Copyright © 1997 by the Genetics Society of America

The Mouse *Clock* Mutation Behaves as an Antimorph and Maps Within the W^{19H} Deletion, Distal of *Kit*

David P. King,*,[1] Martha Hotz Vitaterna,*,[1] Anne-Marie Chang,* William F. Dove,† Lawrence H. Pinto,* Fred W. Turek* and Joseph S. Takahashi*

**National Science Foundation Center for Biological Timing, and Department of Neurobiology and Physiology, Northwestern University, Evanston, Illinois 60208-3520 and †McArdle Laboratory for Cancer Research and Laboratory of Genetics, University of Wisconsin, Madison, Wisconsin 53706*

Manuscript received September 10, 1996
Accepted for publication March 21, 1997

ABSTRACT

Clock is a semidominant mutation identified from an *N*-ethyl-*N*-nitrosourea mutagenesis screen in mice. Mice carrying the *Clock* mutation exhibit abnormalities of circadian behavior, including lengthening of endogenous period and loss of rhythmicity. To identify the gene affected by this mutation, we have generated a high-resolution genetic map (>1800 meioses) of the *Clock* locus. We report that *Clock* is 0.7 cM distal of *Kit* on mouse chromosome *5*. Mapping shows that *Clock* lies within the W^{19H} deletion. Complementation analysis of different *Clock* and W^{19H} compound genotypes indicates that the *Clock* mutation behaves as an antimorph. This antimorphic behavior of *Clock* strongly argues that *Clock* defines a gene centrally involved in the mammalian circadian system.

FORWARD genetics has been a powerful tool for understanding the mechanism of circadian rhythms (HALL 1990; DUNLAP 1993; TAKAHASHI 1995). Mutagenesis screens have led to the molecular characterization of essential clock genes in both *Drosophila melanogaster* and *Neurospora crassa*. In Drosophila, two genes that are central to the circadian clock, *period* (*per*) and *timeless* (*tim*), have been identified as the result of ethyl methane sulfonate and transposable *P*-element mutagenesis screens, respectively (KONOPKA and BENZER 1971; SEHGAL *et al.* 1994). Similarly, the *frequency* (*frq*) gene was identified following a nitrosoguanidine mutagenesis screen in Neurospora (FELDMAN and HOYLE 1973). All three of these genes were subsequently cloned and the mutations causing the aberrant circadian phenotypes have been identified (BAYLIES *et al.* 1987; YU *et al.* 1987; MCCLUNG *et al.* 1989; MYERS *et al.* 1995). Because orthologues (DICKINSON 1995) of these genes have not been found in mammals, and because other strategies to identify mammalian clock genes have not yet been successful, we initiated an ENU (*N*-ethyl-*N*-nitrosourea) mutagenesis screening strategy to isolate clock mutations in the mouse (TAKAHASHI *et al.* 1994). We identified a mutation, which we designated *Clock*, that has several effects on the circadian behavior of mice (VITATERNA *et al.* 1994). *Clock*, which segregates as a single autosomal locus and behaves as a semidominant mutation, lengthens the period of the circadian rhythm by ~1 hr in *Clock*/+ heterozygotes. In *Clock*/*Clock* homozygotes, period lengthens by ~4 hr upon initial transfer to constant darkness, after which these mice lose persistent circadian rhythms.

As an initial step toward the molecular identification of the *Clock* gene, we have constructed a high-resolution genetic map of the *Clock* region of mouse chromosome *5*, using meioses from six intraspecific and two interspecific crosses. By linkage analysis, we have mapped *Clock* just distal (0.7 cM) of the *Kit* (*W, Dominant white spotting*) locus. This region of mouse chromosome *5* contains a cluster of three receptor tyrosine kinase genes: *Pdgfra* (platelet derived growth factor receptor, alpha subunit), *Kit*, and *Flk1* (fetal liver kinase) (GEISSLER *et al.* 1988b; MATTHEWS *et al.* 1991; SMITH *et al.* 1991; STEPHENSON *et al.* 1991; BRUNKOW *et al.* 1995). We have used simple sequence length polymorphism (SSLP) content to map the extent of two deletion mutations of the *Kit* region, W^{19H} and *Patch* (*Ph*), which has allowed us to place *Clock* within the W^{19H} deletion, but distal to the *Ph* deletion. Complementation analyses involving these two deletion mutants and *Clock* provide results consistent with the genetic and deletion mapping of this region of chromosome *5*. Based on the phenotypes of various *Clock* and W^{19H} compound genotypes, we conclude that *Clock* behaves as an antimorph, one type of dominant negative mutation. The mutant allele thus competes with the wild-type allele of the gene in the generation of circadian rhythms, providing strong evidence that the *Clock* gene is an essential component of the mammalian circadian clock system.

MATERIALS AND METHODS

Source of animals: All *Clock* mutant mice used in this study were bred in our colony. These mice were all derived from a

Corresponding author: Joseph S. Takahashi, Department of Neurobiology and Physiology, Northwestern University, 2153 North Campus Dr., Evanston, IL 60208-3520. E-mail: j-takahashi@nwu.edu

[1] These authors contributed equally to this work.

C57BL/6J (B6) founder animal (no. 25) born at the University of Wisconsin (VITATERNA *et al.* 1994). Mice of the following strains were obtained from the Jackson Laboratory: BALB/cJ, CAST/Ei, C57BL/6J, C3H/HeJ, C3HeB/FeJ, C57BL/6J-*Ph*, WBB6F1/J, and WBB6F1/J-*Kit*^{W}/*Kit*^{Wv}. J. BARKER (The Jackson Laboratory, Bar Harbor, ME) provided mice of the C57BL/6J-*rs/rs* and C3HeB/FeJ-*W*^{19H}/+ strains. Before behavioral testing, animals were maintained on a light:dark cycle of 12 hr light:12 hr dark (LD12:12). All of the animal care and experimental treatments were performed in accordance with institutional guidelines at Northwestern University.

Phenotyping of circadian behavior: Animals were phenotyped essentially as described (VITATERNA *et al.* 1994). Briefly, mice were individually housed in cages equipped with running wheels within ventilated, light-tight chambers with timer-controlled lighting. Activity data (wheel revolutions) were collected using an on-line PC computer system (Stanford Software Systems, Chronobiology Kit). Mice were kept for at least 1 week on an LD12:12 light:dark cycle. Transfer to constant darkness (DD) was accomplished by allowing the lights to go out at the usual time and then leaving them off on the following day. The mice remained in DD for at least 3 weeks, during which time the circadian behavior of locomotor activity was recorded.

For analysis of "steady-state" circadian period, a 10-day interval in which the circadian period was stable during exposure to DD was examined. Period was estimated from this interval with the Stanford Software Systems Chronobiology Kit using the chi-square periodogram program.

Genetic mapping crosses: Animals used for genetic mapping of the *Clock* locus were obtained from eight crosses, described in Table 1. The *Clock* mutation originated on a B6 background and was backcrossed to wild-type B6 mice for four generations (N_4) to reduce the number of ENU-induced mutations unlinked to *Clock*. BALB/cJ (BALB) was used as a counterstrain. Intraspecific backcross and intercross animals were generated in the following manner. Male B6 mice heterozygous for the *Clock* mutation, from either the N_2 or N_4 generation following mutagenesis, were crossed to wild-type female BALB mice to generate F_1 progeny. These animals were then phenotyped for circadian behavior. The F_1 mice (of both sexes) that carried the *Clock* mutation were then either backcrossed to wild-type B6 mice or intercrossed to generate progeny for genetic mapping. Interspecific backcross animals were generated using the same backcross strategy described above and *Mus castaneus* mice of the strain CAST/Ei.

Genotyping and linkage analysis: High-molecular weight genomic DNA was extracted from liver tissue by standard proteinase K digestion and phenol/chloroform extraction methods, using protocols adapted from AUSUBEL *et al.* (1995). High molecular weight genomic DNA was also extracted from tail tips, using an adaptation of a proteinase K digestion and phenol/chloroform extraction protocol (kindly provided by J. KORNHAUSER).

Clock was mapped using simple sequence length polymorphisms (SSLPs) (COPELAND *et al.* 1993; DIETRICH *et al.* 1994, 1996) obtained from Research Genetics Inc. SSLP genotyping methods were modified from those described by DIETRICH *et al.* (1992). Briefly, PCR reactions were carried out in 10 μl volumes, using ~25 ng (liver) or ~40 ng (tail tip) template DNA in 5 μl dd$H_2$0, 0.25 units of Amplitaq DNA (Perkin Elmer), 200 nM each dNTP, 0.85× GeneAmp PCR buffer II (1× buffer: 50 mM KCl, 10 mM Tris-HCl pH 8.3), 1 μg/μl BSA, and 1.275 mM MgCl. One hundred ten nanomolar of each primer was used, with all of the forward primer aliquot for each reaction labeled with [γ-^{32}P]ATP (specific activity 6000 Ci/mmol, Du Pont/NEN), using T4 polynucleotide kinase. PCR reactions were carried out on either a 96- or 192-well PTC-100 thermal cycler (MJ Research). The thermocycling profile was as follows: 94° for 3 min, followed by 27 cycles of 94° for 15 sec, 55° for 2 min, and 72° for 2 min, which was then followed by a single extension step at 72° for 7 min. PCR products were separated on 7% denaturing acrylamide sequencing gels and visualized by autoradiography.

