than the subjective night ($X^2 = 39.0, 69.3, 23.6$ respectively for wild-type flies, and $X^2 = 6.25, 33.924, 3.846$, respectively for the *ap^{56f}* flies; Figure 6). These data are consistent with a similar circadian rhythm for eclosion in both wild-type and *ap^{56f}* mutant flies. The *ap^{56f}* flies were significantly more rhythmic in both the 12:12 LD cycle and in DD than the wild-type flies (X^2 -contingency = 12.6 for LD ($p < 0.001$) and 19.0 for DD ($p < 0.0001$)).

Experiment 6: transcript levels of clock genes in CS+ and *ap^{56f}* flies

Consistent with previous findings (Hardin, 2005), wild-type flies showed significant upregulation of *per* and *tim* transcripts at ZT12 compared to ZT6 (Figure 7; *per* – 3.47 ± 0.81 -fold, $p=0.008$; *tim* – 5.04 ± 0.94 -fold, $p=0.001$). Similar to wild-type, *ap^{56f}* flies in 12:12 LD also showed significant accumulation of *per* and *tim* transcripts from ZT6 to ZT12 (Figure 7; *per* – 2.45 ± 0.34 -fold, $p=0.001$; *tim* – 4.62 ± 0.62 -fold, $p=0.0001$). However, *ap^{56f}* flies showed remarkably lower accumulation of both *per* and *tim* transcripts at ZT12 compared to wild-type flies (Figure 8; *per* – 0.78 ± 0.13 -fold, $p=0.09$; *tim* – 0.82 ± 0.06 -fold, $p=0.01$).

Discussion

Here we show that the *ap^{56f}* mutation in *Drosophila* is associated with a significant deficit in the expression of circadian locomotor activity rhythms. The majority of *ap^{56f}* mutants were arrhythmic in both a light-dark cycle and constant darkness, compared to the CS+ wild-type strain where most of the flies displayed circadian rhythms. This effect was not due to the absence of wings in the *ap^{56f}* mutants since *vg¹* mutants, also lacking wings, displayed circadian rhythms comparable to the wild-type strain in constant darkness where a circadian clock generates the rhythm.

The effect of the *ap^{56f}* mutation on the circadian activity rhythm was expressed in F2 flies as a recessive trait and was not observed in F1 hybrid offspring heterozygous for the *ap^{56f}* mutation. This pattern of heredity is consistent with a single-locus, autosomal mode of inheritance of the *ap^{56f}* mutation.

Abnormal circadian activity rhythms segregated with the *ap^{56f}* mutant wing phenotype in F2 flies possessing a mixed genetic background derived equally from the original wild-type and *ap^{56f}* parent strains. This result is consistent with an effect of the *ap^{56f}* mutation on circadian activity rhythm expression. It is possible, however, that a mutation at a locus closely linked to the *ap^{56f}* mutation, present in the original *ap^{56f}* strain, might be causing the abnormal circadian phenotype. We showed that the *ap^{blt}* strain, carrying an *apterous* mutation derived independently from *ap^{56f}* in a different genetic background, also shows impaired circadian activity rhythm expression, which argues against the latter interpretation.

The most direct confirmation that wild-type *apterous* function is necessary for robust circadian locomotor activity would be direct mutation of the *apterous* locus. Shimada et al. (2016) subjected wild-type flies to *n-Synaptobrevin* pan neuronal RNA interference designed to knock down *apterous* gene expression, but they observed wild-type like circadian locomotor activity in DD in these flies. This approach requires further investigation using other methods of directed mutagenesis.

Further research is also required to determine the extent to which circadian abnormalities associated with the *apterous* mutation represent effects on the core molecular clock mechanism, or effects on mechanisms peripheral to the clock linking locomotor activity to the core clock mechanism (Wulbeck, Grieshaber, and Helfrich-Forster 2008; Helfrich-Forster et al. 2007). Our results, however, show that core circadian clock genes *period* and *timeless* are expressed rhythmically in *ap^{56f}* flies. However, *timeless* gene expression was

significantly reduced at ZT12, which suggests that the *apterous* locus may play a role in regulating clock gene expression. Previous research linking *apterous* gene expression to s-LNV clock neurons that express PDF (Shimada 2016), and to neurons in the subesophageal ganglion that express LK (Herrero et al. 2003), two neuropeptides linked to output pathways from circadian clock neurons, argue in favor of the *apterous* locus playing a key role in circadian locomotor activity expression downstream from the core circadian clock mechanism.

We show that *ap^{56f}* flies display a circadian rhythm for eclosion, and for *per* and *tim* clock gene expression. These results suggest that wild-type *apterous* gene expression is not essential for circadian clock function, but instead plays a key role in the output pathways that connect the core circadian clock oscillation to output pathways that regulate locomotor activity.

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Figure 1.

Actogram depicting activity pattern of a typical CS+ fly with over 48 hours shown on the x-axis and successive days on the y-axis for 4 days in a light-dark cycle (12:12 LD) followed by five days of constant dark (DD).

