AC005639, 30036–28714. Accession numbers for the other genes are as follows (data are as of 5 January 2000) (complete sequences are available for the first four and only partial sequences are available for the remaining genes; LU, location unknown): transcript GR1F.1, accession number AL035632, range 7301–8711; GR47F.1, AC005653, 42838–44204; GR68D.1, AC006492, 46040–44916; GR77E.1, AC006490, 104929–103117; GR28A.1, AC008354, 66711–66973; GR57B.1, AC007837, 102661–103185; GR65C.1, AC004251, 23136–24215; GR93F.1, AC012873, 35043–35228; GR93F.2, AC012892, 2781–2650; GR93F.3, AC012892, 4271–4143; GR93F.4, AC012892, 6482–5559; GR94E.1, AC008200, 72472–72308; GR97D.1, AC007984, 121300–121977; GR98B.1, AC007817, 45506–46916; GR98B.2, AC007817, 10695–10784; GR98B.3, AC007817, 45189–45284; GR98B.4, AC007817, 39658–39765; GRLU.1, AC017438, 22141–21398; GRLU.2, AC017138, 10997–11122; GRLU.3, AC015395, 43210–43612; GRLU.4, BACR28P1-T7, 28–129; GRLU.5, BACR28P1-T7, 388–734; GRLU.6, BACR06I03-T7, 1028–48; and GRLU.7, AC012799, 8212–8123.
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26. The amount of each tissue used to prepare cDNA was that determined to give approximately the same signal with a pair of positive control primers, CGGATCCCTATGTCAAGGTG and GAAGAGCT TCGTGCTGGTCT, representing the *Drosophila synaptotagmin* gene (*23*). Specifically, the amount of tissue used in each cDNA preparation was as follows: 50 labella, 5 heads from which taste organs (the labellum, the LSO, the dorsal cibarial sense organ, and the ventral cibarial sense organ) had been surgically removed, 20 thoraces, 20 abdomens, 200 legs, and 20 anterior wing margins (the portion of the wing containing chemosensory sensilla). Complementary DNA preparation and PCR were performed as in (*2*). For all genes, primer pairs (*24*) that span introns were used to distinguish bands amplified from cDNA from those amplified from any remaining genomic DNA. All negative results were confirmed by testing at least one additional primer pair.
27. We thank J. Kim for providing candidate transmembrane domain sequences and helping to analyze them, G. Fitzgerald for expert technical assistance, K. Kimura for the *poxn* mutant, and J. Nathans for comments on the manuscript. We are very grateful to the personnel of the BDGP for their efforts. Supported by grants from NIH (DC-02174) and the Human Frontier Science Program to J.R.C.

3 November 1999; accepted 27 January 2000

Correlates of Sleep and Waking in *Drosophila melanogaster*

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Drosophila **exhibits a circadian rest-activity cycle, but it is not known whether fly rest constitutes sleep or is mere inactivity. It is shown here that, like mammalian sleep, rest in *Drosophila* is characterized by an increased arousal threshold and is homeostatically regulated independently of the circadian clock. As in mammals, rest is abundant in young flies, is reduced in older flies, and is modulated by stimulants and hypnotics. Several molecular markers modulated by sleep and waking in mammals are modulated by rest and activity in *Drosophila*, including cytochrome oxidase C, the endoplasmic reticulum chaperone protein BiP, and enzymes implicated in the catabolism of monoamines. Flies lacking one such enzyme, arylalkylamine *N*-acetyltransferase, show increased rest after rest deprivation. These results implicate the catabolism of monoamines in the regulation of sleep and waking in the fly and suggest that *Drosophila* may serve as a model system for the genetic dissection of sleep.**

Sleep is ubiquitous in mammals and birds and must serve a fundamental biological function that is as yet unknown (*1*). Both vertebrates and invertebrates often display a prominent circadian organization of rest and activity. But do invertebrates, such as *Drosophila*, sleep? If this were known, powerful genetic tools could be used to investigate sleep mechanisms and functions.

