Genes that control a temperature-compensated ultradian clock in *Caenorhabditis elegans*

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ABSTRACT Substantial progress has been made in understanding the genetic basis of temperature-compensated circadian clocks. Ultradian rhythms, with a period shorter than 24 h, are at least as widespread as circadian rhythms. We have initiated genetic analysis of defecation behavior, which is controlled by an ultradian clock in *Caenorhabditis elegans*. The defecation motor program is activated every 45 sec, and this rhythm is temperature compensated. We describe mutations in 12 genes that either shorten or lengthen the cycle period. We find that most of these mutations also disrupt temperature compensation, suggesting that this process is an integral part of the clock. These genes open the way for molecular genetic dissection of this ultradian clock.

Rhythmic activities are found universally from single-celled organisms to multicellular eukaryotes (1–6). Genetic and molecular analyses of one type of biological rhythm, the circadian rhythm, have been successfully carried out in several organisms, including cyanobacterium (7), *Drosophila* (2–4), *Neurospora* (2–4), mouse (8), and *Arabidopsis* (9, 10). Circadian clocks have well-defined characteristics (1–4); the 24-h rhythm persists without environmental cues; the rhythm can be entrained by environmental cues; the oscillation phase can be reset by external stimuli, such as light or temperature; and the rhythm is nearly constant at different temperatures (temperature compensation).

Ultradian rhythms, with a period of less than 24 h, are widely observed (5, 6). Some ultradian rhythms, including those of heart pacemaker cells and central pattern generator neurons, have been studied electrophysiologically (5, 11). The courtship song of *Drosophila* varies rhythmically with a period of 50 to 65 sec. This rhythm is temperature-compensated and has been reported to be affected by *per* circadian clock mutations (12). To systematically investigate the genetic basis of an ultradian clock, we studied defecation behavior, which, in the nematode *Caenorhabditis elegans*, is controlled by a temperature-compensated ultradian clock (13). With abundant food, a defecation motor program is activated every 45 sec, the rhythm persists in the absence of motor program activation, the oscillation phase can be reset by sensory stimuli, and the rhythm is nearly constant at temperatures from 19°C to 30°C (13).

Here, we report that the defecation clock can be genetically dissected. We found that mutations in 12 genes cause abnormal defecation cycle periodicity (Dec phenotype). These mutations fall into two major groups: short Dec (Dec-s) and long Dec (Dec-ℓ). Since most Dec mutations affect the cycle period differently at different temperatures, it is likely that these mutations affect temperature compensation rather than causing thermolabile gene products. This nematode defecation system is an excellent genetic model for studying an ultradian clock and temperature compensation.

MATERIALS AND METHODS

Genetics. All strains were derivatives of the Bristol *C. elegans* strain N2, and maintenance and handling of these strains were described previously (14). This paper follows the standard *C. elegans* nomenclature (15). To isolate *dec* mutants, young adult N2 hermaphrodites were treated with 50 mM ethyl methanesulfonate (14). Five mutagenized animals were picked and placed on a fresh 60-mm plate seeded with *Escherichia coli*. After these parents laid 20–30 eggs each, the parents were removed from the plate. When the F_1 progeny became young adults, they were transferred to fresh 60-mm plates seeded with *E. coli* (five animals per plate). After these five animals laid 20–30 eggs each, the F_1 animals were removed. From each plate, five F_2 progeny at the young adult stage were briefly observed by using a dissecting microscope. If an individual showed a potentially abnormal defecation cycle period, either shorter than 36 sec or longer than 72 sec, it was picked and placed on a fresh plate as a mutant candidate. Progeny from such candidates were examined more extensively. A total of 7000 F_2 progeny of 7000 F_1 animals were screened, a number equivalent to approximately 3000 mutagenized haploid genomes (16). Some mapping results for newly isolated genes are summarized below. A complete set of map data has been submitted to the *Caenorhabditis* Genetic Center and to the *C. elegans* data base (ACeDB). To avoid marker effects, recombinants with markers were crossed with Dec males and cycles were scored in cross progeny.

