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Possible involvement of temperature-entrainable timing system in arrhythmic mutant flies in *Drosophila melanogaster*

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In *Drosophila melanogaster*, it is known that the circadian clock consists of an autoregulatory feedback loop, which includes so-called clock genes, such as *per, tim, dClk* and *cyc* and produces periodical expression of *per*. It is recently suggested, however, that the circadian oscillation without the rhythmical expression of *per* is involved in the regulation of circadian locomotor rhythms. In the present study, we examined the existence and the property of the possible *per*-less oscillation using arrhythmic clock mutant flies carrying per^{01}, tim^{01}, $dClk^{Jrk}$ or cyc^{01}. When temperature cycles consisting of 25°C and 30°C with varying periods (T = 8~32 hr) were given, they showed rhythms synchronizing with the given cycle under constant darkness (DD). per^{01} and tim^{01} flies always showed a peak around 7 hr after the onset of thermophase irrespective of Ts of temperature cycles, while $dClk^{Jrk}$ and cyc^{01} flies did not. In addition, several days were necessary to establish a clear temperature entrainment in per^{01} and tim^{01} flies, when they were transferred from a constant temperature to a temperature cycle under DD. These results suggest that per^{01} and tim^{01} flies have a temperature-entrainable weak oscillatory mechanism. The fact that $dClk^{Jrk}$ and cyc^{01} flies did not show any sign of the endogenous oscillation suggests that the *per*-less oscillatory mechanism may require CLK and CYC.

Key words: *Drosophila melanogaster*, circadian clock, temperature cycle, clock mutant, *per*-less oscillation

INTRODUCTION

The circadian clock is an endogenous timing mechanism governing various physiological functions. In *Drosophila melanogaster*, the clock system is thought to consist of an autoregulatory feedback loop including so-called clock genes, such as *per*, *tim*, *dClk* and *cyc*, which code PER, TIM, dCLK and CYC, respectively [1-5]. It is recently suggested, however, that the circadian oscillation without the rhythmical expression of *per* is involved in the regulation in circadian locomotor rhythms by behavioral studies, in which per^{01} flies were entrained by light cycles of various periods [6]. In this study, to clarify the existence and property of the possible *per*-less oscillator, we examined the entrainability of locomotor rhythms of arrhythmic mutant flies carrying per^{01}, tim^{01}, $dClk^{Jrk}$ or cyc^{01} to another powerful zeitgeber, temperature cycles. Their locomotor activity rhythms were recorded under temperature cycles (30°C: 25°C) of various periods (T=8~32 hr) in constant darkness (DD). The results indicate the existence of a timing system including a temperature entrainable weak oscillator in per^{01} and tim^{01} flies.

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MATERIAL AND METHODS

Adult flies, *Drosophila melanogaster*, were used. They were raised on standard cornmeal-glucose-yeast medium at 25°C under a light cycle of 12hr light and

12hr dark. The fly strains that we used for behavioral assay were *period*^{01} (*per*^{01}), *timeless*^{01} (*tim*^{01}), *dClock*^{Jrk} (*dClk*^{Jrk}) and *cycle*^{01} (*cyc*^{01}).

Male flies of about five days old were individually housed in transparent acrylic rectangular tubes (3×3×70mm). The tube was plugged at one end with agar/glucose medium as food and was sealed with Parafilm, and at the other end with a silicon tube filled with damped absorbent cotton as water source. A moving fly interrupted an infrared beam and the number of interruptions during each 6 min were recorded using a computerized system [7]. Temperature cycles were set by built-in thermostat driven by an electronic timer. Temperature steps-up and –down were finished within 15 min. Temperature cycles used were composed of an equal duration of thermophase (30°C) and cryophase (25°C) with periods varying from 32 hr (T=32 hr) to 8 hr (T=8 hr) at an interval of 4 hr.