After genotyping several hundred mice ($N = 527$) that had been phenotyped first, the mapping process was accelerated by *first* genotyping mice, using two pairs of SSLPs that flank *Clock* (in duplex reactions that included both a proximal and distal SSLP, usually *D5Mit135* with *D5Mit114,* and *D5Mit235* with *D5Mit236*), with DNA extracted from tail tips. Mice with chromosomes recombinant within this interval were selected for behavioral testing and were also selected for high resolution genetic mapping, using all polymorphic SSLP markers close to *Clock*.

Pdgfra was mapped in intraspecific backcross progeny using PCR primers derived from sequence of the 3′-untranslated region of the gene, and the same protocols as for the SSLP markers. The primers used for this PCR reaction were as follows: forward, 5′ TTC CCA TTC TAG TCA ACG TGG 3′; reverse, 5′ GGA TGC TCC TGA TAG CCT ACC 3′. We detected a mobility shift in the PCR products of DNA samples heterozygous at this locus (perhaps due to the formation of heteroduplexes) that was easily scored.

To identify restriction fragment length polymorphisms (RFLPs), a Southern blot of DNA samples extracted from C57BL/6J, BALB/cJ, and C3H/HeJ strains of mice and restriction digested with 18 different restriction enzymes was probed with a random-primed labeled (Promega) rat *Kit* cDNA clone (kindly provided by K. MAYO). The cDNA clone was ~2.8-kb *Eco*RI-*Xba*I fragment including bases 1464–4246 of the *Kit* gene from a rat placental cDNA library in λGEM4 (O. K. PARK and K. MAYO), from which a ~1.5-kb *Eco*RI-*Bgl*II fragment was used as probe. We identified *Kit* RFLPs between the B6 and BALB strains with several enzymes, including *Pvu*II and *Hinc*II. To map the *Kit* locus, 28 animals with recombinations between *D5Mit83* and *D5Mit12* were selected, DNA from these animals was restriction digested with *Pvu*II, and RFLP analysis was carried out using standard protocols (AUSUBEL *et al.* 1995). Subsequently, DNA from the intraspecific backcross animals with recombinations between *D5Mit201/D5Mit235* and *D5Mit307* were analyzed in the same way. A similar strategy was used to identify an RFLP in the *Flk1* gene, using a 1.2-kb *Hin*dIII fragment of a mouse *Flk1* cDNA clone, kindly provided by M. BUCAN.

Genetic mapping data were analyzed using the program MapManager, version 2.6 (MANLY 1993).

Deletion mapping: F_1 progeny were produced to map the extent of the *W*^{19H} and *Ph* deletions, using SSLPs. The *W*^{19H} deletion was originally induced in a (101 × C3HeB/FeJ)F_1 mouse (LYON *et al.* 1984) and is maintained on a C3HeB/FeJ congenic background. The *Ph* deletion originated (GRUNEBERG and TRUSLOVE 1960) and is maintained on the C57BL/6J strain background. The *W*^{19H} deletion mapping was performed using progeny from a (C3HeB/FeJ-*W*^{19H} × CAST/Ei)F_1 interspecific cross. The *Ph* deletion mapping was performed using progeny from a (C57BL/6J-*Ph* × CAST/Ei)F_1 interspecific cross. Interspecific crosses were used to increase the number of informative SSLPs in the region. The animals with either chromosome 5 deletion were identified by the presence of white spots on their coats. The spotting phenotype observed in the offspring of both deletion crosses is probably due to disruption of *Kit* gene expression (LYON *et al.* 1984; DUTTLINGER *et al.* 1995). Although the *Ph* deletion does not include the *Kit* gene [the distal endpoint of the deletion is proximal from *Kit* (BRUNKOW *et al.* 1995)], recent evidence suggests that this deletion affects *Kit* 5′ regulatory sequences, resulting in misexpression of the gene within cells involved

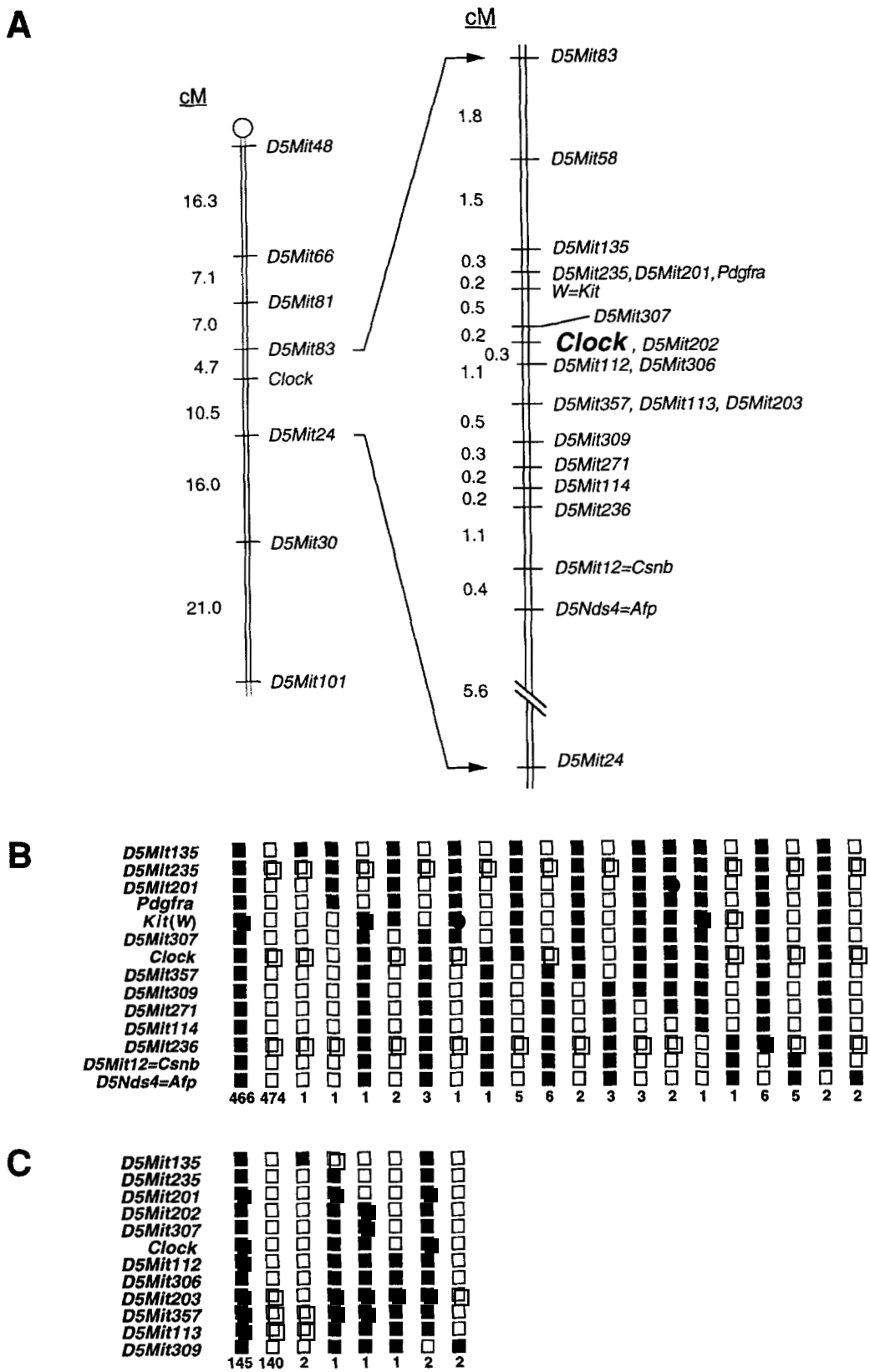

FIGURE 1.—(A) Genetic map of the *Clock* region of chromosome 5, using the recombination fractions of Table 1. Genetic distances were determined using combined recombination fractions of all eight mapping crosses. Genetic mapping did not resolve the markers just distal to *Clock* (*D5Mit112, D5Mit306, D5Mit357, D5Mit113,* and *D5Mit203*). Although all of these markers map distal of *Clock*, no recombinations between these markers have been detected. But *D5Mit112* and *D5Mit306* lie within the W^{19H} deletion, whereas *D5Mit357, D5Mit113,* and *D5Mit203* do not (see Figure 2 and *Deletion mapping results*). Thus, we are able to place *D5Mit112* and *D5Mit306* proximal from *D5Mit357, D5Mit113* and *D5Mit203*. (B) Haplotypes of the 988 intraspecific backcross progeny. □, the BALB/cJ strain background; ■, the C57BL/6J strain background. Indicated below each haplotype is the number of meioses exhibiting that pattern of inheritance. (C) Haplotypes of the 294 interspecific backcross progeny. □, the CAST/Ei strain background; ■, the C57BL/6J strain background. Indicated below each haplotype is the number of meioses exhibiting that pattern of inheritance.

in early melanogenesis (DUTTLINGER *et al.* 1995). DNA was extracted from tail-tips of F_1 progeny exhibiting white spotting, as well as control animals (with no spots), the parental strains (B6, C3HeB/FeJ-W^{19H}, and CAST/Ei) and C3HeB/FeJ. The spotted animals were tested for the presence or absence of the deletion strain alleles of SSLPs in the region of the deletion. SSLPs that lacked the deletion strain allele were considered to lie within the deletion.