All Dec-ℓ mutants grew slowly. This slow-growing phenotype was used for mapping and in each case was confirmed to be linked to the Dec-ℓ mutation. *dec-2* maps at the extreme right of chromosome III, to the right of *unc-25*. A total of 163 slow-growing progeny were picked from *dpy-18(e364) unc-25(e156)/dec-2*: 126/163 segregated no Dpy and no Unc, 4/163 segregated Dpy Unc, and 33/163 segregated Dpy non-Unc. *dec-4* maps between *unc-5* and *dpy-20* on chromosome IV. A total of 89 slow-growing progeny were picked from *unc-5(e53) dpy-20(e1282)/dec-4*: 79/89 segregated no Unc and no Dpy, 3/89 segregated Unc non-Dpy, and 7/89 segregated Dpy non-Unc. *dec-7* maps between *unc-32* and *unc-47* on chromosome III: from *unc-32(e189) dpy-18(e364)/ dec-7*, 2/8 Unc non-Dpy recombinants segregated Dec-s and 4/5 Dpy non-Unc animals segregated Dec-s; from *sma-2(e502) unc-47(e307)/dec-7*, 2/7 Unc non-Sma recombinants segregated Dec-s and 2/3 Sma non-Unc animals segregated Dec-s. *dec-9* maps between *daf-14* and *dpy-20* on chromosome -IV: from *unc-24(e138) daf-14(m77) dpy-20(e1362)/dec-9*, 8/8 Unc non-Daf non-Dpy recombinants segregated Dec-s and 1/3 Unc Daf-c non-Dpy recombinants segregated Dec-s. *dec-10* maps between *unc-115* and *egl-15* on chromosome X: from *unc-115(e2225) egl-15(n484)/dec-10*, 1/2 Unc non-Egl recombinants segregated Dec-s and 3/4 Egl non-Unc recombinants segregated Dec-s. *dec-11* maps

Abbreviations: Dec, abnormal defecation cycle periodicity; Dec-s, short Dec; Dec-ℓ, long Dec; pBoc, posterior body-wall muscle contraction; aBoc, anterior body-wall muscle contraction; Exp, expulsion.
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1 intercycle
2 pBoc
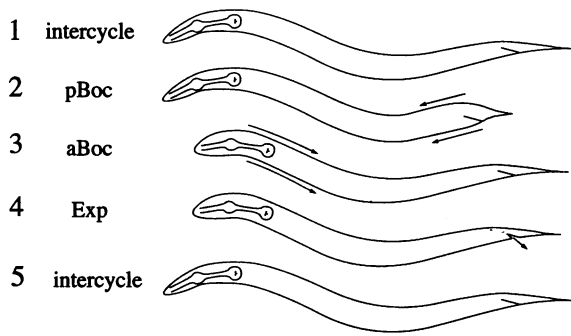3 aBoc
4 Exp
5 intercycle

FIG. 1. Schematic drawing of the defecation motor program.

on the right arm of chromosome IV: from *dpy-13(e184) unc-30(e191)/dec-11*, 2/22 slow growers segregated Dpy non-Unc and 2/4 non-slow growers segregated Unc non-Dpy; from *unc-26(e205) egl-23(n601sd)/dec-11*, 9/9 non-Egl non-slow growers segregated non-Unc. *dec-12* maps left of *dpy-5* and right of *bli-3* on chromosome I: from *dpy-5(e61) unc-75(e950)/dec-12*, 39 slow-growing progeny were picked and 24/39 segregated non-Unc and non-Dpy, 8/39 segregated Dpy Unc, and 7/39 segregated Unc non-Dpy; from *bli-3(e767) lin-17(n677)/dec-12*, 19/33 slow-growing progeny segregated non-Bli non-Lin, 14/33 segregated Bli (Lin phenotype was difficult to score in the *bli-3* background). *flr-1* X and *flr-4* IV: the *sa96* and *sa201* mutations were mapped to the regions of the *flr-1* and *flr-4* genes, respectively (data not shown). The *sa96* and *sa201* mutants grew slowly and were fluoride resistant. We found that *flr-1(ut11)* and *flr-4(ut7)* had short defecation cycles (17) and failed to complement *sa96* and *sa201*, respectively.