In mammals, sleep is distinguished from inactivity both behaviorally and electrophysiologically. In invertebrates, the identification of sleep-like states depends primarily on the behavioral analysis of quiescence, increased arousal threshold, and increased rest after prolonged waking (a criterion that indicates that rest is under homeostatic control) (*2*). Recently, molecular screening has revealed that sleep and waking also differ in the expression of several neural genes (*3*). We therefore evaluated whether *Drosophila* has sleep-like states by investigating both behavioral and molecular characteristics of its rest-activity cycle.

Continuous, high-resolution measurement of fly behavior (5-day-old virgin females, Canton-S) was achieved with an ultrasound activity monitoring system (*4*). This system detects fine movements of the fly's head, wings, and limbs, in good agreement with visual observation (*5*). Flies subjected to 12 hour:12 hour light/dark cycles exhibited sustained periods of activity and quiescence, with >90% of quiescence (henceforth referred to as rest) occurring during the dark period (Fig. 1A) (*6*). To monitor rest-activity patterns in large numbers of flies, we used an infrared activity monitoring system, which confirmed a robust circadian organization of activity and showed good correspondence with the ultrasound system (*7*).

To determine whether periods of rest are associated with increased arousal thresholds, we subjected flies to vibratory stimuli of increasing intensity [0.05*g* (acceleration), $n = 12$; 0.1*g*, $n = 10$; and 6.0*g*, $n = 8$] (*8*). Flies that had been behaviorally awake readily responded to intensities of 0.05*g* and 0.1*g* (90% of trials). Flies that had been behaviorally quiescent for 5 min or longer rarely showed a behavioral response to these stimuli (<20% of trials; $P <$

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0.001, χ^2). However, when the intensity of the stimulus was increased to 6*g*, all flies quickly responded regardless of behavioral state ($P > 0.1$, χ^2). Thus, like sleep in mammals, sustained periods of quiescence in *Drosophila* are characterized by increased arousal thresholds.

We next investigated whether the amount of rest in *Drosophila* is homeostatically regulated. Flies were deprived of rest individually by gentle tapping for 12 hours during the dark period (i.e., manual rest deprivation). During the following 12-hour light period, flies exhibited a large increase in rest compared to baseline (Fig. 1B). Additionally, an automated system was used to deprive large numbers of flies of rest during the 12-hour dark period, resulting in an increase in rest over baseline values during the first 6 hours of the following light period (Fig. 1B) (*8*). In the first 24 hours after manual rest deprivation, flies recovered 50% of the rest that was lost, a value comparable to the sleep rebound seen in mammals after short-term sleep deprivation.

Recordings with the ultrasound system showed that the rest rebound after deprivation was characterized by actual immobility, as opposed to an increase in stationary waking activities (such as eating or grooming) that may result in reduced infrared beam crossing. Moreover, the increase in rest was not accounted for by levels of prior activity (Fig. 1C). Consistent with this result, when flies were stimulated in the apparatus during the 12-hour light period, rest not only failed to increase, but was actually reduced by 16 ± 4% during the first 6 hours of recovery (Fig. 1D). Thus, the increase in rest is not due to physical exhaustion induced by forced activity (*8*). To investigate whether the homeostatic response is separable from circadian factors, we examined per^{01} mutants (*4*), which are arrhythmic under constant darkness. In the absence of a circadian rest-activity rhythm, per^{01} flies showed a robust homeostatic response after 12 hours of rest deprivation (Fig. 1E). This indicates that, as in mammals, rest is homeostatically regulated and can be dissociated from circadian control (*9*).

In mammals, sleep is prominent in the very young, stabilizes during adolescence and adulthood, and declines during old age (*10*). Rest in *Drosophila* follows a similar pattern. On the first full day after eclosion, the amount of rest was high but declined steadily until day 3, when it reached an adult pattern (Fig. 2A). As the flies aged, the amount of rest during the night declined, and by 33 days of age it was significantly below that found in young adults (Fig. 2B). Several studies indicate that the homeostatic regulation of sleep is preserved in older humans (*10*). When 33-day-old flies were deprived of rest, they exhibited a rest rebound similar to young flies.