Complementation crosses: To test for complementation, progeny were generated in the following manner: heterozygous deletion mutants (W^{19H} and *Ph*) were crossed to *Clock/Clock* homozygotes (obtained from the intercrosses described

TABLE 1

Recombination fractions of markers flanking *Clock*

Genetic interval	Intraspecific backcrosses				
	$B6^{Clock/+}N_2$[a]		$B6^{Clock/+}N_4$[b]		
	F_1 sire[c]	F_1 dam[d]	F_1 sire[e]	F_1 dam[f]	Total
D5Mit83-D5Mit58	3/52	0/47	—	—	3/99
D5Mit58-D5Mit135	1/52	1/47	—	—	2/99
D5Mit135-D5Mit235/201[g]	0/571	0/193	1/214	0/10	1/988
D5Mit235/201-Pdgfra	0/571	0/193	0/214	0/10	0/988
Pdgfra-Kit	1/571	1/193	0/214	0/10	2/988
Kit-D5Mit307	1/571	2/193	2/214	0/10	5/988
D5Mit307-Clock	1/571	1/193	0/214	0/10	2/988
Clock-D5Mit357	6/571	2/193	3/214	0/10	11/988
D5Mit357-D5Mit309	4/571	0/193	1/214	0/10	5/988
D5Mit309-D5Mit271	0/571	2/193	0/214	1/10	3/988
D5Mit271-D5Mit114	0/571	1/193	1/214	0/10	2/988
D5Mit114-D5Mit236	2/571	0/193	0/214	0/10	2/988
D5Mit236-Csnb	6/571	2/193	3/214	0/10	11/988
Csnb-Afp	2/571	1/193	1/214	0/10	4/988
Afp-D5Mit24	3/58	3/51	—	—	6/109

Genetic interval	Intercrosses[h]		
	$B6^{Clock/+}N_2$[a,i]	$B6^{Clock/+}N_4$[b,j]	Total
D5Mit83-D5Mit58	1/180	—	1/180
D5Mit58-D5Mit135	2/180	—	2/180
D5Mit135-D5Mit235/201	1/428	0/94	1/522
D5Mit235/201-D5Mit307	5/428	0/94	5/522
D5Mit307-Clock	1/428	0/94	1/522
Clock-D5Mit357	4/428	1/94	5/522
D5Mit357-D5Mit309	1/428	0/94	1/522
D5Mit309-D5Mit271	1/428	0/94	1/522
D5Mit271-D5Mit114	1/428	0/94	1/522
D5Mit114-D5Mit236	1/428	0/94	1/522
D5Mit236-Csnb	6/254	—	6/254
Csnb-Afp	2/254	—	2/254
Afp-D5Mit24	20/254	—	20/254

Genetic interval	Interspecific backcrosses[k]		
	F_1 sire[l]	F_1 dam[m]	Total
D5Mit135-D5Mit235/201	1/68	2/226	3/294
D5Mit235/201-D5Mit202	0/68	1/226	1/294
D5Mit202-D5Mit307	0/68	0/226	0/294
D5Mit307-Clock	0/68	0/226	0/294
Clock-D5Mit112	0/68	1/226	1/294
D5Mit112-D5Mit306	0/68	0/226	0/294
D5Mit306-D5Mit203	0/68	0/226	0/294
D5Mit203-D5Mit357	0/68	0/226	0/294
D5Mit357-D5Mit113	0/68	0/226	0/294
D5Mit113-D5Mit309	0/68	4/226	4/294
D5Mit309-D5Mit271	0/62	0/197	0/259
D5Mit271-D5Mit114	0/51	0/172	0/223
D5Mit114-D5Mit236	0/51	1/172	1/223

[a] $B6^{Clock/+}N_2$ animals were two backcross generations removed from the ENU-treated animal.
[b] $B6^{Clock/+}N_4$ animals were four backcross generations removed from the ENU-treated animal.
[c] Animals were the progeny of the following backcross: $[B6^{+/+} \times (BALB^{+/+} \times B6^{Clock/+}N_2)F_1^{Clock/+}]$.
[d] Animals were the progeny of the following backcross: $[(BALB^{+/+} \times B6^{Clock/+}N_2)F_1^{Clock/+} \times B6^{+/+}]$.
[e] Animals were the progeny of the following backcross: $[B6^{+/+} \times (BALB^{+/+} \times B6^{Clock/+}N_4)F_1^{Clock/+}]$.
[f] Animals were the progeny of the following backcross: $[(BALB^{+/+} \times B6^{Clock/+}N_4)F_1^{Clock/+} \times B6^{+/+}]$.
[g] We detected no recombinations between *D5Mit235* and *D5Mit201* in 1804 meioses.
[h] Only F_1 progeny carrying the *Clock* mutation were selected for mating in these intercrosses.
[i] Animals were the progeny of the following intercross: $(BALB^{+/+} \times B6^{Clock/+}N_2)F_2$.
[j] Animals were the progeny of the following intercross: $(BALB^{+/+} \times B6^{Clock/+}N_4)F_2$.
[k] Both interspecific crosses began with $B6^{Clock/+}N_2$ animals.
[l] Animals were the progeny of the following backcross: $[B6^{+/+} \times (CAST^{+/+} \times B6^{Clock/+}N_2)F_1^{Clock/+}]$.
[m] Animals were the progeny of the following backcross: $[(CAST^{+/+} \times B6^{Clock/+}N_2)F_1^{Clock/+} \times B6^{+/+}]$.

in Table 1) to generate F_1 animals, about half of which carried the deletion (see Figure 4A). As with the deletion mapping, the animals with a chromosome-*5* deletion were identified by the presence of white spots on their coats. All of the mice carry the *Clock* mutation on the homologue opposite the deletion.

To generate *Clock/Clock* mice in a strain background comparable to the W^{19H}/*Clock* compound heterozygotes, W^{19H}/*Clock* compound heterozygotes were intercrossed to generate progeny of two types: *Clock/Clock* homozygotes, as well as additional W^{19H}/*Clock* compound heterozygotes [W^{19H} homozygotes are embryonic lethal (LYON *et al.* 1984)].

RESULTS

Genetic mapping of the *Clock* locus: To identify a chromosomal region in which to focus our physical mapping and molecular cloning efforts, we have generated a high-resolution genetic map of the *Clock* region, using SSLPs (Figure 1A) (COPELAND *et al.* 1993; DIETRICH *et al.* 1994, 1996). *Clock* originated on the C57BL/6J strain background. We used the BALB/cJ strain as counterstrain to generate intraspecific backcross and intercross mice for linkage analysis. *M. castaneus* mice were used to generate interspecific backcross mice. In the course of this mapping, we placed *Clock* ~3 cM proximal to *Csnb,* which contains within it an SSLP (*D5Mit12*). This placed *Clock* close to the *Kit* locus (GEISSLER *et al.* 1988b). To place *Kit* on our map, we identified, using a *Kit* partial cDNA clone as probe, several restriction enzymes that detected RFLPs between the B6 and BALB strains. Using the restriction enzyme *Pvu*II and DNA from animals with chromosomes recombinant between *D5Mit83* and *D5Mit24,* we initially placed *Kit* ~1 cM (three recombinants/276 meioses) proximal from *Clock,* between *D5Mit201/235* and *D5Mit307.* Subsequent high resolution genetic mapping, using all backcross meioses recombinant between *D5Mit201/235* and *D5Mit307,* placed *Kit* 0.7 cM (seven recombinants/988 meioses) proximal from *Clock* (Figure 1A). Although it is unlikely that *Clock* is an allele of the *Kit* locus based on this genetic mapping information, it is important to note that our probe for the RFLP analysis was obtained from sequence toward the 5′ end of the gene. Since the 3′ end of the gene is distal to the 5′ end on chromosome *5* and the gene may extend over a large genomic region (BRUNKOW *et al.* 1995), it is possible that the *Clock* mutation affects sequence in the 3′ region of this gene. We have also placed the *Pdgfra* gene on our map, using a polymorphism detected in a PCR product derived from the 3′-untranslated region of the gene. This provides the first genetic mapping information placing *Pdgfra* proximal from *Kit.* In previous genetic mapping efforts, *Pdgfra* and *Kit* have not been resolved by meiotic recombination (0/589 meioses total) (KOZAK *et al.* 1996). We have attempted to place *Flk1* (MATTHEWS *et al.* 1991), which is distal of *Kit* (BRUNKOW *et al.* 1995), on our map, but have thus far detected no polymorphism between the B6 and BALB strains that would allow us to do so.

We have now genotyped 1804 meioses from eight crosses (see Table 1), using SSLPs, as well as genetic markers for *Pdgfra* and *Kit,* that map to the midportion of chromosome *5.* Haplotypes of the 988 meioses genotyped from the intraspecific backcrosses are shown in Figure 1B. Haplotypes of the 294 meioses genotyped from the interspecific backcrosses are shown in Figure 1C. We have placed *Clock* within a ~0.5-cM interval, 0.2 cM (three recombinants/1804 meioses) distal of *D5Mit307* and 0.3 cM (one recombinant/294 meioses) proximal from *D5Mit112/D5Mit306* (Figure 1A). The location of this distal recombination has been confirmed in test-cross progeny. There are four cloned genes that have been mapped to chromosome *5,* between *Kit* and *Csnb: Flk1, Ste* (sulfurtransferase, estrogen preferring), *Gnrhr* (gonadotropin releasing hormone receptor), and *Csna* (alpha casein). The location of these genes relative to *D5Mit307* and *D5Mit112/D5Mit306* is not known. This represents an initial list of candidate genes for the *Clock* mutation, which awaits further genetic and physical mapping information.

