EVOLUTION of CIRCADIAN CLOCK

Edited by
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Preface

The theme of 5th Sapporo Symposium was "Evolution of Circadian Clock" which consisted of prize-winner's lecture, plenary lectures, 5 sessions of various topics, poster session, and satellite symposium, covering the topics from the level of genes, cells, organs to individuals and even to social life.

This symposium was of triple significance to us; firstly because this marked, I would say, the 10th Anniversary of the Sapporo Symposium. Secondly because the title of the symposium was well-timed in view of the state-of-the art of this research field. The last, but not the least, on the occasion of this symposium Professor Doctor Jürgen Walther Ludwig Aschoff was conferred upon the honorary degree from the Hokkaido University for his great contributions not only to the development of rhythm research in the world, but to the initiation and cultivation of the Sapporo Symposium. Thanks to having every leading speaker of each topic join us, this memorable symposium was certainly stimulating and fruitful. We are also grateful to those who were concerned and also who have consistently supported the Symposia, especially Professor Dr. Jürgen Aschoff, Dr. Keizo Honma and others. Personally, special thanks are due to Professor Ken-ichi Honma and his staff for their efforts to have maintained the Symposia.

I should here mention remarks of Professor J. Woodland Hastings at the closing session of the symposium. He remarked the importance of future research on evolutionary aspects of circadian clocks and evaluated our timely selection of the topic. However, so far, less was presented than what was expected, and he hoped much to be done in the near future.

A decade of activity, I should say, is an achievement of something, in a sense that, as Goethe wrote in his Faust,

Das erste steht uns frei!
beim zweiten sind wir Knechte. (line, 1412)
(Literally this means, In the first one we are free of, but in the second we are slave to.)

Certainly, the first one is full of ambition and energy, but it is usually difficult to keep the level of activity (vigor) thereafter. Thanks to the enormous fervor and devotion of all who were concerned, the Sapporo Symposium survived a period of decade and looks more promising for years to come.

Since this is my last opportunity of chairmanship of the symposium, I would thank again all the people and participants for their cooperation for the past decade. Now, taking advantage of this opportunity, I would add a few words

about my expectation of the future of the symposium. As I understand it, the biological oscillation is the very key process in the functional and structural organization of the living organism. In the past, many great pioneers in the field of rhythm study paved the way to understanding of the most basic oscillation of the organism. And we still continue to walk on the paved road, leading to elucidation of the top secrets of circadian temporal organization.

I suppose, however, the time will come soon when we have to deal with biological oscillations of more irregular and random fashion and to think out their physiological implications, and at the same time, we have to clear up the basic relation between random and regular fluctuations. This query may be somehow related to an evolution of circadian clocks. What sort of theory is needed, then? And what kind of technique for analysis should be devised?

Anyway, if this is successfully achieved, the Sapporo Symposium on Biological Rhythm will transform into the "Sapporo Symposium on Biological Oscillation". I believe this theme will be one of the hottest topics during the coming century. And I do hope that, by so doing, Sapporo be a center of excellence in biological research.

Tsutomu Hiroshige, M. D., Ph. D.
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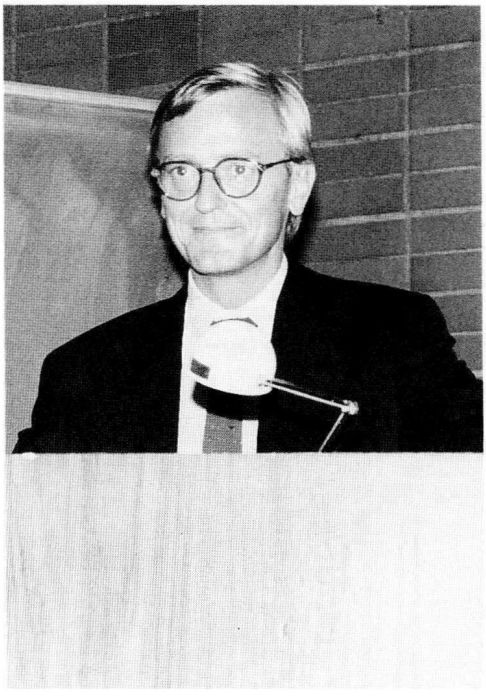

Dr. Till M. A. Roenneberg was born in May 1953 in Munich Germany. He studied photobiology at the Institute for Medical Psychology University of Munich and received his Ph.D in 1983. He became Assistant Professor in 1988 at the Institute for Medical Psychology, Medical Faculty University of Munich, and finished Habilitation in 1993. At present, he is Associate Professor at the same institute.