Behavioral Analysis. Animals were grown at a constant temperature and were scored at young adult stage by using a dissecting microscope kept at the same temperature. The temperatures 20°C and 25°C were chosen because we had the facility to both grow and observe animals at each of these temperatures. Individual animals were observed for a fixed number of cycles, and behavior was recorded by using a computer program as described (13). The anterior body-wall muscle contraction (aBoc) step was not recorded in most observations to accurately measure the interval between the posterior body-wall muscle contraction (pBoc) and expulsion (Exp) steps. A cycle period was defined as the interval between the initiation of two successive pBoc steps. When the periodicity of an ethogram was obvious, the cycle period was based directly on the ethogram. When a periodicity was not obvious because of frequent skipping of motor program steps, periodicities were calculated by using the MESA program (18), which calculates the best-fit periodicity for such records.

Strain Constructions. *mDf7/dec-9* heterozygotes were constructed as follows. *unc-24 dec-9/+ +* heterozygous males were mated with *mDf7 dpy-13/nT1* hermaphrodites. F_1 animals that segregated Unc progeny and rare Dpy progeny were inferred to be *unc-24 dec-9/mDf7 dpy-13*, and non-Unc non-Dpy F_2 progeny were scored for defecation cycle periods. The presence of *mDf7* was confirmed by complementation testing with *daf-14(m77) dpy-20(e1362)*. Construction of *mDf7/+* heterozygotes was identical, except that *unc-24/+* males were used to initiate the cross. All strains for *dec-9* contained + *unc-24/dpy-13* +. *nDf19/dec-10* and *nDf19/+* heterozygotes were constructed in an analogous fashion by mating *lon-2(e678) dec-10* or *lon-2* males with *nDf19/lon-2 szT1* hermaphrodites. The presence of *nDf19* was confirmed by complemen-

A wild type

```
            10        20        30        40
...................p...x...................
..................p...x....................
..................p....x...................
...................p...x...................
..................p...x....................
.................p....x....................
.................p....x....................
.................p...x.....................
.................p...x.....................
.................p....x....................
.............p...........................
.....................p....x....stop
```

B *flr-1*

```
                10
.............
..............
.........p.x..
..............
.p..........
.....p......
.....p......
.......p...
.........p...
.........p...
.............
..p.....x.....
..............
p.....x...stop
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C *dec-7*

