pear at the late gastrula stage (stages 11 to 13) after the onset of embryonic transcription at the mid-blastula transition (stage 8) (*11*). At this time, however, a larger transcript of ~7 kb predominated. On longer exposures, the ~7-kb transcript was visible in RNA from oocytes and diminished in abundance in early cleavage stage embryos. The relation of the ~7-kb transcript to the smaller species is unknown. It may be an incompletely spliced form or precursor of the ~3-kb mRNA. The presence of multiple A chain transcripts in *Xenopus* oocytes and embryos is reminiscent of the expression of transcripts ranging from 1.9 kb to 3 kb in human cells (*3, 7*).

Although a *Xenopus* PDGF B chain gene was detected by DNA blotting with a v-*sis* probe, RNA blot analysis of RNA samples identical to those shown in Fig. 2 revealed no evidence of B chain mRNA (*5*). This result, combined with the lack of B chain cDNA clones in the oocyte and gastrula stage libraries, indicates that the B chain mRNA is present at a much lower level than the A chain mRNA within the early embryo, if at all.

Abundance of the A chain mRNA was estimated by its frequency of recovery from oocyte and gastrulation stage cDNA libraries. Each mature oocyte contained about 90 ng of polyadenylated mRNA corresponding to about 2×10^{10} mRNA molecules (*12*). Based on a representation of four copies per 300,000 clones in the cDNA library, a mature oocyte would contain about 5×10^5 A chain mRNA transcripts.

The isolation of the long form of A chain from an oocyte library and the short form from the gastrula library does not necessarily imply a developmental program of alternative splicing. Both forms are present in human cell lines expressing A chain (*7*) and in early mouse embryos (*5*). Furthermore, RNA protection data from experiments with a hybridization probe spanning the splice junction show that the short form predominates in both oocytes and stage 11 to 13 embryos (*5*). It is not yet known what, if any, functional differences distinguish the two forms.

Recently, two other growth factors have been implicated in the control of early *Xenopus* development. Early embryos contain a maternal mRNA containing sequences complementary to fibroblast growth factor (FGF) (*13*). *Vg1*, which encodes a molecule related to transforming growth factor–β (TGF-β), is also maternally encoded (*14*). Thus, growth factors may act during oogenesis or the first stages of embryogenesis. Growth factors may promote the rapid cell division seen in the early embryo, and purified FGF and TGF-β can mimic some aspects of the induction of mesoderm which normally occurs at gastrulation (*13, 15*). While these studies did not use proteins isolated from early *Xenopus* embryos, they suggest that endogenous growth factors may regulate differentiation. Recently, a paradigm for the control of differentiation by PDGF has come from studies of rat astrocytes (*16*). PDGF-like molecules have been shown to inhibit differentiation by promoting the division of a progenitor cell. It is conceivable, therefore, that PDGF A chain controls differentiation in *Xenopus* embryos by a similar mechanism. The experimental accessibility of the *Xenopus* embryo in conjunction with recent advances in antisense nucleotide technology will aid in determining the role of PDGF in development.

Note added in proof: The PDGF A chain mRNA has been observed in mouse eggs and preimplantation embryos (*17*).

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A Mutation of the Circadian System in Golden Hamsters

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A mutation has been found that dramatically shortens the period of the circadian locomotor rhythm of golden hamsters. The pattern of inheritance of this mutation suggests that it occurred at a single, autosomal locus (*tau*). Wild-type animals have rhythms with free-running periods averaging about 24 hours; animals heterozygous for the mutation have periods of about 22 hours, whereas homozygous animals have rhythms with periods close to 20 hours. Animals that carry the mutant alleles exhibit abnormal entrainment to 24-hour light:dark cycles or are unable to entrain.

GENETIC MUTATIONS THAT AFFECT the period (τ) of circadian rhythms have been studied and characterized extensively in nonvertebrate organisms and have provided a useful approach to the study of the molecular and biochemical mechanisms that generate and control rhythmicity. "Clock" mutants have been described in *Drosophila, Neurospora*, and *Chlamydomonas* (*1, 2*). However, single gene mutations that affect τ have not been described for any vertebrate.