Sleep in mammals is modulated by stimulants and hypnotics. For example, caffeine increases waking and motor activity, whereas antihistamines reduce sleep latency (*11*). Flies given caffeine showed a dose-dependent decrease in rest (Fig. 2C). By contrast, hydroxyzine, an antagonist of the H1 histamine receptor, increased rest and reduced its latency (Fig. 2, D and E). Thus, two agents that modulate waking and sleep in mammals also modulate vigilance states in *Drosophila*.

We performed a systematic screening of gene expression in *Drosophila* by using mRNA differential display combined with ribonuclease protection assays (RPA) (*12*). RNA was extracted from whole heads of flies that (i) had been spontaneously resting for 3 hours during the dark period, (ii) had been rest-deprived for 3 hours at the same circadian time, or (iii) had been spontaneously awake for 3 hours during the light period, thereby allowing us to distinguish between changes associated with behavioral state and those associated with circadian time (Fig. 3A) (*13*).

As in the rat (*3*), only ~1% of the transcripts examined in *Drosophila* were modulated by behavioral state (*14*). A transcript whose expression was higher after periods of rest is shown in Fig. 3A ("Rest"). As confirmed using RPA, expression of this mRNA was 45% higher during rest than during rest deprivation. None of the rest-related transcripts matched any published sequence. By contrast, several known genes were expressed at higher levels during waking than during rest, irrespective of circadian time (Fig. 3A, "Waking"). One, with high homology to *Fatty acid synthase* (*Fas*) (*15*), was increased after 3 hours of spontaneous waking or rest deprivation relative to rest (Fig. 3B). This transcript was localized throughout the fly brain, including the optic lobes (Fig. 3C), but not in the eye (*16*). Although the role of this enzyme in the fly brain is unclear, fatty acids are modulators of neural activity (*17*). Cytochrome P450 (*Cyp4e2*), a member of a