```
            10        20        30
...............p...x...............
................p....x.............
...............p...x..............
...............p..................
...............p....x.............
................p...x.............
...............p....x.............
................p...x.............
.................p...x...........
................p....x.......stop
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D *dec-2*

```
         10        20        30        40        50        60        70        80
..........................................p.....x.................................
..........................................p....x.................................
.........................................p.....x................................
.........................................p.....x................................
..........................................p...x.................................
...........................................p.....x..............................
.........................................p.....x................................
.........................................p....x.................................
.........................................p....x.................................
....................................................p.....x.......stop
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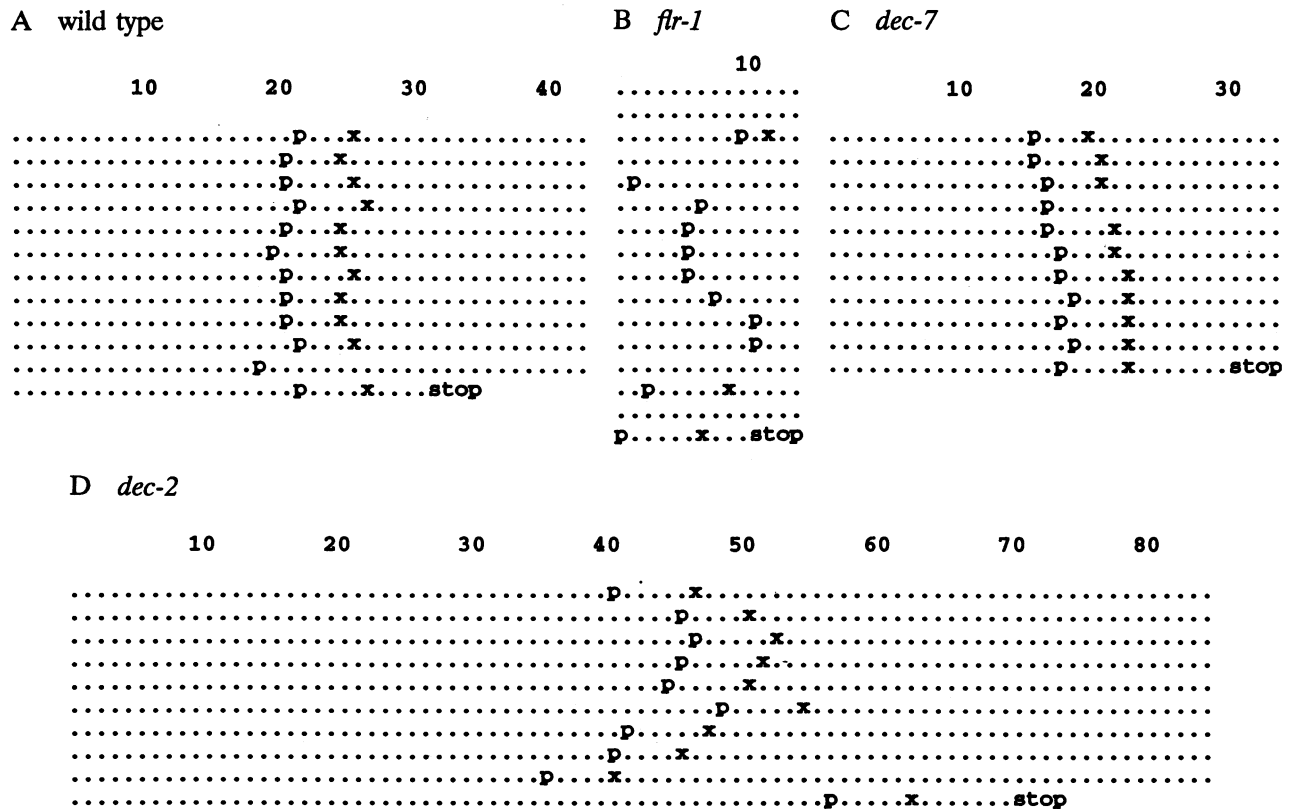
FIG. 2. Ethograms of defecation behavior in the wild type and some *dec* mutants. Each dot or character represents 1 sec. Seconds elapsed are indicated above each ethogram. p and x represent pBoc and Exp, respectively. The aBoc step was omitted from each record for clarity. (*A*) Wild type at 20°C. The mean cycle period in this assay was 43.1 sec, with an SD of 1.4 sec. (*B*) *flr-1(sa96)* at 20°C. The best-fit mean cycle period was 15.2 sec, as calculated by MESA (18) (see Fig. 4). (*C*) *dec-7(sa296)* at 20°C. The mean cycle period was 34.3 ± 0.7 sec. (*D*) *dec-2(sa89)* at 20°C. The mean cycle period was 86.7 ± 7.7 sec.

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tation testing with egl-15(n484). All strains for dec-10 contained lon-2/+.

RESULTS AND DISCUSSION

The C. elegans defecation motor program consists of a stereotyped series of three muscle contractions (19) (Fig. 1). First, posterior body-wall muscles contract and then relax, causing the gut contents to accumulate near the anus. Three to four seconds later, anterior body-wall muscles contract to pressurize the gut contents. Finally, specialized enteric muscles contract to expel the contents from the anus. These motor program steps were recorded and displayed as an ethogram (Fig. 2) by using a computer program (13).

To investigate how the defecation cycle is genetically controlled, we screened for mutations that can cause Dec phenotype (Fig. 3). A previous genetic screen for defecation mutants with a constipated phenotype identified a single Dec gene,
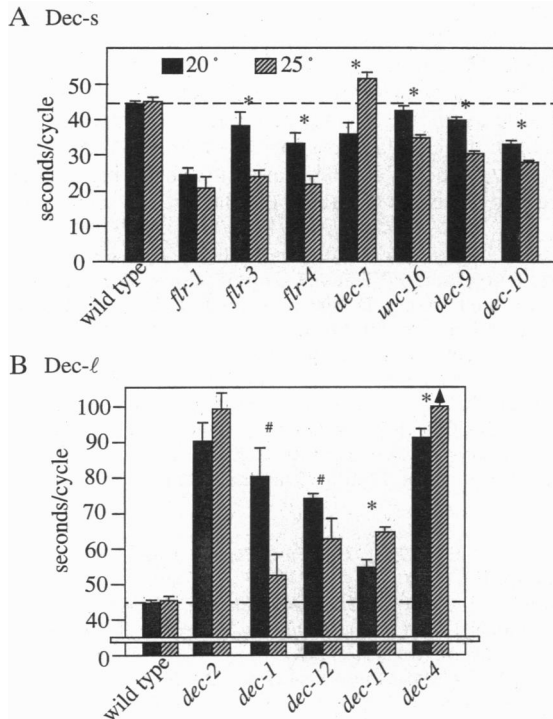