Figure 2.

Composite actograms for experiment 1. The ap^{56f} strain shows weak circadian rhythmicity in LD and DD, compared to robust rhythms for the CS+ wild-type strain and a wingless vg^I mutant strain.

Figure 3.

Composite actograms for experiment 2. F1 hybrids between CS+ wild-type and ap^{56f} strains display circadian rhythms in LD and DD similar to wild-type parents, demonstrating that the ap^{56f} circadian phenotype is recessive.

Figure 4.

Composite actograms for experiment 3. F2W flies with wild-type wing phenotypes display circadian activity rhythms similar to wild-type flies (CS+), but F2A flies with *ap*^{56f} phenotypes display weak circadian rhythmicity similar to the *ap*^{56f} parent strain, demonstrating that the weak circadian activity trait segregates with the *ap*^{56f} wing phenotype.

Figure 5. Composite actograms for experiment four comparing ap^{56f} and ap^{blt} effects on circadian activity rhythms. Both *apterous* alleles show lower amplitude circadian activity rhythm expression than wild-type flies.

Figure 6.

Composite actograms of eclosion data from experiment five. (A) CS+ in LD; (B) CS+ in DD; (C) *ap^{56f}* in LD and (D) *ap^{56f}* in DD. Both wild type (CS) and *ap^{56f}* flies were significantly rhythmic in LD and DD, and *ap^{56f}* flies showed a significantly stronger rhythmicity and wild-type in both conditions.

Figure 7. Changes in transcription from the *period* and *timeless* loci between ZT6 and ZT12 in *ap^{56f}* versus wild-type flies. Both wild-type (*CS*) and *apterous* show robust (significant) increases in *per* and *tim* transcripts at ZT12 vs. ZT6.

Figure 8.

Transcription from the *period* and *timeless* loci in *ap^{56f}* versus wild-type flies at ZT6 and ZT12. *ap^{56f}* flies show a reduction in *per* (trending) and *tim* (significant) transcripts at ZT12 compared to wild-type (*CS*).

Table I.

Percentage of flies with statistically significant circadian activity rhythms in the 12:12 light-dark photoperiod (RLD) and constant darkness (RDD), mean activity levels in LD (XLD) and DD (XDD) (beam crossings per 10-minute interval), amplitude in DD (QPDD) and circadian period for rhythmic flies in DD (TAUDD) $\pm$ SEM. Asterisks denote significant differences from CS+ wild-type.

	N	RLD	RDD	XLD	XDD	QPDD	TAUDD
Exp 1							
CS+	23	87	87	5.5 $\pm$ 0.5	8.6 $\pm$ 0.9	303 $\pm$ 20	24.2 $\pm$.1
<i>ap⁵⁶</i>	28	14*	7*	3.8 $\pm$ 0.7*	5.8 $\pm$ 0.8*	190 $\pm$ 04*	24.4 $\pm$.8
<i>vg^l</i>	28	56*	67	7.5 $\pm$ 0.7*	8.2 $\pm$ 1.3	242 $\pm$ 13*	23.8 $\pm$.1*
Exp 2							
CS+	27	93	78	7.2 $\pm$ 0.6	8.4 $\pm$ 0.6	268 $\pm$ 17	24.0 $\pm$.2
<i>ap⁵⁶</i>	28	43*	14*	6.6 $\pm$ 1.2	7.5 $\pm$ 1.7	183 $\pm$ 06*	23.7 $\pm$.6
F1	30	80	80	6.1 $\pm$ 0.5	6.9 $\pm$ 0.5	257 $\pm$ 10	24.0 $\pm$.2
Exp 3							
CS+	32	94	84	5.5 $\pm$ 0.3	7.7 $\pm$ 0.6	274 $\pm$ 10	24.3 $\pm$.2
<i>ap⁵⁶</i>	32	31*	16*	3.8 $\pm$ 0.5*	4.1 $\pm$ 0.8*	198 $\pm$ 03*	23.6 $\pm$.2*
F2W	39	77	64	7.1 $\pm$ 0.6	8.1 $\pm$ 0.8	233 $\pm$ 08*	24.4 $\pm$.2
F2A	36	25*	22*	3.5 $\pm$ 0.6*	4.2 $\pm$ 0.9*	199 $\pm$ 04*	24.2 $\pm$.5
Exp 4							
CS+	29	93	92	7.7 $\pm$ 0.7	10.4 $\pm$ 0.7	303 $\pm$.3	24.7 $\pm$.2
<i>ap⁵⁶</i>	29	31*	23*	6.6 $\pm$ 1.0	6.6 $\pm$ 1.0	191 $\pm$ 04*	24.2 $\pm$.7
<i>ap^{blt}</i>	29	48	36*	3.4 $\pm$ 0.7	4.6 $\pm$ 0.3	197 $\pm$ 03*	24.7 $\pm$.3