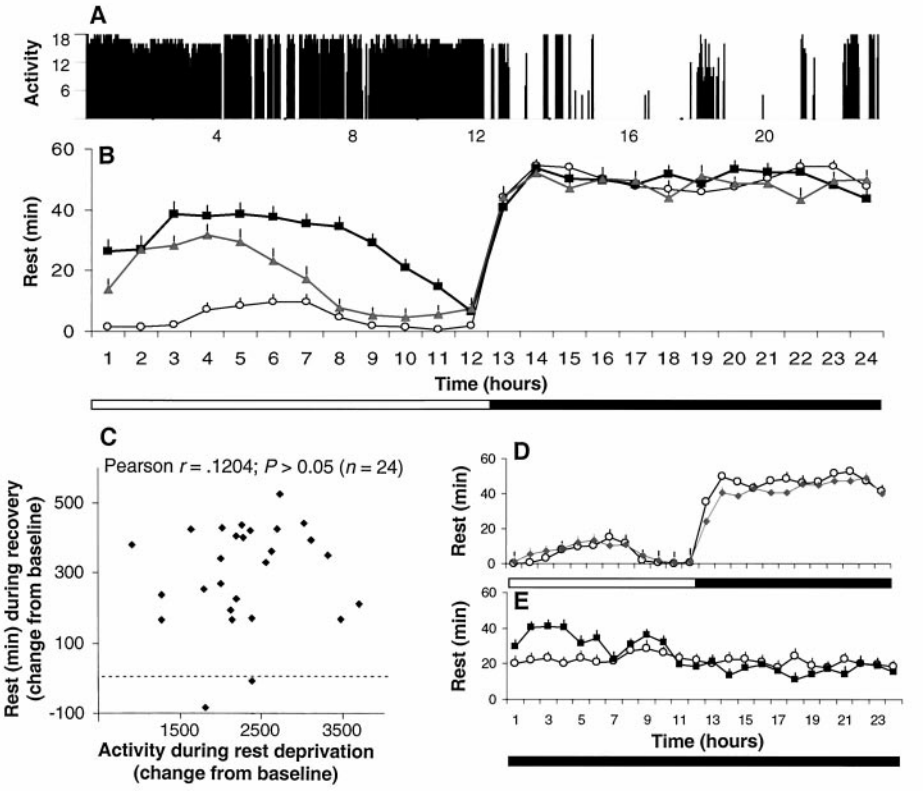

Fig. 1. (**A**) Activity record of flies maintained on a 12 hour:12 hour light (horizontal open bar) /dark (horizontal solid bar) cycle monitored with the ultrasound system. Activity counts indicate the number of perturbations of the ultrasound standing wave detected over 2-s bins. (**B**) The rest-activity cycle monitored with the infrared system (mean ± SEM, $n = 24$). Baseline values are shown in circles. After manual rest deprivation (not shown), flies exhibited a large increase in rest during the subsequent light period (squares; $P < 0.001$; Wilcoxon signed-ranks test). Flies deprived of rest by the automated system also showed an increase in rest during the subsequent light period (triangles; $n = 25$, $P < 0.001$). This finding was replicated in 10 independent experiments ($n = 286$). (**C**) The amount of rest during the 12-hour recovery period was not correlated with the amount of activity during rest deprivation. (**D**) Stimulation of the flies during the light period did not result in a compensatory increase in rest during recovery (diamonds) with respect to baseline (circles). (**E**) Under constant darkness, per^{01} flies had the same amount of rest as under light-dark conditions ($P > 0.05$), but this was evenly distributed across the 24 hours (open circles). Twelve hours of automated rest deprivation resulted in a significant increase in rest during the first 6 hours of recovery (squares) compared to baseline (circles; $n = 25$, $P < 0.001$). Because rest is evenly distributed in per^{01} flies, rest deprivation only eliminated ~50% of daily rest, compared with 90% in wild-type flies.

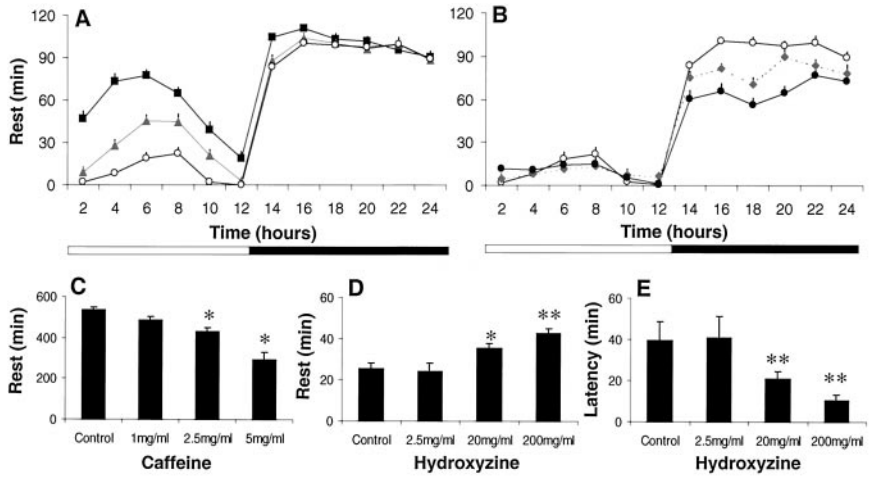

Fig. 2. (**A**) Rest was pronounced during the first full day after eclosion (squares), decreased on day 2 (triangles), and reached adult values by day 3 (circles; $P < 0.001$, ANOVA, Tukey post hoc). The amount of rest remained stable across days 3, 5, and 7 (ANOVA, $P = 0.92$). (**B**) By 16 days of age (diamonds), rest began to decline during the night and was significantly below day 3 values (open circles) by 33 days of age (solid circles; $P < 0.001$). (**C**) Flies given caffeine obtained less rest during the dark period in a dose-dependent fashion ($n = 36$ per dose, $^*P < 0.0001$). Drugs dissolved in food were continuously available beginning in the final hour of the light period. Hydroxyzine, an H1 antagonist, increased the percentage of rest (**D**) and decreased its latency (**E**) during the first hour of the dark period ($n = 40$ per dose; $^*P = 0.056$, $^{**}P < 0.001$). The increase in rest was not associated with an impairment of fly behavior. The activity per waking minute was unchanged during the dark period, including the first hour, as was the total amount of activity during the light period. Responsiveness to arousing stimuli was preserved.