FIG. 3. Mean defecation cycle periods of the wild type and dec mutants at 20°C and 25°C. Genes are shown below each bar. The y axis shows mean cycle period (seconds/cycle). The standard error of the mean is indicated at the top of each bar. * indicates significant difference between the means at 20°C and 25°C for a given mutant (P < 0.01 when using the two-tailed t test and the Mann–Whitney test). # indicates a marginal significance (P < 0.05 when using the Mann–Whitney test for data sets deviating from the normal distribution). The horizontal dashed line in each panel shows the wild-type mean at 20°C. At least 19 wild-type animals were observed for at least 10 cycles each at each temperature. For each mutant, at least 10 animals were observed for 10 cycles each at each temperature. (A) Comparison among the wild type and Dec-s mutants. Gene names and alleles are as follows: flr-1(sa96), flr-3(ut9), flr-4(sa201), dec-7(sa296), unc-16(e109), dec-9(sa293), and dec-10(sa294). For second alleles, five animals were observed for five cycles each. Mean periods (seconds/cycle) of the second alleles are as follows: flr-1(ut11), 45.8 ± 1.3 at 20°C and 23.4 ± 2.2 at 25°C; flr-3(ut8), 22.6 ± 2.2 at 20°C and 30.5 ± 5.8 at 25°C; flr-4(ut7), 50.4 ± 7.8 at 20°C and 24.5 ± 2.8 at 25°C; dec-7(sa92), 32.6 ± 1.0 at 20°C and 49.9 ± 5.9 at 25°C; and unc-16(n730), 44.3 ± 1.0 at 20°C and 36.4 ± 1.5 at 25°C. (B) Comparison among the wild type and Dec-ℓ mutants. The y axis is truncated between 0 and 40 seconds/cycle. Gene names and alleles are as follows: dec-2(sa89), dec-1(sa48), dec-12(sa295), dec-11(sa292), and dec-4(sa73). The arrow on the dec-4 mutant at 25°C indicates rare activation of the motor program (see text).

dec-1 (19). However, most such mutations do not strongly affect defecation periodicity (19). Therefore, we employed direct cycle observation in our Dec screen. We identified mutations in 12 genes that can mutate to cause a Dec phenotype. Mutations in these genes fall into two major groups, Dec-s and Dec-ℓ.

Mutations in the flr-1, flr-3, or flr-4 genes, previously identified on the basis of a fluoride-resistant phenotype (17), resulted in Dec phenotypes very similar to one another. Our screen identified one allele each of the flr-1 and flr-4 genes, prompting us to examine mutations in the phenotypically very similar flr-3 gene (17). Two alleles of each flr gene were examined in detail for the Dec phenotype (see Fig. 3 legend). Mutations in each gene were recessive and caused a very short mean cycle period, especially pronounced at 25°C (Figs. 2B and 3A). We found that the shorter the cycle period, the less frequently the Exp step was activated (Fig. 4). We also noticed that pBoc clearly became weaker as the cycle period shortened, though this change was more difficult to measure. These observations suggest depletion of an important factor by a high frequency of motor program activation. It is unlikely that missing the Exp step causes short cycles, because previously isolated aex and exp mutants (Exp defective, see ref. 19) do not have such short cycles. We also examined mutants for the other flr genes, flr-2 and flr-5, and found no Dec phenotypes in these mutants (data not shown).