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We found a single male hamster (*Mesocricetus auratus*; Charles River Breeding Labs) that exhibited an abnormally short τ in constant dark (DD). Whereas the normal τ_{DD} for golden hamsters averages about 24.1 hours and is rarely shorter than 23.5 hours (*3, 4*), the free-running period of the abnormal male was 22.0 hours and was stable for 3 weeks. When it was exposed to a light:dark cycle (LD) that consisted of 14 hours of light (100 lux) and 10 hours of dark (LD 14:10), the animal entrained. However, entrainment was abnormal; activity onsets occurred about 4 hours earlier than those of normal animals. We bred this animal with three female hamsters with normal freerunning periods (τ = 24.01, 24.03,

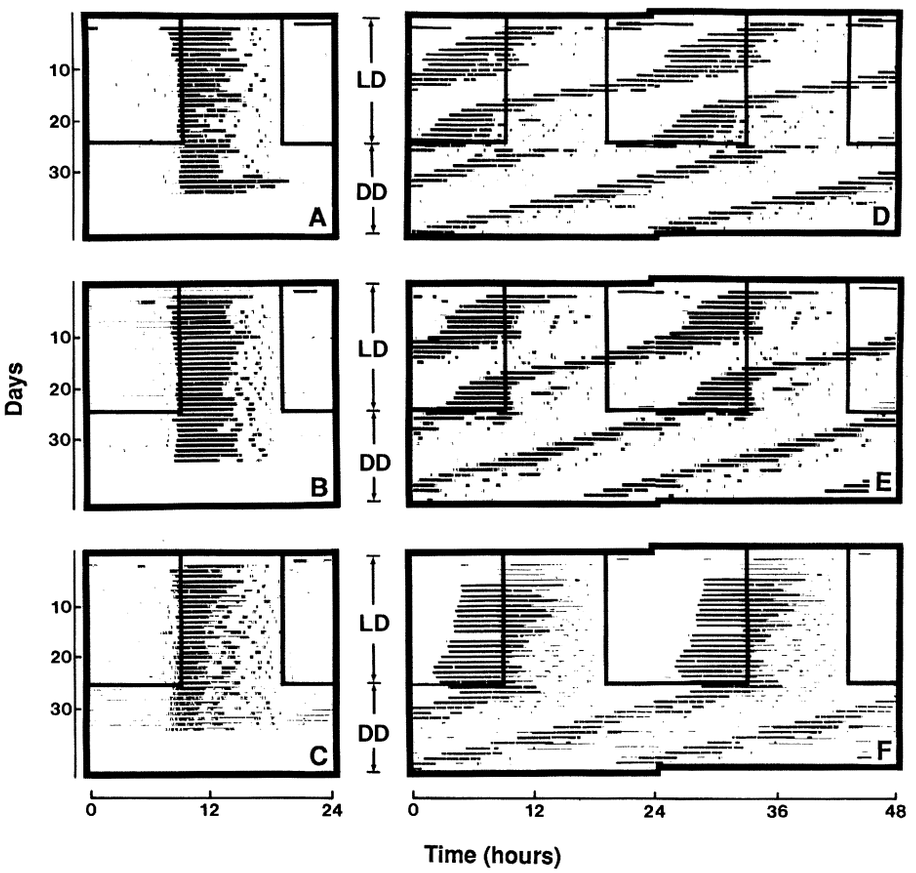

Fig. 1. Activity records (raw data) of six male F_1 littermates. Panels D to F are double plotted (as shown by the time axis). Boxes superimposed on each panel enclose the light portion (14 hours) of a light:dark cycle. LD, 14 hours of light in 100 lux; 10 hours of dark. DD, constant dark. Wheel running activity is indicated by the dark bands on each day.