Deletion mapping: Although *Clock* is not likely to be an allele of the *Kit* locus on the basis of genetic mapping, there are mutations in the *Kit* region, including deletions and inversions, that may affect the *Clock* locus. We sought to determine whether *Clock* lay within the deletions near *Kit,* especially W^{19H}, which is a large deletion (LYON *et al.* 1984). Although the precise extent of the W^{19H} deletion is unknown, it is estimated to be ~3 cM in length (GEISSLER *et al.* 1988a). To determine if *Clock* is within the W^{19H} deletion, we mapped its SSLP content, using the F_1 progeny of an interspecific cross between C3HeB/FeJ-W^{19H} mice and *M. castaneus* (CAST/Ei). The parental strains were polymorphic for all of the markers that we tested, including SSLP loci that detected multiple alleles in a single parental strain (*i.e.,* the strain is not isogenic; see below). We detected loss of the C3HeB/FeJ-W^{19H} allele in the spotted F_1 progeny for several markers flanking *Kit,* including the closest genetic marker proximal from *Clock, D5Mit307,* as well as two markers distal of *Clock, D5Mit112* and *D5Mit306* (Figure 2). Thus, *Clock* maps within this deletion. The interspecific crosses did not produce any meioses recombinant between the closest five markers distal of *Clock* (*D5Mit112, D5Mit306, D5Mit203, D5Mit357, D5Mit113;* see Table 1). We have placed *D5Mit112* and *D5Mit306* closer to *Clock* on the genetic map because they fall within the W^{19H} deletion, whereas the remaining three markers are outside of this deletion. The W^{19H} deletion extends at least from *D5Mit58* to *D5Mit112/D5Mit306,* a genetic distance of ~2.8 cM. This is in close agreement with the original estimate of its size (GEISSLER *et al.* 1988a). The mapping of this deletion may be useful for investigating other genes within W^{19H}. For example, LYON *et al.* identified a recessive lethal locus within the W^{19H} deletion, distal to *Kit* (LYON *et al.* 1984). We can now place this locus proximal to *D5Mit203, D5Mit357,* and *D5Mit113.*

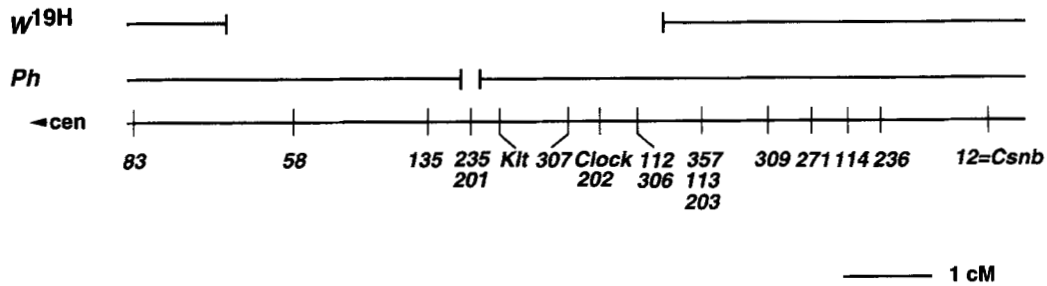

FIGURE 2.—SSLP content map of the *Ph* and W^{19H} deletions on chromosome 5. Numbers on the map refer to D5Mit markers, in all cases.

For the SSLPs that most closely flank the deletion, *D5Mit83* and *D5Mit357,* we detected two alleles in the C3HeB/FeJ-W^{19H} strain. This is not surprising, however, considering that this is a congenic strain, originating from a (101 × C3HeB)F_1 hybrid (LYON *et al.* 1984). One of the alleles for each of these two markers corresponds to the allele of the C3HeB/FeJ inbred strain. The other we presume to originate from the 101 strain (the size of the *D5Mit83* "101" allele is ~170 bp; the *D5Mit357* "101" allele is ~124 bp). This would suggest that the original deletion occured on the 101 chromosome, and indeed, the alleles segregate in the F_1 animals, with the C3HeB allele of both *D5Mit83* and *D5Mit357* detected in the wild-type (non-spotted) progeny, and the "101" allele of both markers detected in the deletion (spotted) progeny.

A similar SSLP content mapping strategy was used to determine the extent of the *Ph* deletion. Unlike the W^{19H} deletion, the *Ph* deletion is quite limited, containing only *D5Mit201* and *D5Mit235* (Figure 2). Thus, *Clock* is distal of this deletion. These data regarding the *Ph* deletion are consistent with those reported by BRUNKOW *et al.* (1995), who additionally confirmed the results of STEPHENSON *et al.* (1991) and SMITH *et al.* (1991), that *Pdgfra* lies within this deletion.

Circadian phenotypes of *Kit* region mutations: To determine whether mutations in the *Kit* region affect the circadian behavior of mice and thus perhaps affect the *Clock* locus, we measured circadian activity rhythms in the following mutant strains: *Patch* (*Ph*/+), Kit^W/Kit^{Wv} (a compound heterozygote), W^{19H}/+, and recessive spotting (*rs*/*rs*) (Table 2). These strains were chosen either because they have a *Kit* region deletion (W^{19H} and *Ph*) or because they are viable when homozygous (*rs*/*rs*) or as a compound heterozygote (Kit^W/Kit^{Wv}). Representative activity records of mice carrying the *Ph*, Kit^W/Kit^{Wv}, and W^{19H} mutations are shown in Figure 3, with activity records of wild-type mice of comparable strain backgrounds included for comparison. Both the *Ph* and W^{19H} mutations are homozygous lethal (GRUNEBERG and TRUSLOVE 1960; LYON *et al.* 1984); thus, the activity records shown are of heterozygous mutants. A

TABLE 2

Comparison of free-running periods of various chromosome 5 genotypes

Genotype	Strain	*N*	Mean	SEM
	Mutant strain comparisons			
+/+	C57BL/6J	12	23.6	0.06
Ph/+	C57BL/6J	3	23.5[a]	0.14
rs/*rs*	C57BL/6J	3	23.7[a]	0.04
+/+	WBB6F1/J	3	23.2	0.18
Kit^W/Kit^{Wv}	WBB6F1/J	3	23.3[a]	0.14
+/+	C3H/HeJ	12	23.6	0.06
W^{19H}/+	C3HeB/FeJ	5	23.8[a]	0.06
	Complementation crosses			
+/+	[C3H × (BALB × B6)F_2]F_1	7	23.6	0.04
W^{19H}/+	[C3H × (BALB × B6)F_2]F_1	5	23.7[a]	0.10
Clock/+	[C3H × (BALB × B6)F_2]F_1	23	24.2	0.05
Clock/W^{19H}	[C3H × (BALB × B6)F_2]F_1	17	25.6[b]	0.15
Clock/W^{19H}	[C3H × (BALB × B6)F_2]F_2	1	26[a]	
Clock/*Clock*	[C3H × (BALB × B6)F_2]F_2	5	26.9	0.56
Clock/+	[B6 × (BALB × B6)F_2]F_1	13	24.7	0.09
Clock/*Ph*	[B6 × (BALB × B6)F_2]F_1	21	24.5[a]	0.07

[a] Not significant at $P \leq 0.05$, Student's *t*-test comparison to control genotype listed above.
[b] $P < 10^{-7}$.

