Intrinsic control of rhabdom size and rhodopsin content in the crab compound eye by a circadian biological clock

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Summary. Under conditions of constant darkness, rhabdom volume and the amount of visual pigment chromophore show circadian changes in the compound eye of the crab *Hemigrapsus sanguineus*. The present results indicate that an intrinsic circadian biological clock is involved in the control of the changes.
Key words. Grapsid crab; *Hemigrapsus sanguineus*; compound eye; circadian rhythm; rhabdom; visual pigment chromophore.

In many arthropod species, periodic breakdown and synthesis of photoreceptive membrane occur in synchrony with light and dark cycles. In general, the breakdown occurs mainly around dawn and the incorporation of new membrane into the rhabdom starts just after dusk, so that the rhabdom volume is larger at night than during the day[2,3]. We previously demonstrated that the rhabdom volume of the grapsid crab *Hemigrapsus sanguineus* increased by about 8 times at night over that during the day at 20 °C and that this anatomical change was accompanied by a change in the total amount of visual pigment chromophore[4].

In vertebrate rod and cone outer segments, shedding of the photoreceptive membrane shows a circadian rhythmicity under conditions of constant darkness or constant illumination[5]. Although it has been shown that the turnover processes of crab photoreceptive membrane are controlled by both the ambient light conditions and an endogenous factor[6-8], circadian involvement of the phenomenon has not been demonstrated either in crabs or in any other invertebrate photoreceptors.

In this report, we show that the changes in the amount of photoreceptive membrane and visual pigment chromophore in the grapsid crab have a long-lasting circadian rhythmicity.

Materials and methods. Adult *Hemigrapsus sanguineus* of both sexes (carapace width 20–25 mm) were collected in the Nojima Park, Yokohama City, Japan. Before the crabs were used for experiments, they were kept in a 12:12 light/dark regime (L = 09.00–21.00 h) for at least 2 weeks at 20 °C in a environment controlling system (Koitotron). Experiments under various light conditions were also carried out in the same system. The light intensity of the illumination period was about 2000 lux; a similar brightness to the natural habitat.

Compound eyes were dissected under a dim red light (during the dark period) or room light (during the light period) and were embedded in Epon 812 following a conventional fixing procedure. To compare the change in rhabdom size, rhabdom occupation ratios of individual ommatidial retinulae (ROR) were measured from light micrographs. Cross-sections through 16–20 hexagonally arranged ommatidia were examined from the forward looking eye region at the nuclear layer of the retinula cells. Rhabdom area and inter-ommatidial distances were measured by an image-analyzing system connected to a computer (NEC 9801E). The mean value of all inter-ommatidial distances for an eye was taken as the mean ommatidial eye diameter for the ROR calculation.

For measurements of the chromophore content by high-performance liquid chromatography (HPLC), the compound eyes were dissected out and isolated from the eye stalks under a dim red light at 03.00 and 15.00 h. All procedures for quantifying the chromophore content in a single compound eye by HPLC have already been described in a previous paper[4].

Each point in the following figures indicates the mean value (± SE) of data from at least five individuals.

Results and discussion. We already reported that the ROR of *H. sanguineus* was 8 times larger at night than during the day at 20 °C[4]. As shown in figure 1, the daily change in the ROR under an LD cycle continues under DD condition for at least 10 days. Such a long-lasting free-running rhythmicity has not been demonstrated in any crab before, and clearly indicates that the endogenous factor which controls the rhab-

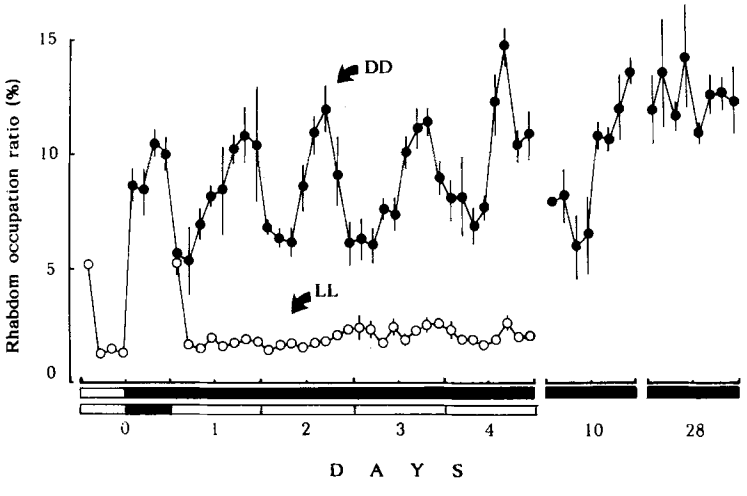

Figure 1. Change in the rhabdom occupation ratio (ROR) under a normal LD light regime followed by constant darkness (DD) for 28 days (filled circle) or constant light (LL) for 4 days (open circle). Each data point represents the mean ROR value of 5 different individuals, with a standard error bar. Circadian rhythmicity of the ROR change is obvious after at least 10 days of total darkness whereas no periodical change is observed under LL condition.