Mutations in two other genes, dec-7 and unc-16, were recessive and caused moderately short cycle periods (Fig. 2C). Two mutations in the dec-7 gene were identified in our screen and both caused the same temperature-sensitive phenotype (Fig. 3A). These dec-7 mutations were isolated at a frequency expected for strong loss-of-function mutations (14). It is unlikely that mutations causing thermolabile products would be generated at this frequency, since specific base changes are necessary to create thermolabile products. We conclude that temperature-sensitivity is characteristic of mutations in this gene and not due to a thermolabile dec-7 gene product. Similarly, two mutations in unc-16, previously identified on the basis of uncoordinated locomotion and egg-laying-defective
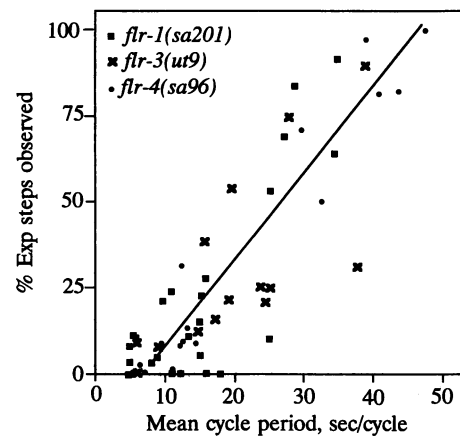

FIG. 4. Correlation between mean cycle period and presence of the Exp step in the flr-1, flr-3, and flr-4 mutants. Most periodicities plotted on this graph were calculated by using the MESA program (18) since flr mutants frequently skipped motor program steps (see Fig. 2). The x axis shows mean cycle periods for individual animals (seconds/cycle) and the y axis shows the frequency of Exp steps observed [100 × (number of Exp steps observed/number of motor programs predicted on the basis of the cycle period)]. To assure accuracy in observation of pBoc and Exp steps, we intentionally did not observe aBoc steps. When no motor programs were skipped and the periodicity of an ethogram was obvious, the cycle period was determined directly from the ethogram. In 13 cases, data were omitted from the graph because no MESA correlation peak higher than a spectrum value of 100 was detected over 5 sec.
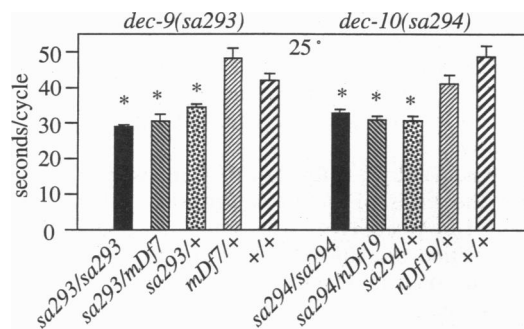
FIG. 5.   Dominance of the *dec-9(sa293)* and *dec-10(sa294)* mutations. The relevant genotype of each strain is shown below each bar. The *y* axis shows the mean defecation cycle period (seconds/cycle). All strains were grown and scored at 25°C. For each strain, at least six animals were scored for at least five cycles each. * indicates a significant difference between the wild type and a given mutant strain ($P < 0.01$ when using the two-tailed *t* test). The difference in the mean period between the *sa293* and *sa293/+* strains is statistically significant ($P < 0.01$ when using the two-tailed *t* test). *mDf7* and *nDf19* delete the *dec-9* and *dec-10* genes, respectively, on the basis of their genetic map positions. Mean cycle periods (± SEM) (seconds/cycle) of *dec-9* mutant strains are as follows: *sa293/sa293*, 28.8 ± 0.8; *sa293/mDf7*, 30.3 ± 1.8; *sa293/+*, 34.7 ± 0.9; *mDf7/+*, 47.8 ± 2.9; and *+/+*, 42.3 ± 1.7. Mean cycle periods for *dec-10* mutant strains are as follows: *sa294/sa294*, 32.7 ± 0.9; *sa294/nDf19*, 31.0 ± 0.7; *sa294/+*, 31.0 ± 0.9; *nDf19/+*, 41.4 ± 2.3; and *+/+*, 48.6 ± 2.7.

phenotypes (14, 19), caused temperature-sensitive defecation cycle period defects (Fig. 3*A*). In contrast, their uncoordinated phenotype is not temperature sensitive (14, 19), suggesting that the temperature sensitivity of their Dec phenotype also is not due to a thermolabile gene product.