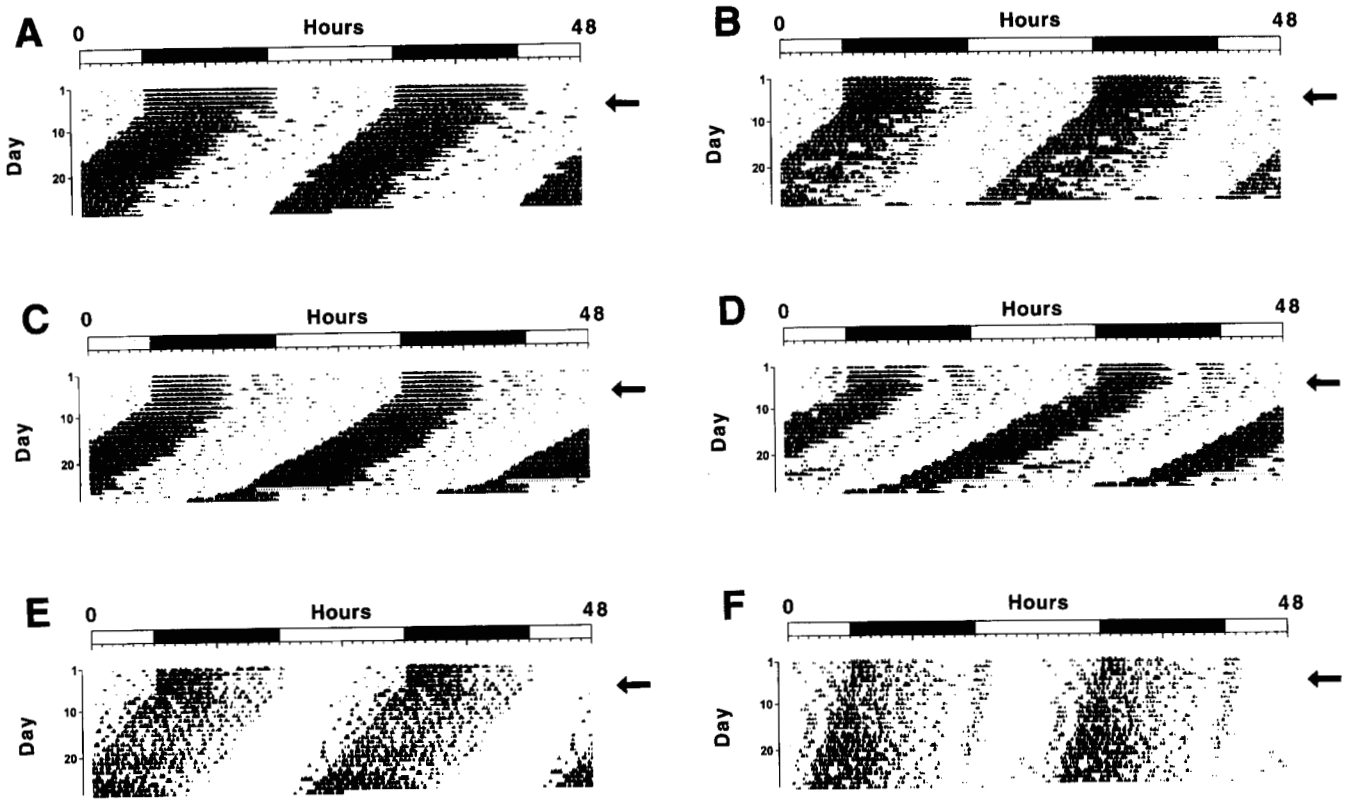

FIGURE 3.—Locomotor activity records of representative *Kit* (*W, Dominant white spotting*) region mutant mice. All records are double plotted, with 48 hr presented on each horizontal trace, so that each day is presented to the right and beneath the preceding day. Times of activity are indicated in black. Mice were kept on a 12 hr light:12 hr dark cycle (LD12:12) for the first 6 days shown (indicated by the bar at the top) and transferred to constant darkness at the usual lights-off time for the remaining days. The day of transfer is indicated by an arrow. (A) Wild-type (+/+) C57BL/6J female. (B) Heterozygote (*Ph*/+) *Patch* deletion mutant C57BL/6J female. (C) Wild-type (+/+) WBB6F1/J male. (D) Compound heterozygote (Kit^{W}/Kit^{Wv}) *Kit* mutant male. (E) Wild-type (+/+) C3H/HeJ male. (F) Heterozygote (W^{19H}/+) *Kit* region deletion mutant C3H/FeJ male.

typical activity record of a C57BL/6J female is shown in Figure 3A. The free-running period, in constant darkness, is ~23.7 hr. A representative activity record of a C57BL/6J female mouse carrying the *Ph* deletion is shown in Figure 3B. Activity records of a wild-type mouse of the appropriate strain background (WBB6F1/J) and a Kit^{W}/Kit^{Wv} compound heterozygote mouse are shown in Figure 3, C and D, respectively. The W^{19H} deletion, which originated in a (C3HeB/FeJ × 101)F_1 hybrid (LYON *et al.* 1984), is maintained, as a congenic strain, on a C3HeB/FeJ (C3HeB) background. The activity record of a wild-type mouse of the closely related strain, C3H/HeJ (C3H), is shown in Figure 3E. Shown in Figure 3F is the activity record of a W^{19H}/+ mouse. For all of these mutations, no significant differences in circadian period length measured in constant darkness were detected between mutant and wild-type mice (Table 2). In addition, there were no obvious differences in entrainment behavior to LD 12:12 or pattern of the activity rhythm between mutant mice and their respective wild-type controls.

Complementation crosses: To determine the effect of the W^{19H} deletion on the *Clock* phenotype, we performed a complementation test. Mice heterozygous for the W^{19H} deletion were mated with (BALB × B6)F_2 hybrid *Clock*/*Clock* mice to generate F_1 progeny, about half of which were compound heterozygotes (Figure 4A). We determined the free-running periods of these F_1 animals by recording their wheel-running activity (see MATERIALS AND METHODS). The F_1 mice that carried the W^{19H} deletion were distinguished by the presence of white spotting on their coats. Representative activity records of F_1 progeny from the complementation cross are shown in Figure 4, B–D. The activity record of a *Clock*/+ mouse (with no spots) from this cross is shown in Figure 4B. On this [C3HeB × (BALB × B6)F_2]F_1 hybrid genetic background, *Clock*/+ heterozygotes behaved as expected, with an average period of 24.2 hr (Table 1). In contrast, *Clock*/W^{19H} compound heterozygotes (with white spots) expressed longer circadian periods than *Clock*/+ mice (Figure 4B and C), and in addition, some compound heterozygotes also displayed disrupted activity rhythms, similar to, but not as severe as, the activity rhythms of *Clock*/*Clock* homozygotes (Figure 4D). A histogram displaying the periods of all of the F_1 progeny is shown in Figure 5A. The average period of the *Clock*/W^{19H} compound heterozygotes was 25.6 ± 0.15 hr. This period length was intermediate between

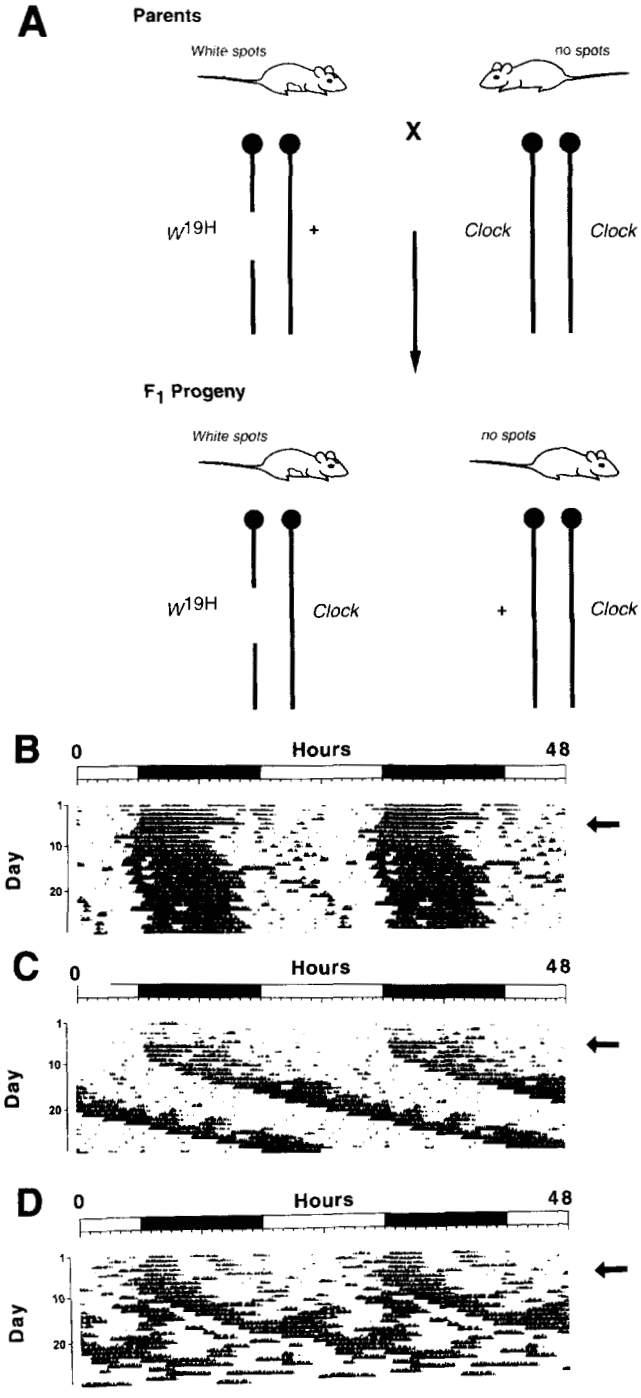

FIGURE 4.—Complementation analysis of *Clock* and W^{19H}. (A) The complementation test cross. Heterozygous females carrying either the W^{19H} deletion were bred with *Clock/Clock* F_2 or F_3 males. Because W^{19H} heterozygotes have a white spotted phenotype, compound heterozygous F_1 progeny from such a mating can be identified. (B) Locomotor activity record of a *Clock*/+ heterozygote F_1 from the W^{19H} cross. (C) Locomotor activity record of a *Clock*/W^{19H} compound heterozygote F_1 from the W^{19H} cross. (D) Locomotor activity record of a *Clock*/W^{19H} compound heterozygote F_1 from the W^{19H} cross, with an activity record that resembles the activity records of *Clock* homozygous mice.