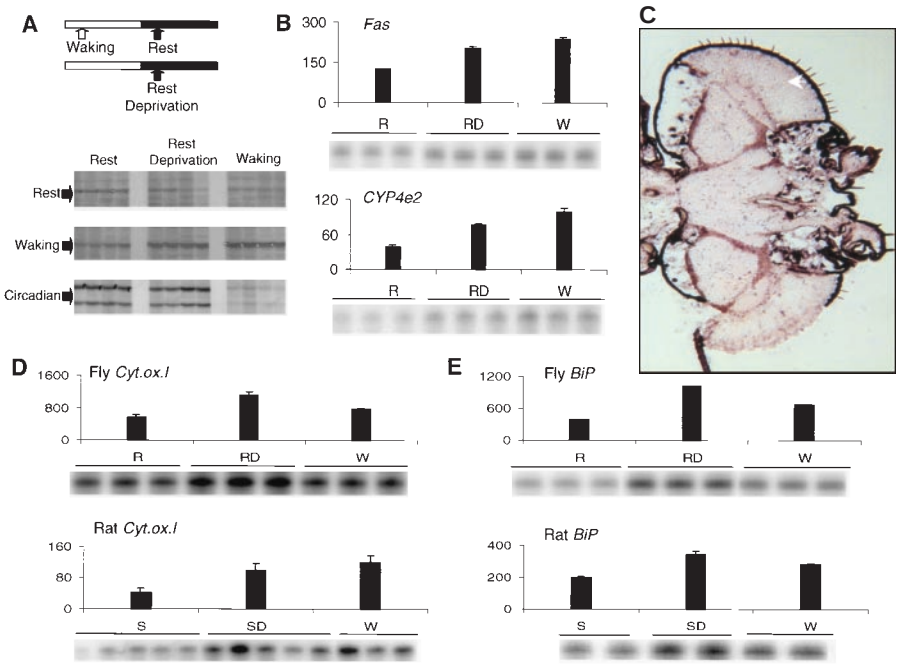

Fig. 3. (**A**) Examples of transcripts identified with differential display that are expressed differentially depending on behavioral state and circadian time. The waking band corresponds to a gene with high homology to *Fas*. (**B**) RPA confirmed the differential display results. Messenger RNA levels of *Fas* and *Cyp4e2* are higher during waking (W) and rest deprivation (RD) compared to rest (R) ($P < 0.01$, ANOVA, Tukey post hoc). Densitometric analysis was performed with a PhosphorImager. (**C**) In situ hybridization shows that *Fas* mRNA is present in the central nervous system but not in the eye (arrow). (**D**) *Cytochrome oxidase C*, subunit I, and (**E**) *BiP* mRNA levels are higher during waking in both fly and rat ($P < 0.01$, ANOVA, Tukey post hoc).

family of detoxifying enzymes, was also increased in waking and rest deprivation relative to rest in the fly (Fig. 3B) (*18*).