Single dominant Dec-s mutations in each of two genes (*dec-9* and *dec-10*) were identified. The *dec-9(sa293/+)* heterozygote had a cycle period intermediate between the homozygote and the wild type (Fig. 5). Since a heterozygous deficiency of *dec-9* (*mDf7/+*) showed a normal cycle period, we conclude that *dec-9(sa293)* is a gain-of-function mutation (Fig. 5). Similarly, the *dec-10(sa294)* mutation was dominant and of the gain-of-function type (Fig. 5). Both *dec-9* and *dec-10* mutations affected cycle periods less severely at 20°C (Fig. 3*A*).

All five identified Dec-ℓ mutations were recessive, and each Dec-ℓ gene was defined by a single mutation. Unlike most Dec mutants, the *dec-2* mutant did not show a temperature-sensitive phenotype (Figs. 2*D* and 3*B*). The Dec-ℓ phenotypes of *dec-1* (19) and *dec-12* were cold sensitive, while those of *dec-11* and *dec-4* were heat sensitive (Fig. 3*B*). At 25°C the defecation motor program was rarely initiated in the *dec-4* mutant: 5 of 10 animals observed had no motor program at all during 10 min of observation each (Fig. 3*B*). The recently reported *clk-1* mutant, among its many pleiotropies, also has a Dec-ℓ phenotype (20).

In general, biochemical reactions depend upon temperature, occurring faster as temperature increases. However, the defecation cycle period remains constant over a range of temperatures in the wild type (13). This fact implies that a specific mechanism compensates the cycle period at different temperatures. Most *dec* mutants had temperature-dependent cycle periods. The high frequency of temperature-dependent *dec* mutations implies that their temperature-sensitive phenotypes do not result from thermolabile gene products but that these *dec* genes are integral parts of both the clock mechanism itself and the temperature-compensation process. We suggest that temperature compensation is an intrinsic feature of the clock mechanism rather than a separate circuit that regulates cycle periodicity. In *Drosophila* and *Neurospora*, mutations in the *per* and *frq* genes cause temperature-compensation defects in addition to abnormal circadian clock rhythms (21–26), suggesting that this clock oscillation is also integrated with temperature compensation.

The cellular basis for some ultradian oscillators has been investigated by electrophysiological methods. There are two major types of oscillators: the neural-network type and the single-neuron type. For example, a network oscillator is found in the central pattern generator for the sea slug (*Tritonia*) escape swimming (27), while a single-neuron type is seen for lobster (*Panulirus interruptus*) pyloric movements (28, 29). When individual cells or groups of cells in the network are perturbed, network oscillators often retain rhythmicity with altered periodicity, suggesting considerable redundancy in function. Thus far, we have isolated no mutations that strongly disrupt the defecation rhythm, including those in previous screens for a constipated phenotype (19); instead all *dec* mutants maintain rhythmicity with altered cycle periods. In the previous screens, many severely constipated mutants with no active expulsions were