and 24.04). These crosses yielded three litters totaling 21 animals (*5*). Six individuals from the oldest litter were exposed to LD 14:10 to examine entrainment. The remaining animals were placed in DD as soon as they were given access to running wheels. Three of the six animals exposed to the LD cycle showed normal entrainment with activity onset occurring close to the lights-off transition (Fig. 1, A to C). Two animals did not entrain (Fig. 1, D and E). The activity rhythms for these animals showed relative coordination with the light cycle (*6*), indicating that their circadian systems were affected by the light, but not sufficiently to enable entrainment. One animal (Fig. 1F) appeared to entrain; however, activity onsets occurred at an unusually early phase with respect to the light cycle (four or more hours before the light offset). The entrainment was also unstable, with activity onsets occurring earlier over time. After 24 days in LD, the animals were released into DD and allowed to free-run. The free-running period for each individual was consistent with the characteristics of its entrainment behavior in LD; animals with normal entrainment had τ values that were within the normal circadian range; animals that did not entrain or that showed abnormal entrainment had short τ values, which were similar to that of the original abnormal male.

The free-running period was determined for 20 of the 21 animals in the F_1 generation (the record of one female was uninterpretable). Animals could be divided into two distinct groups, T_s (mean τ = 22.31 ± 0.15 hours) and T_n (mean τ = 24.03 ± 0.07 hours), each containing eight males and two females (*7*). The F_1 ratio of T_s:T_n (50:50 for both sexes) is what one would predict given a mutation at a single, autosomal locus if one parent were wild type and the other heterozygous for the trait. If this interpretation is correct, then T_s animals in the F_1 generation are heterozygous for this trait and T_n animals are wild type.

On the basis of this interpretation, we sought to produce homozygous mutants and to determine their phenotype. After performing a number of outcrosses to increase the colony size, we crossed T_s animals (putative heterozygotes). Initially these animals were closely related because of the small population size; however, more recently the parents have been less closely related. Offspring from $T_s \times T_s$ crosses fell into three distinct phenotypic groups: the τ value of one group averaged about 24 hours; that of a second group about 22 hours; and that of a third group was about 20 hours.

We hypothesized that the group of animals with τ values close to 20 hours were homozygous for the mutation (T_{ss}). To test this and to test further the proposition that the 22-hour period is the phenotype of heterozygous animals, we performed an array of crosses among the three phenotypes. The frequency distributions of τ values for the offspring from these crosses are shown in Fig. 2. Chi square analysis indicates that in only one case (WT × T_s) do the observed phenotypic frequencies among the offspring differ significantly from those expected, given a semidominant mutation at a single locus ($P < 0.05$). The meaning of this difference is not clear; it might result from any of several factors including differential viability of phenotypes before weaning or it may be due to chance. Mann-Whitney analysis indicates that the frequency distribution for each phenotype does not depend to a statistically significant extent on the type of cross from which it was produced. This finding indicates that the periods of the parents' circadian rhythms do not significantly influence the period of their offspring; offspring with the same phenotype as their mothers are indistinguishable from offspring of mothers with different phenotypes.

We conclude from these data that a mutation has occurred at a single, autosomal locus, *tau*, that affects the circadian period in hamsters. The mutant trait is partially dominant and has the primary behavioral effect of creating a short circadian period. It remains to be seen to what extent the mutation has affected other physiological or developmental factors.

Although the period of the circadian oscillator is altered substantially by the mutation, the entrainment data presented here do not establish whether other aspects of the circadian system are also affected. Entrainment is thought to occur primarily through light-induced phase shifts (delays and advances) of the underlying circadian oscillation (*8*). The phase dependency of these responses, for any species, is described by its phase response curve (PRC). In wild-type hamsters, the magnitudes of delay and advance responses are sufficient to enable entrainment to LD cycles that are close to 24 hours; however, entrainment fails if τ and the period of the entraining cycle differ by more than an hour (*9, 10*). However, many of the heterozygous animals in our experiments are able to entrain to a 24-hour LD

cycle even though τ differs from 24 by 2 hours or more. In *Drosophila* and *Neurospora*, period mutations are associated with changes in PRC amplitude (*2*). Preliminary data from phase-shifting experiments on mutant hamsters indicate that the amplitude of the PRC for heterozygous animals has been increased. The mutation may cause, in addition to the shortening of τ, a compensatory change in the PRC that may account for the ability of some heterozygous mutant animals to entrain.