Clock/+ mice (24.2 ± 0.05 hr) and *Clock/Clock* mice (26.9 ± 0.56 hr), on the same hybrid genetic background (Table 2). The clear segregation in the free-

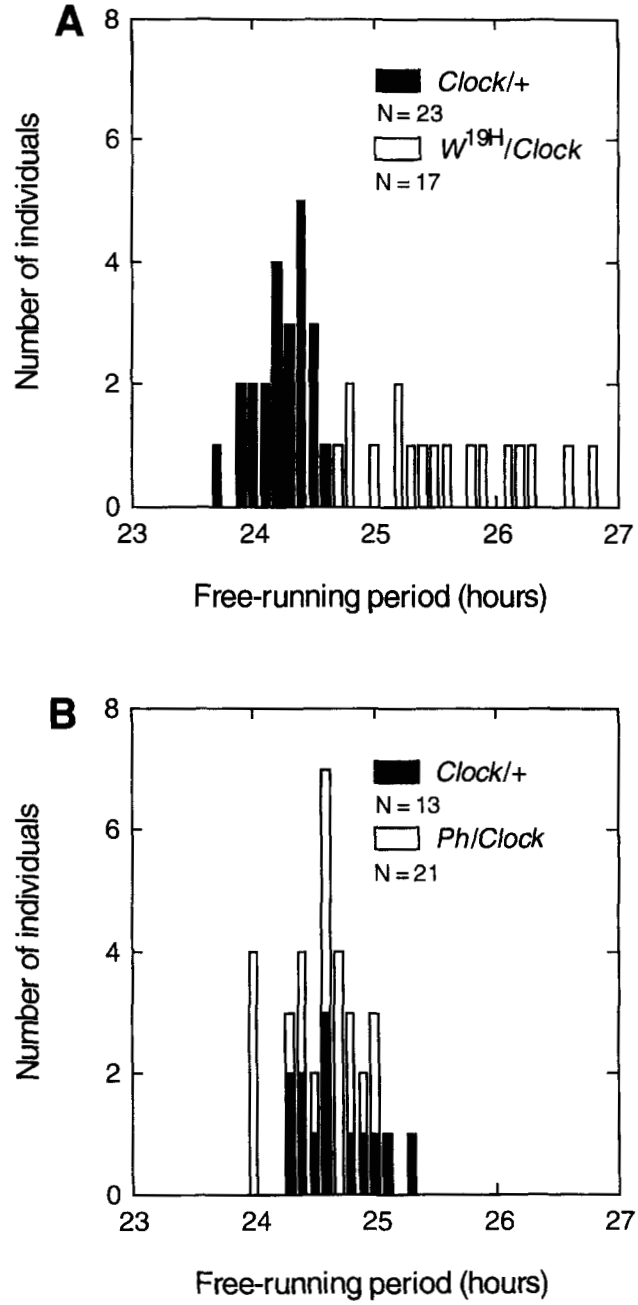

FIGURE 5.—Phenotypic distributions of the free-running period of locomotor activity in constant darkness of F_1 progeny from the two complementation crosses. (A) Distribution of period of W^{19H} cross progeny. (B) Distribution of period of *Ph* cross progeny.

running periods of *Clock*/W^{19H} compound heterozygotes and *Clock*/+ heterozygotes (Figure 5A) indicates that W^{19H} fails to complement *Clock*. This is consistent with the placement, within the W^{19H} deletion, of genetic markers that flank the *Clock* locus.

By contrast, a complementation test using the same mating strategy described above indicates that the *Ph* deletion fully complements *Clock* (Figure 5B). Again, this is consistent with the deletion mapping. Note, however, that the average period of the [B6 × (BALB × B6)F_2] *Clock*/+ heterozygotes is 24.7 hr (Table 2),

which is 0.5 hr longer than that seen in the [C3HeB × (BALB × B6)F_2] *Clock*/+ mice (Student's *t*-test, $P < 0.001$). This is one example of the contribution of genetic background to circadian period in different hybrids, underscoring the importance of appropriate strain background controls in quantitative experiments.

Finally, the *Clock* mutation had no effect on the extent or pattern of coat color spotting in the compound heterozygotes of either W^{19H} or *Ph*. Thus, we did not find any evidence that *Clock* expresses any *Kit*-like phenotypic effects on coat color.

DISCUSSION

Our initial analysis of the *Clock* mutation indicated that the mutation exhibited a semidominant phenotype (VITATERNA *et al.* 1994). There are several possible causes of a semidominant phenotype (DUNLAP 1993). Indeed, the possibility exists that the mutation was induced in a gene that otherwise is not involved in the generation of circadian rhythms, but when mutated, interferes with the normal generation of these rhythms. Access to a null allele of *Clock* (in the form of a deletion that encompasses the gene) has allowed us to analyze further the phenotypic effect of this mutation. MULLER's classic allelomorph analysis (MULLER 1932), as well as more recent analysis of dominant mutations in *Caenorhabditis elegans* (PARK and HORVITZ 1986), provide us with a framework in which to analyze the *Clock* mutation. MULLER described five types of mutant alleles, or "allelomorphs," resulting from the analysis of several classical Drosophila mutations: hypomorph, amorph, hypermorph, antimorph, and neomorph alleles. Allelomorph analysis is possible when one is able to manipulate the copy number of the mutant and wild-type alleles, and observe the resultant phenotypes. The W^{19H} deletion has provided us with the ability to observe the effect of a single copy of the *Clock* mutant allele in the absence of a wild-type allele. This *Clock*/deletion phenotype that we observed is more severe than the phenotype of *Clock*/+ heterozygotes, but less severe than the phenotype of *Clock/Clock* homozygotes. In addition, the +/deletion hemizygous phenotype is indistinguishable from the homozygous wild-type phenotype. Thus the null allele is recessive to wild type. The relative severity of these mutant phenotypes is shown in Figure 6. This order of phenotypic severity is the defining characteristic of an antimorphic allele (MULLER 1932). The essential comparison for this determination is that of the *Clock*/+ to the *Clock*/deletion phenotypes (MULLER 1932; PARK and HORVITZ 1986). That the *Clock*/deletion phenotype is significantly more severe than the *Clock*/+ phenotype indicates that the wild-type allele is interacting with the *Clock* mutant allele to ameliorate the severity of the *Clock* mutant phenotype. This is in contrast to what would be expected of a neomorph mutation, in which case the wild-type allele would have no effect on the expression or severity of the mutant allele. Thus, with the caveat that we are dealing

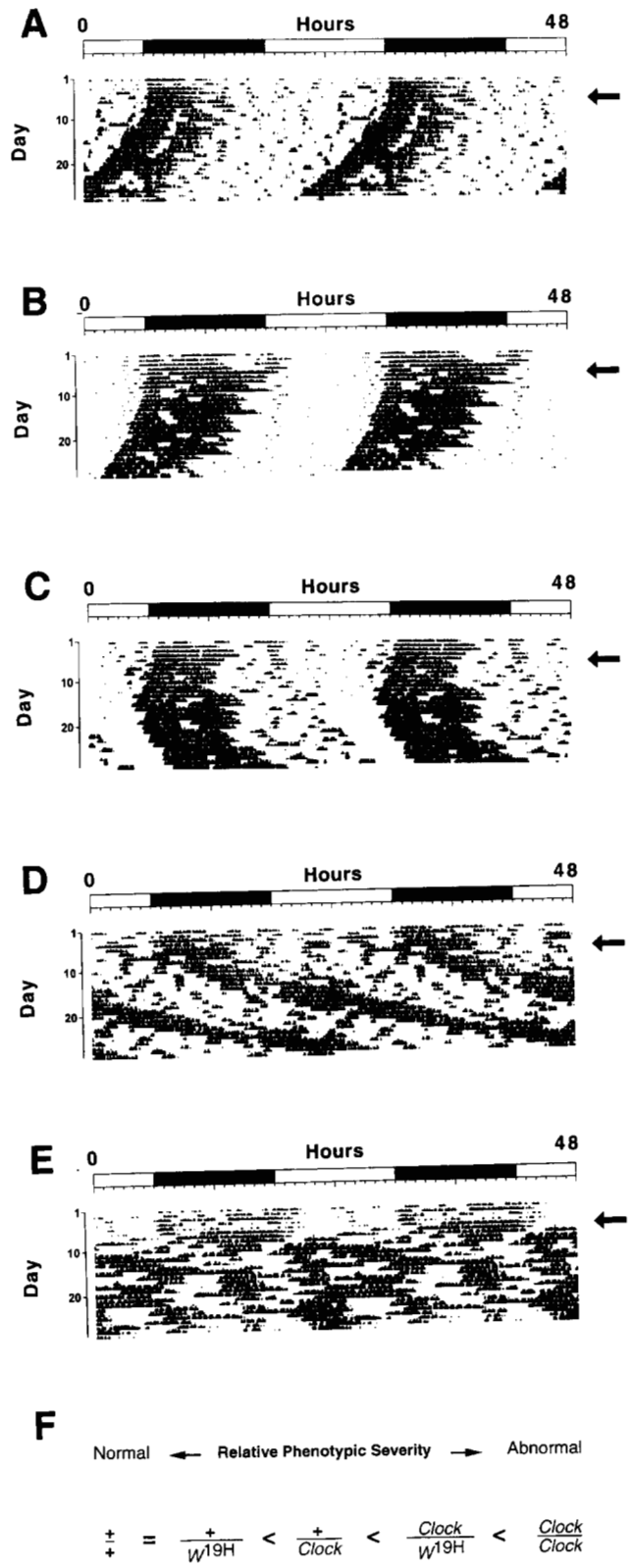

FIGURE 6.—Locomotor activity records showing the relative circadian phenotypic severity of different *Clock* genotypes. Records are from [C3H W^{19H}/+ × (BALB × B6)F_3 *Clock*/*Clock*]F_1 or F_2 progeny or from [C3H W^{19H}/+ × (BALB × B6)F_3 +/+] F_3 progeny. (A) Wild type (+/+). (B) Deletion heterozygote (W^{19H}/+). (C) *Clock* heterozygote (*Clock*/+). (D) Compound heterozygote (*Clock*/W^{19H}). (E) *Clock* homozygote (*Clock/Clock*). (F) Relative circadian phenotypic severity of genotypes depicted in 6A-6E.

with a large deletion that could cause complex interactions that are not obvious, we conclude that *Clock* behaves as an antimorph. Furthermore, because the W^{19H} deletion is large (~2.8 cM) and because multiple loci, both proximal from and distal of *Clock*, lie within the deletion, it would seem unlikely that the breakpoints of the deletion interact directly with the *Clock* gene.