Several "waking" genes in the fly corresponded to "waking" genes in the rat. For example, the mitochondrial gene *Cytochrome oxidase C,* subunit I, showed a rapid increase in expression during the first few hours of waking (Fig. 3D), likely a local response of nervous tissue to the increased metabolic requirements of waking (*3*). Another "waking" gene in both *Drosophila* and rat is *BiP* (*Hsc70-3*), an endoplasmic reticulum chaperone protein that may promote the structural changes necessary for the establishment of long-term memory (Fig. 3E) (*19*). Finally, mRNA levels of arylalkylamine *N*-acetyltransferase (*Dat*), an enzyme involved in the catabolism of monoamines (*20*), were increased by 48% after 2 to 3 hours of waking relative to rest. In rats, waking is associated with a marked increase in brain mRNA for arylsulfotransferase, another enzyme implicated in the catabolism of monoamines (*3*). These findings are of importance because waking is associated with high central monoaminergic activity, whereas a reduction of such activity is a hallmark of sleep (*21*). This has led to the suggestion that sleep may serve to counteract the effects of continued monoaminergic discharge. According to this hypothesis, an impaired catabolism of monoamines should result in an increased need for sleep (*22*).

To evaluate this possibility, we examined a *Drosophila* mutant in which the transcriptional level and activity of the *Dat* enzyme is deficient (Dat^{lo}) (*20*). By both infrared and ultrasound measurements, flies homozygous for the Dat^{lo} mutation did not differ from wild-type flies in the percentage and circadian distribution of rest and waking (Fig. 4A) and showed normal amounts and patterns of activity (Fig. 4B). However, after 12 hours of rest deprivation during the dark period, homozygous Dat^{lo} flies displayed a rest rebound that was greater than in rest-deprived controls (Fig. 4C). To confirm that this phenotype maps to the *Dat* locus and to assay for gene dosage effects, we crossed Dat^{lo} homozygotes with flies carrying a deficiency (*Df*) of the *Dat* locus, *Df(2R)Px1* (*20, 23*). The resulting Dat^{lo}/Df flies did not differ from wild-type flies or Dat^{lo} homozygotes in the percentage and circadian distribution of rest and waking (Fig. 4A). Dat^{lo}/Df flies showed not only an increased rest rebound during the first 6 hours of recovery relative to wild-type flies (Fig. 4C), but also a persistent rebound during the second 6 hours of recovery (Fig. 4D). These results indicate that the more severely mutant the fly is at the *Dat* locus, the greater the rebound. Although the mechanisms responsible for the increased homeostatic response to rest deprivation are currently unclear, these results suggest a linkage between the catabolism of monoamines and the regulation of sleep and waking in *Drosophila*.

In conclusion, behavioral, pharmacological, molecular, and genetic investigations indicate that *Drosophila* rest shares many critical features with mammalian sleep. The identification of molecular correlates of sleep and waking that are conserved across evolution offers a new

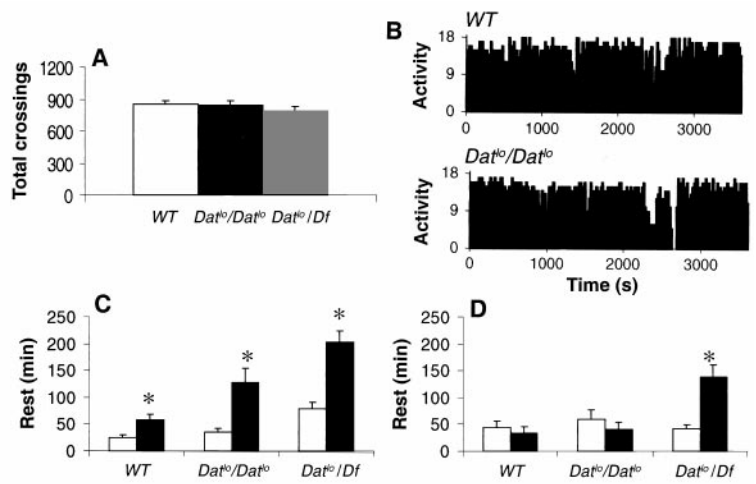

Fig. 4. (**A**) The number of infrared beam crossings per day is similar in wild-type, Dat^{lo}/Dat^{lo}, and Dat^{lo}/Df flies ($P > 0.05$, $n = 25$). (**B**) Activity patterns (ultrasound system, units as in Fig. 1A) are similar in all three *Drosophila* genotypes (two representative records for 1 hour during the light period are shown). (**C**) The amount of rest during the first 6 hours of recovery (solid bars) compared to baseline (open bars) was higher in Dat^{lo}/Dat^{lo} and Dat^{lo}/Df flies than in wild-type flies ($^*P < 0.005$, Wilcoxon test). (**D**) In Dat^{lo}/Df flies, rest rebound persists into the second 6 hours of recovery ($^*P < 0.005$).