The *tau* mutation described here provides a tool for investigations into the generation and control of mammalian circadian rhythmicity and entrainment at many levels of organization. Furthermore, because the mutation behaves as though it has occurred at a single locus, it may be possible to identify and study a single gene product that is intimately involved in the regulation of the circadian period in mammals.

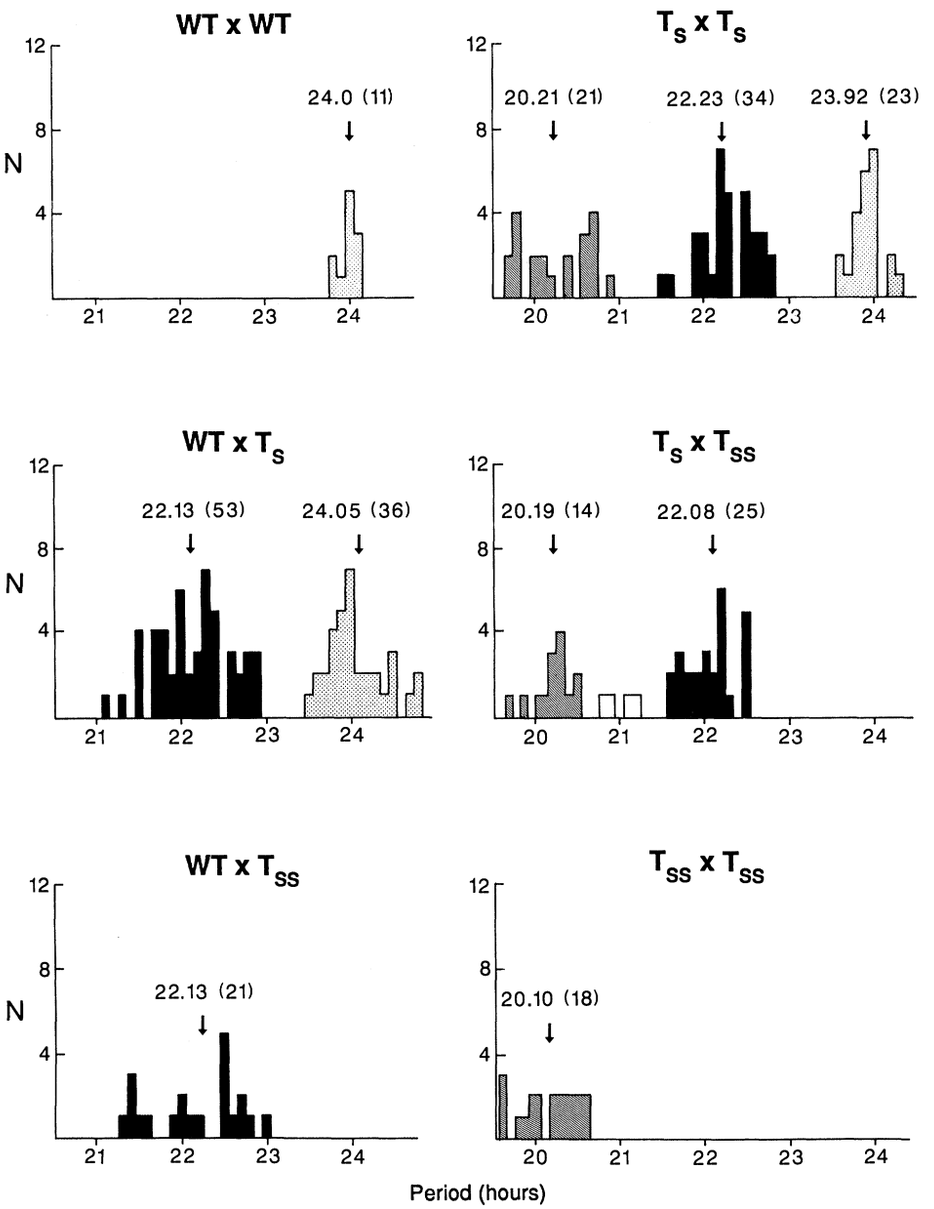

Fig. 2. Frequency distribution of τ from various crosses. After recording entrainment for 5 days, the animals were released into DD. Period was determined from the slope of a line fit by eye or regression analysis through the onsets of activity between day 4 and day 14 in DD. For statistical analysis, animals were assigned to particular groups on the basis of their phenotype: T_{ss} ($\tau \leq 21.00$ hours); T_s ($21.00 < \tau \leq 23.25$ hours); WT (23.25 hours $< \tau$). Our current data indicate that the ranges of the three groups do not overlap. Animals with τ values close to the extreme of the range for a group are routinely tested in backcrosses before genotype is inferred. Furthermore, many animals represented in this figure have been used in our breeding program, and in no case have the distributions of τ from their offspring indicated that we have been incorrect in assigning animals to a particular group. The four animals represented by the open histograms (in the $T_s \times T_{ss}$ panel) could not be assigned to a group based on phenotype alone and have not produced sufficient offspring to infer genotype; therefore, they have not been included in the statistical analysis. WT, wild type ($\tau \approx 24$ hours); T_s, putative heterozygote phenotype ($\tau \approx 22$ hours); and T_{ss}, putative homozygote mutant phenotype ($\tau \approx 20$ hours). Average τ for each group is indicated; number (*n*) of animals for each group is shown in parentheses; N is number of animals with a given free-running period.

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4. In experiments conducted in our laboratory between June 1982 and June 1986 in which the free-running period of golden hamsters has been measured (>1000 animals), the shortest period recorded before we found the T_s male was 23.5 hours.