The antimorphic behavior of the *Clock* allele provides us with some clues about the nature of this mutation. Antimorphic behavior suggests that the mutant allele generates a molecule that competes with the wild-type function. This, and the observation that *Clock*/deletion and *Clock*/+ have much more severe phenotypes than +/deletion, allow us to conclude that the *Clock* mutation is unlikely to be either a null mutation (amorph), or a partial loss of function (hypomorph). Further, because +/deletion has no circadian differences from wild type, the *Clock* phenotype does not appear to be the result of haplo-insufficiency. We now expect that the mutation conferring the altered behavior in *Clock* mutant mice is within the coding region of the gene, due to its ability to interfere with the function of the wild-type allele (although other interpretations are possible, this is the most likely way that *Clock* could be an antimorph). Because *Clock* behaves as an antimorph rather than a neomorph, competitively inhibiting the wild-type function of the gene, we conclude that the gene in question is a component of the normal circadian system.

The phenotypic analysis of the *Clock*/+ mice obtained from the two complementation tests revealed that these mice have a strain dependent difference in circadian period length (Table 2). This suggests that there are other, polymorphic, loci affecting the endogenous period. It is of particular interest that it is on a *Clock*/+ background that this difference is revealed. Wild-type mice of differing strain backgrounds do not have significant differences in period length (Table 2). Thus, it appears that *Clock*/+ mice are more sensitive to modifiers that affect circadian behavior. A similar effect has been observed in mice carrying the *Min* (*Multiple intestinal neoplasia*) mutation, another ENU-induced mutation (MOSER *et al.* 1990). On an appropriate strain background (in this case, AKR) the strain-dependent difference observed was largely due to a single locus (*Modifier of Min-1, Mom-1*), which was mapped to chromosome *4* (DIETRICH *et al.* 1993). Loci could potentially be identified that similarly modify the *Clock* phenotype.

There is an intriguing similarity between the *Clock* mutation and previously identified mutations affecting circadian rhythms in other organisms. Mutations that change the pace of the clock, either lengthening or shortening the endogenous period, usually have a semidominant phenotype, at least with regard to period length (DUNLAP 1993). Although not explicitly stated, an examination of the literature regarding the classic semidominant mutations of *per* (KONOPKA and BENZER 1971; SMITH and KONOPKA 1982) and *frq* (FELDMAN and HOYLE 1976; LOROS *et al.* 1986) reveals that they also appear to behave as antimorphs. A recently isolated *per* mutation conferring to the fly a very short period length (~16 hr) also appears to be antimorphic (KONOPKA *et al.* 1994). Interestingly, both long and short period alleles of *per* (per^s, per^l) and *frq* (frq^1, frq^4, frq^7) are antimorphic. In addition, all of the antimorphic mutations identified at the molecular level in *per* and *frq* occur within the coding regions of these genes (BAYLIES *et al.* 1987; YU *et al.* 1987; ARONSON *et al.* 1994). The model of the biological clock that is emerging from the detailed molecular analysis of these "canonical" clock genes, and the recently identified *tim* gene (SEHGAL *et al.* 1994), is one of an autoregulatory feedback loop, in which the proteins encoded by "clock" genes affect, either directly or indirectly, the rate of the clock genes' own transcription (DUNLAP 1996). In Drosophila, much recent work has focused on the PER:TIM protein interaction, which is required for the nuclear localization of PER. In tim^{01} null mutants, *per* does not localize to the nucleus and the fly is arrhythmic. While it is too early to conclude that *Clock* is a component of a molecular feedback loop similar to these canonical clock genes, the fact that the period-changing mutations in Neurospora, Drosophila and the mouse are all antimorphic is suggestive and encouraging.

The *tau* mutation in the golden hamster, *Mesocricetus auratus* (RALPH and MENAKER 1988), is the only other known genetic mutation affecting the mammalian circadian system. *tau* expresses a semidominant phenotype as does *Clock* (except that the *tau* mutation causes the circadian rhythm to be shorter than 24 hr, rather than longer), but current genetic resources in the golden hamster (OKAZAKI *et al.* 1996) make it impossible to determine if this mutation behaves as an antimorph. Therefore *Clock*, by virtue of being an antimorphic allele, is the first mammalian example of a mutation shown to define a gene that functions within the normal circadian system.

Using over 1800 meioses obtained from six intraspecific and two interspecific crosses, we have mapped *Clock*, by linkage analysis, to the midportion of mouse chromosome *5*, distal to the *Kit* gene, flanked by the SSLP markers, *D5Mit307* and *D5Mit112*/*D5Mit306*. *Clock* is the first mammalian circadian gene to be placed on a high resolution genetic map. This genetic mapping includes meioses obtained from several different crosses, both intra- and interspecific. Although the interspecific crosses did not include a large part of our collected meioses, they allowed us to test all SSLPs of interest in the region. Our genetic map is in close agreement with the standard SSLP map generated by the Whitehead Institute and displayed on their World Wide Web site (release 14, May 1997; http://www.genome.wi.mit.edu/). Of course, we have been able to separate many more markers because our genetic map is of a higher resolution (Table 1), but none of the markers that we tested mapped to a differ-

ent genetic interval than that reported by the Whitehead Institute. Thus, within this genetic region, the Whitehead map appears to have few, if any, ordering errors. This is limited, however, to markers that are reported to map to the interval in which we are interested. If any loci that belonged in this interval were erroneously placed elsewhere on the Whitehead map, we would not have detected this error.

The genetic interval containing *Clock, D5Mit307-D5Mit112/D5Mit306* is sufficiently small (~0.5 cM) to be amenable to long range physical mapping and the development of contigs spanning the region. In fact, the mouse genome physical mapping effort underway at the Whitehead Institute (also displayed on their WWW site) has already provided us with some preliminary physical information. This physical mapping is in the form of YAC contigs, generated using a new large insert (>800 kb) YAC library with roughly 10-fold coverage of the mouse genome, and sequence-tagged sites derived from the SSLP genetic mapping efforts (HALDI *et al.* 1996). Although these efforts are in their early stages and there is not a "doubly-linked" contig (in which markers are linked by more than one YAC clone) spanning the *Clock* region, there does appear to be a single YAC clone (362-D-6) that includes two of the markers most closely flanking *Clock, D5Mit307* and *D5Mit112.* Unfortunately, this YAC clone is chimeric, as it includes two loci that map ~5 cM away from *D5Mit307* and *D5Mit112,* as well as one locus from chromosome *1.* If the segment of this YAC clone from D5Mit307 to D5Mit112 is nonchimeric and intact, then it contains the *Clock* locus.

The placement of *Clock,* distal to *Kit* and proximal from *Afp,* allows us to predict the map position of a human orthologue of *Clock,* should such a gene exist. The human orthologue of *Kit* maps to chromosome *4q12,* and the human orthologue of *Afp* maps to the *q11-q13* interval of chromosome *4* (DEBRY and SELDIN 1996). In addition, no human orthologues of loci on mouse chromosome *5* between *Kit* and *Afp* have been placed outside of this region of human chromosome *4* (DEBRY and SELDIN 1996), suggesting that the entire interval shows conserved synteny between human and mouse. The human orthologue of *Clock* should therefore map to the *4q12–4q13* interval of chromosome *4.* A human orthologue of *Clock* could be involved in clinical disorders having a circadian component, such as delayed sleep phase syndrome and affective disorders (WEHR and ROSENTHAL 1989; VIGNAU *et al.* 1993). Thus, this region of human chromosome *4* should be investigated in human linkage studies of such disorders.

Clock is the first antimorphic mutation affecting circadian rhythms to be identified in a mammal. This, combined with the powerful tools available for positional cloning in the mouse (TAKAHASHI *et al.* 1994), make *Clock* an excellent candidate for identifying a molecule centrally involved in the mechanism of mammalian circadian rhythms. Because *Clock* was isolated on an isogenic background and because ENU-induced alleles have been found to represent single base pair changes, one can be optimistic about the prospects of identifying the *Clock* gene by positional cloning.

We thank K. SHIMOMURA, T. STEEVES, and G. SUYEOKA for assistance with SSLP genotyping, J. KORNHAUSER for assistance with tail-tip DNA extraction protocols, K. MAYO for the rat *Kit* cDNA clone, M. BUCAN for the mouse *Flk1* cDNA clone, and J. BARKER for providing W^{19H} and *rs/rs* mutant mice. This work was supported by grants from the National Science Foundation Center for Biological Timing and the Bristol-Myers Squibb Foundation to J.S.T.

Note added in proof: As anticipated for the coisogenic strategy involved in the genetics of *Clock* (DOVE W. F., Genetics **116:** 5–8, 1987), *Clock* has now been cloned (KING, D. P. *et al.*, Cell **89:** 641–653, 1997; ANTOCH, M. P. *et al.*, Cell **89:** 655–667, 1997).

LITERATURE CITED

ARONSON, B. D., K. A. JOHNSON, J. J. LOROS and J. C. DUNLAP, 1994 Negative feedback defining a circadian clock: autoregulation in the clock gene *frequency.* Science **263:** 1578–1584.

AUSUBEL, F. M., R. BRENT, R. E. KINGSTON, D. D. MOORE, J. G. SEIDMAN *et al.*, 1995 *Current Protocols in Molecular Biology,* John Wiley & Sons, New York.

BAYLIES, M. K., T. A. BARGIELLO, F. R. JACKSON and M. W. YOUNG, 1987 Changes in abundance or structure of the *per* gene product can alter periodicity of the *Drosophila* clock. Nature **326:** 390–392.