isolated, suggesting that mutants that strongly disrupt the rhythm should be viable (19). These observations suggest that the nematode defecation rhythm is controlled by redundant pathways, perhaps by a neural network. Genetic mosaic analysis of *dec* mutations and laser-ablation of candidate rhythm-generating neurons can be used to establish the cellular basis for this rhythm. Thus, nematode defecation can be an excellent model for studying the cellular and genetic basis for ultradian oscillators.

1.   Bunning, E. (1973) *The Physiological Clock* (Springer, New York), Ed. 3.
2.   Dunlap, J. C. (1993) *Annu. Rev. Physiol.* **55,** 683–728.
3.   Edmunds, L. N. (1988) *Cellular and Molecular Bases of Biological Clocks* (Springer, New York).
4.   Hall, J. C. (1990) *Annu. Rev. Genet.* **24,** 659–697.
5.   Dekin, M. S. & Haddad, G. G. (1990) *J. Appl. Physiol.* **69,** 809–821.
6.   Rapp, P. E. (1979) *J. Exp. Biol.* **81,** 281–306.
7.   Kondo, T., Tsinoremas, N. F., Golden, S. S., Johnson, C. H., Kutsuna, S. & Ishiura, M. (1994) *Science* **266,** 1233–1236.
8.   Vitaterna, M. H., King, D. P., Chang, A.-M., Kornhauser, J. M., Lowrey, P. L., McDonald, J. D., Dove, W. F., Pinto, L. H., Turek, F. W. & Takahashi, J. S. (1994) *Science* **264,** 719–725.
9.   Millar, A. J., Carr'e, I. A., Strayer, C. A., Chua, N. H. & Kay, S. A. (1995) *Science* **267,** 1161–1163.
10.   Millar, A. J., Straume, M., Chory, J., Chua, N. H. & Kay, S. A. (1995) *Science* **267,** 1163–1166.
11.   Reuter, H. (1984) *Annu. Rev. Physiol.* **46,** 473–484.
12.   Kyriacou, C. P. & Hall, J. C. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 6729–6733.
13.   Liu, D. W. C. & Thomas, J. H. (1994) *J. Neurosci.* **14,** 1953–1962.
14.   Brenner, S. (1974) *Genetics* **77,** 71–94.
15.   Horvitz, H. R., Brenner, S., Hodgkin, J. & Herman, R. K. (1979) *Mol. Gen. Genet.* **175,** 129–133.
16.   Ellis, R. E. & Horvitz, H. R. (1991) *Development (Cambridge, U.K.)* **112,** 591–603.
17.   Katsura, I., Kondo, K., Amono, T., Ishihara, T. & Kawakami, M. (1994) *Genetics* **136,** 145–154.
18.   Dowse, H. B. & Ringo, J. M. (1989) *J. Theor. Biol.* **139,** 487–515.
19.   Thomas, J. H. (1990) *Genetics* **124,** 855–872.
20.   Wong, A., Boutis, P. & Hekimi, S. (1995) *Genetics* **139,** 1247–1259.

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Proc. Natl. Acad. Sci. USA 92 (1995)   10321

21. Loros, J. J. & Feldman, J. F. (1986) *J. Biol. Rhythms* **1,** 187–198.
22. Konopka, R. J., Pittenrigh, C. & Orr, D. (1989) *J. Neurogenet.* **6,** 1–10.
23. Ewer, J., Hamblen-Coyle, M., Rosbash, M. & Hall, J. C. (1990) *J. Neurogenet.* **7,** 31–73.
24. Konopka, R. J., Hamblen-Coyle, M. J., Jamison, C. F. & Hall, J. C. (1994) *J. Biol. Rhythms* **9,** 189–216.
25. Aronson, B. D., Johnson, K. A. & Dunlap, J. C. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 7683–7687.
26. Huang, Z. J., Curtin, K. D. & Rosbash, M. (1995) *Science* **267,** 1169–1172.
27. Getting, P. A. (1983) *J. Neurophysiol.* **49,** 1017–1035.
28. Miller, J. P. & Selverston, A. I. (1982) *J. Neurophysiol.* **48,** 1378–1391.
29. Marder, E. (1984) *J. Exp. Biol.* **112,** 147–167.