5. The offspring (16 males, 5 females) were weaned at 21 days and were introduced to running wheel cages between 4 and 6 weeks of age. All animals were maintained in LD 14:10 before recordings were made. For recording activity, animals were housed individually in cages in light-tight boxes (six animals to a box) in which the light cycle could be controlled. Wheel running activity was recorded with Esterline Angus event recorders. For recording individual locomotor activity, each revolution of the running wheel closed a microswitch mounted to the outside of the cage. This is recorded as a pen deflection on one channel of an Esterline Angus chart recorder. Continuous activity appears as a thick black line. The record for each 24-hour period is mounted on a card directly beneath that from the previous day. The result is an uninterrupted record of activity with activity from a single day reading from left to right and consecutive days reading from top to bottom. In some cases (Fig. 1, D to F), records are double-plotted so that each day appears twice and activity for a 48-hour interval can be seen on a single line.
6. Relative coordination to a zeitgeber is a phenomenon that occurs when the period of the zeitgeber is outside the range of entrainment for the circadian rhythm. The circadian rhythm will free-run with a period that is modulated by the zeitgeber according to the relative phases of the two oscillators. A rhythm may appear to entrain for a while before breaking away from the zeitgeber [E. von Holst, *Ergeb. Physiol.* **42**, 228 (1939); R. Wever, *J. Theor. Biol.* **36**, 119 (1972); J. Aschoff, in *Handbook of Behavioral Neurobiology: Biological Rhythms*, J. Aschoff, Ed. (Plenum, New York, 1981), vol. 4, pp. 81–93].
7. The slight difference in variability of τ values between the two groups can be attributed to the increase in measurement error when τ is much shorter (or longer) than 24 hours. Free-running period is measured from an eye-fitted line, which is drawn through at least seven consecutive onsets of activity. The angle that this line makes with a horizontal line (measured clockwise from the horizontal line) depends on the period of the rhythm. The period is proportional to the tangent of this angle; therefore, the error due to measurement increases considerably as the free-running period deviates from 24 hours.
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