approach for studying the phylogeny of sleep. Most important, the demonstration that a mutation modifies the homeostatic regulation of sleep-like states opens the way for gene discovery through mutant screening and validates the use of *Drosophila* as a model system for elucidating the functions of sleep.

Note added in proof: While this paper was in review, another group reported that rest in *Drosophila* is a sleep-like state (*24*).

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4. Flies were cultured at 25°C, 50 to 60% humidity, 12 hour:12 hour light/dark cycle, on yeast, dark corn syrup, and agar food. We obtained per^{01} flies from J. C. Hall (Brandeis University) and Dat^{lo} and $Df(2R)Px1/In(2LR)SM5$, al^2 Cy lt^v sn^2 sp^2 flies from the Bloomington Drosophila Stock Center. For details about the ultrasound monitoring system, see *Science* Online (www.sciencemag.org/feature/data/1047207.shl).
5. Five behaviors were visually scored in 2-s bins by an observer blind to the output of the ultrasound system on 18 independent trials for a total of 8 hours during the light period. The correspondence rates were as follows: locomoting, 99%; inactive, 97%; grooming anterior limbs, 94%; grooming posterior limbs, 98%; and eating, 97%.
6. Rest was defined as uninterrupted behavioral quiescence lasting for at least 5 min.
7. *Drosophila* Activity Monitoring System (Trikinetics) [M. Hamblen *et al*., *J. Neurogenet.* **3**, 249 (1986)]. The system was validated by visual observation for 17.75 hours ($n = 7$). Flies were awake but did not cross the infrared beam in 5 of 213 bins (miss rate = 2.35%).
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13. The behavioral state was determined individually for each fly; only flies that satisfied specific criteria were selected for analysis. A fly was considered awake if it was active for at least 90% of the 3-hour light period and 100% of the hour before killing. A fly was resting if it was inactive for at least 66% of the 3-hour dark period and 100% of the hour before killing. Only about 60 to 70% of the flies examined satisfied these criteria. Failure to specifically identify rest and waking results in samples containing a mixture of behavioral states.
14. An estimated ~5000 RNA species were screened. For additional data, see *Science* Online (www.sciencemag.org/feature/data/1047207.shl).
15. The sequence matched a *Drosophila* P1 clone (AC005554). Analysis using Genescan indicated that the proposed peptide has a 49% homology with rat *Fas*.
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15 November 1999; accepted 8 February 2000

Genetic Suppression of Polyglutamine Toxicity in *Drosophila*

Parsa Kazemi-Esfarjani* and Seymour Benzer

A *Drosophila* model for Huntington's and other polyglutamine diseases was used to screen for genetic factors modifying the degeneration caused by expression of polyglutamine in the eye. Among 7000 P-element insertions, several suppressor strains were isolated, two of which led to the discovery of the suppressor genes described here. The predicted product of one, dHDJ1, is homologous to human heat shock protein 40/HDJ1. That of the second, dTPR2, is homologous to the human tetratricopeptide repeat protein 2. Each of these molecules contains a chaperone-related J domain. Their suppression of polyglutamine toxicity was verified in transgenic flies.

Expanded polyCAG tracts in the genes for Huntington's disease (HD) and at least seven other disorders are associated with hereditary neurodegeneration (*1*). The polyCAGs are translated to polyglutamines, which form cytoplasmic and/or nuclear aggregates and produce toxic effects (*1*, *2*). One approach to the identification of proteins that can modify polyglutamine aggregation and toxicity is the isolation of enhancer and suppressor genes.