BRUNKOW, M. E., D. L. NAGLE, A. BERNSTEIN and M. BUCAN, 1995 A 1.8-Mb YAC contig spanning three members of the receptor tyrosine kinase gene family (*Pdgfra, Kit,* and *Flk1*) on mouse chromosome 5. Genomics **25:** 421–432.

COPELAND, N. G., N. A. JENKINS, D. J. GILBERT, J. T. EPPIG, L. J. MALTAIS *et al.* 1993 A genetic linkage map of the mouse: current applications and future prospects. Science **262:** 57–66.

DEBRY, R. W., and M. F. SELDIN, 1996 Human/mouse homology relationships. Genomics **33:** 337–351.

DICKINSON, W. J., 1995 Molecules and morphology: where's the homology? Trends Genet. **11:** 119–121.

DIETRICH, W., H. KATZ, S. E. LINCOLN, H-E. SHIN, J. FRIEDMAN *et al.*, 1992 A genetic map of the mouse suitable for typing intraspecific crosses. Genetics **131:** 423–447.

DIETRICH, W. F., E. S. LANDER, J. S. SMITH, A. R. MOSER, K. A. GOULD *et al.*, 1993 Genetic identification of *Mom-1,* a major modifier locus affecting *Min*-induced intestinal neoplasia in the mouse. Cell **75:** 631–639.

DIETRICH, W. F., J. C. MILLER, R. G. STEEN, M. MERCHANT, D. DAMRON *et al.*, 1994 A genetic map of the mouse with 4006 simple sequence polymorphisms. Nature Genet. **7:** 220–245.

DIETRICH, W. F., J. MILLER, R. STEEN, M. A. MERCHANT, D. DAMRON-BOLES *et al.*, 1996 A comprehensive genetic map of the mouse genome. Nature **380:** 149–152.

DUNLAP, J. C., 1993 Genetic analysis of circadian clocks. Annu. Rev. Physiol. **55:** 683–729.

DUNLAP, J. C., 1996 Genetic and molecular analysis of circadian rhythms. Annu. Rev. Genet. **30:** 579–601.

DUTTLINGER, R., K. MANOVA, G. BERROZPE, T-Y. CHU, V. DELEON *et al.*, 1995 The W^{sh} and *Ph* mutations affect the *c-kit* expression profile: *c-kit* misexpression in embryogenesis impairs melanogenesis in *Wsh* and *Ph* mutant mice. Proc. Natl. Acad. Sci. USA **92:** 3754–3758.

FELDMAN, J. F., and M. N. HOYLE, 1973 Isolation of circadian clock mutants of *Neurospora crassa.* Genetics **75:** 605–613.

FELDMAN, J. F., and M. N. HOYLE, 1976 Complementation analysis of linked circadian clock mutants of *Neurospora crassa.* Genetics **82:** 9–17.

GEISSLER, E. N., S. V. CHENG, J. F. GUSELLA and D. E. HOUSMAN, 1988a Genetic analysis of the dominant white spotting region on mouse chromosome 5; identification of cloned DNA markers near *W.* Proc. Natl. Acad. Sci. USA **85:** 9635–9639.

GEISSLER, E. N., M. A. RYAN and D. E. HOUSMAN, 1988b The dominant white spotting (W) locus of the mouse encodes the c-kit proto-oncogene. Cell **55:** 185–192.

GRUNEBERG, H., and G. TRUSLOVE, 1960 Two closely linked genes in the mouse. Genet. Res. **1:** 69–90.

HALDI, M. L., C. STRICKLAND, P. LIM, V. VANBERKEL, X-N. CHEN *et al.*, 1996 A comprehensive large-insert yeast artificial chromosome library for physical mapping of the mouse genome. Mamm. Genome **7:** 767–769.

HALL, J. C., 1990 Genetics of circadian rhythms. Annu. Rev. Genet. **24:** 659–697.

KONOPKA, R. J., and S. BENZER, 1971 Clock mutants of *Drosophila melanogaster.* Proc. Natl. Acad. Sci. USA **68:** 2112–2116.

KONOPKA, R. J., M. J. HAMBLEN-COYLE, C. F. JAMISON and J. C. HALL, 1994 An ultrashort clock mutation at the *period* locus of *Drosophila melanogaster* that reveals some new features of the fly's circadian system. J. Biol. Rhythms **9:** 189–216.

KOZAK, C. A., M. BUCAN, A. GOFFINET and D. A. STEPHENSON, 1996 Mouse chromosome 5. Mamm. Genome **6:** S97-S112.

LOROS, J. J., A. RICHMAN and J. F. FELDMAN, 1986 A recessive circadian clock mutation at the *frq* locus of *Neurospora crassa.* Genetics **114:** 1095–1110.

LYON, M. F., P. H. GLENISTER, J. F. LOUTIT, E. P. EVANS and J. PETERS, 1984 A presumed deletion covering the *W* and *Ph* loci of the mouse. Genet. Res. **44:** 161–168.

MANLY, K. F., 1993 A Macintosh program for storage and analysis of experimental genetic mapping data. Mamm. Genome **4:** 303–313.

MATTHEWS, W., C. T. JORDAN, M. GAVIN, N. A. JENKINS, N. G. COPELAND *et al.*, 1991 A receptor tyrosine kinase cDNA isolated from a population of enriched primitive hematopoietic cells and exhibiting close genetic linkage to *c-kit.* Proc. Nat. Acad. Sci. USA **88:** 9026–9030.

MCCLUNG, C. R., B. A. FOX and J. C. DUNLAP, 1989 The *Neurospora* clock gene *frequency* shares a sequence element with the *Drosophila* clock gene *period.* Nature **339:** 558–562.

MOSER, A. R., H. C. PITOT and W. F. DOVE, 1990 A dominant mutation that predisposes to multiple intestinal neoplasia in the mouse. Science **247:** 322–324.

MULLER, H. J., 1932 Further studies on the nature and causes of gene mutations. Sixth International Congress of Genetics, Ithaca, NY, Brooklyn Botanic Gardens, pp. 213–255.

MYERS, M. P., K. WAGER-SMITH, C. S. WESLEY and M. W. YOUNG, 1995 Positional cloning and sequence analysis of the *Drosophila* clock gene, *timeless.* Science **270:** 805–808.

OKAZAKI, Y., H. OKUIZUMI, T. OHSUMI, O. NOMURA, S. TAKADA *et al.*, 1996 A genetic linkage map of the syrian hamster and localization of cardiomyopathy locus on chromosome 9qa2.1-b1 using RLGS spot-mapping. Nature Genet. **13:** 87–90.

PARK, E-C., and H. R. HORVITZ, 1986 Mutations with dominant effects on the behavior and morphology of the nematode *Caenorhabditis elegans.* Genetics **113:** 821–852.

RALPH, M. R., and M. MENAKER, 1988 A mutation of the circadian system in golden hamsters. Science **241:** 1225–1227.

SEHGAL, A., J. L. PRICE, B. MAN and M. W. YOUNG, 1994 Loss of circadian behavioral rhythms and *per* RNA oscillations in the *Drosophila* mutant *timeless.* Science **263:** 1603–1606.

SMITH, E. A., M. F. SELDIN, L. MARTINEZ, M. L. WATSON, G. G. CHOUDHURY *et al.*, 1991 Mouse platelet-derived growth factor receptor alpha gene is deleted in W^{9H} and patch mutations on chromosome 5. Proc. Natl. Acad. Sci. USA **88:** 4811–4815.

SMITH, R. F., and R. J. KONOPKA, 1982 Effects of dosage alterations at the *per* locus on the period of the circadian clock of *Drosophila.* Mol. Gen. Genet. **185:** 30–36.

STEPHENSON, D. A., M. MERCOLA, E. ANDERSON, C. WANG, C. D. STILES *et al.*, 1991 Platelet-derived growth factor receptor alpha-subunit gene (*Pdgfra*) is deleted in the mouse patch (*Ph*) mutation. Proc. Natl. Acad. Sci. USA **88:** 6–10.

TAKAHASHI, J. S., 1995 Molecular neurobiology and genetics of circadian rhythms in mammals. Annu. Rev. Neurosci. **18:** 531–553.

TAKAHASHI, J. S., L. H. PINTO and M. H. VITATERNA, 1994 Forward and reverse genetic approaches to behavior in the mouse. Science **264:** 1724–1733.

VIGNAU, J., M. DAHLITZ, J. ARENDT, J. ENGLISH and J. D. PARKES, 1993 Biological rhythms and sleep disorders in man: the delayed sleep phase syndrome, pp. 261–271 in *Light and Biological Rhythms in Man,* edited by L. WETTERBERG. Pergamon Press, Oxford.

VITATERNA, M. H., D. P. KING, A-M. CHANG, J. M. KORNHAUSER, P. L. LOWREY *et al.*, 1994 Mutagenesis and mapping of a mouse gene, *Clock,* essential for circadian behavior. Science **264:** 719–725.

WEHR, T. A., and N. E. ROSENTHAL, 1989 Seasonality and affective illness. Am. J. Psychiatry **146:** 829–839.

YU, Q., A. C. JACQUIER, Y. CITRI, M. HAMBLEN, J. C. HALL *et al.*, 1987 Molecular mapping of point mutations in the period gene that stop or speed up biological clocks in *Drosophila melanogaster.* Proc. Natl. Acad. Sci. USA **84:** 784–788.

Communicating editor: R. E. GANSCHOW