RESULTS AND DISCUSSION

The previous reports suggested that *per*^{01} flies showed locomotor rhythms synchronizing with a temperature cycle of 24 hr [8, 9]. In this study, the entrainability of arrhythmic mutant flies to temperature cycles of various periods (T=8~32 hr) was examined by recording the locomotor activity rhythms in DD. The results showed that the four clock mutant flies synchronized with all tested temperature cycles. Interestingly, in *per*^{01} and *tim*^{01} flies, the phase of the major peaks changed dependent on Ts and gradually advanced as T was shortened from 32 hr to 20 hr and fell in the cryophase in T=8 hr (Fig. 1). T-dependent phase-changes were also observed in the activity trough in *per*^{01} and *tim*^{01} flies. These results suggest that the rhythms of *per*^{01} and *tim*^{01} flies are not direct response to the temperature cycle but driven by an endogenous timing system. On the other hand, *dClk*^{Jrk} and *cyc*^{01} flies did not show the shift of the peak phase and exhibited a large peak about 2.5 hr after the start of thermophase in all Ts. It seems that their rhythms are rather direct responses to the

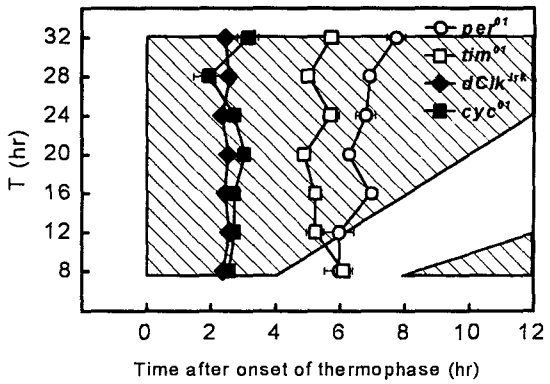

Fig. 1 Mean phase (±SEM) of major peaks of the locomotor rhythm of *Drosophila melanogaster* under various temperature cycles in DD. Shaded area indicates thermophase. Ordinate indicates periods (Ts) of temperature cycles and abscissa the time after the onset of thermophase. Note that the peak consistently occurred at about 2.5 hr after the onset of thermophase in *dClk*^{Jrk} and *cyc*^{01}, while it shifted in *per*^{01} and *tim*^{01} in association with changes in T.

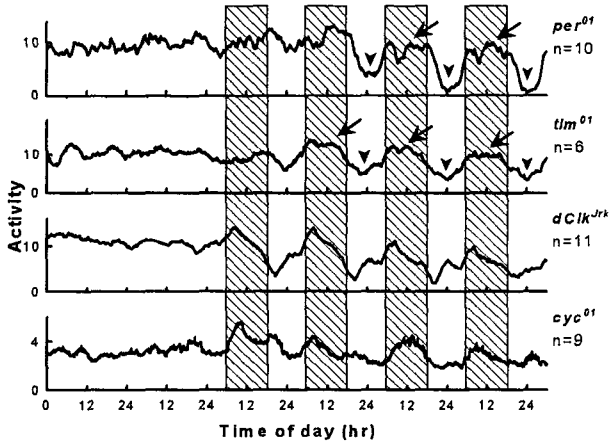

Fig. 2 Averaged longitudinal activity records of *per*^{01}, *tim*^{01}, *dClk*^{Jrk} and *cyc*^{01} flies recorded consecutively in constant 25°C and then in temperature cycle of 30°C 12hr: 25°C 12hr under DD. Shaded areas indicate the thermophase. n indicates number of flies used. Arrows and arrowheads indicate major peaks and troughs, respectively. Note that the activity peak and trough became prominent 2~3 cycles after the transfer to temperature cycles in *per*^{01} and *tim*^{01} flies.

temperature cycles.

To clarify whether the temperature sensitive timing system in per^{01} and tim^{01} flies involves an oscillatory mechanism, we transferred the flies from constant temperature (25°C) to temperature cycles. All mutant flies were arrhythmic during the constant temperature, but became rhythmic when exposed to temperature cycles (Fig. 2). Major peaks of per^{01} and tim^{01} flies needed 2~3 cycles to synchronize with the temperature cycles. Similar tendencies were observed in the activity troughs. In $dClk^{Jrk}$ and cyc^{01} flies, however, the large peaks immediately appeared at the first thermophase, and the activity peak persisted thereafter. These results suggest that per^{01} and tim^{01} flies have a timing system, which includes a temperature entrainable weak oscillatory mechanism. Since $dClk^{Jrk}$ and cyc^{01} flies did not show any sign of an endogenous oscillation, it is suggested that dCLK and CYC are involved in the oscillatory mechanism.

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