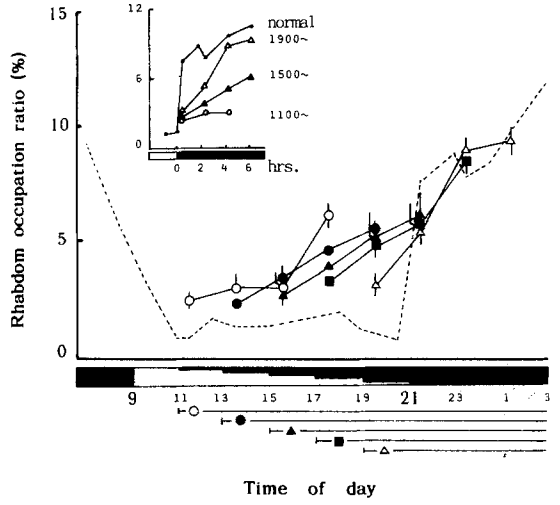

Figure 2. Effects of abrupt cessation of light at various time of the daytime on the ROR recovery. The time of lights-off in each experiment is shown below the abscissa. Each data point represents the mean ROR value for 5 different individuals, with a standard error bar. Inset: same data plotted against the time after lights-off.

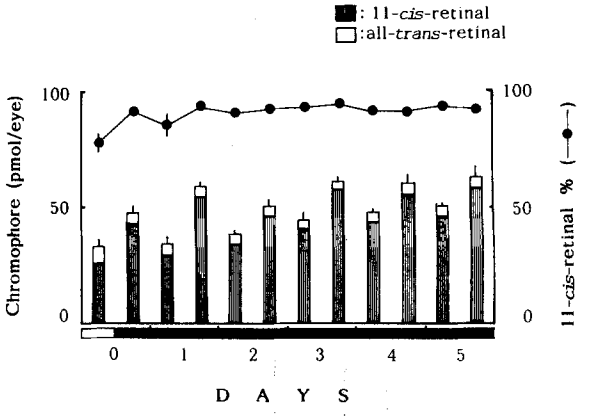

Figure 3. Changes in the total amount of chromophore containing both 11-*cis*-retinal (hatched) and all-*trans*-retinal (unfilled) under total darkness for 5 days. Each column represents the mean value for 5–12 individuals with a standard error bar (left ordinate). Relative amount of the 11-*cis*-retinal is almost constant during the dark period (right ordinate). Each data point represents the mean ROR value of 5 different individuals with a standard error bar.

dom size is strongly coupled with a circadian biological clock as in some vertebrates [5, 9, 10]. On the 28th day of DD, however, ROR remains constant, which indicates that the activities of membrane shedding and synthesis become equal. On the other hand, no periodical change at all is observed under constant illumination, and the ROR stays constant at about 1% (fig. 1). This suggests that the inhibitory (and/or accelerating) effects of light on the activities of membrane synthesis and incorporation (and/or breakdown) exceed those exerted by the circadian clock, or that the activity of the clock itself is suppressed by light.

Figure 2 shows the effects of abrupt cessation of light at various times (11.00, 13.00, 15.00, 17.00, 19.00) during the day on the ROR value. In all cases, the abrupt cessation of light during the daytime causes an increase in the volume of the rhabdomeric membrane. It is noteworthy that the later the lights turned off, the faster the ROR recovered. Considering the morphological observation that many ER, vacuoles and pinocytotic vesicles are found in the retinula cells of *H. sanguineus* just before dusk [4], together with the present results shown in figure 2, it seems very likely that the new membrane components for the night rhabdom could be prepared under the control of the clock during the daytime. This also confirms the circadian nature of the ROR change.

The rhabdom is the site at which the visual pigment rhodopsin is localized. Figure 3 shows the changes in the total amount of chromophores (columns) and the relative content of 11-*cis*-retinal (line) under DD condition. The relative amount of 11-*cis*-retinal at midday of day 0 (76.7% ± 3.51; mean ± SE; n = 5) is significantly lower than that at midnight on day zero (91.1% ± 0.46; mean ± SE; n = 5) ($p < 0.05$, Mann-Whitney's U-test), whereas no significant differences are observed between the data during the prolonged dark period. This indicates that the transformation of the visual pigment is caused only by light. On the other hand, the total amount of chromophore increases during the subjective night and decreases during the subjective day and this lasts at least for 5 days. This indicates that new rhodopsin molecules are synthesized in parallel with the synthesis of the new membrane component under the control of the biological clock. The source of the chromophore molecules of newly synthesized rhodopsin may be retinol or retinyl ester [11]. There must be a number of chemical processes leading from the synthesis of retinal to an eventual increase in the size of the rhabdom. It is clear that at least one of the processes is controlled by the circadian clock although we have no direct evidence to specify which of the processes this is.

The circadian change in the structure and function of the compound eye has been studied in the chelicerate *Limulus* [12]. In *Limulus*, efferent nervous activity is involved in the function of the biological clock, but shedding of photoreceptive membrane is not controlled by efferent nerve activity but only by ambient light conditions [12]. In contrast, the turnover of photoreceptive membrane in *H. sanguineus* is apparently controlled by a circadian biological clock. How the biological clock controls the turnover of the photoreceptive membrane and visual pigment, and where and how it is localized, are questions which await further elucidation.

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