Dr. Till Roenneberg has made major contribution to our understanding of the cellular and molecular mechanism of circadian system, especially of photic entrainment of circadian clock. In his earlier study, he has found a substance which shortened the period of bioluminescence rhythm in a unicellular alga, *Gonyaulax polyedra*, and succeeded to identify the substance as creatine. In the following study, Dr. Roenneber has demonstrated two photoreceptors involved in the circadian clock of *Gonyaulax*. His scientific career was highlighted by the finding of two oscillators in one cell in 1993. This finding provides for the first time a concrete background for the multi-oscillatory model of circadian clock, since Professor Jürgen Aschoff has suggested two oscillatory mechanisms in humans in 1967.

The *Gonyaulax* Circadian System: Evidence for Two Input Pathways and Two Oscillators

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The circadian system is often depicted as a simple input-output diagram with the oscillator in its center. Yet, research on many different organisms has shown it to be far more complex (43). There are, for example, indications for feed-back loops both on the input and the output side. The oscillator can control the sensitivity of its input pathway (9), and the concept of a non-photic phase response curve (PRC) shows that the control of an overt rhythm may feed back on the oscillator itself (27). Although these possible feed-back loops are not believed to be part of the pacemaker's actual rhythm-generating mechanism they may well contribute to changes of the circadian system in response to experimental conditions and they certainly make the interpretation of results more difficult. Further complications arise from the fact that the circadian system of higher organisms may involve several oscillators, be it as putative morning and evening oscillators which both drive the same rhythm (30), as a hierarchical combination of a master and slave oscillators (30), or as equal but coupled circadian oscillators each of which drives different rhythms (2, 7, 22).

Our research on the effect of light on the circadian system of the marine unicellular alga *Gonyaulax polyedra* indicates that the complications described above are not features restricted to multicellular organisms but are also present in the circadian organization of a single cell.

The circadian system of *Gonyaulax*

Gonyaulax displays circadian rhythms in many aspects of its physiology and behavior and is thus an excellent model to study a circadian system which drives many rhythms at different phases within a single cell. The nightly bioluminescence is emitted both as brief flashes of light, which reach a maximum in frequency around CT 18, and a sustained low intensity glow which peaks at the end of the subjective night (10, 13). The molecular machinery of bioluminescence requires enzyme, substrate, and a substrate binding protein (LBP). The levels of all three are controlled by the circadian system (17). This control acts, at least in the case of LBP, at the translational level of protein synthesis (26).

The molecular components necessary for both the glow and the flashes are found during the subjective night in compact organelles called scintillons (28). These are located in tight membrane invaginations of the vacuole. The two rhythms of bioluminescence are, however, believed to be generated by different mechanisms. Flashes are triggered by action potentials across the vacuolar membrane (8) which reach a maximum frequency around CT 18. The sustained glow occurs at the end of the subjective night and is thought to be associated with the degradation of the scintillons (10).

The rate of photosynthesis (11, 44) and the activity of the superoxide dismutase (5) are greater during the day than at night, and cell division is restricted to the early morning hours (14, 46). Like many other unicellular organisms (32), *Gonyaulax* cells show a type of swimming behavior which gives rise to complex bioconvection patterns (35, 37). During the day phase, the cells form aggregations near the water surface, while the cells sink during the subjective night to form a loose "carpet" on the bottom of the container. The fact that some of these different circadian hands can be recorded automatically (37, 49) is a great advantage and many interesting features of the circadian system appear when two rhythms are recorded simultaneously for several weeks.

Tonic effects of light

Light is by far the most important environmental input to all circadian systems, and its effects have been extensively investigated. Curiously, little is known about the transduction mechanisms by which light signals produce changes in the phase and/or period. Both phasic (non-parametric) and tonic (parametric) light effects contribute to entrainment, and it has been suggested that the two effects occur via the same mechanism (6). In most organisms, the period under constant conditions (τ) depends on light intensity (1), with τ either shortening or lengthening as the light intensity is increased. These opposing responses have been attributed to the different PRC-shapes of the pacemakers in different organisms (6). In *Gonyaulax*, τ can be both shortened and lengthened with increasing light intensity, depending on the spectral composition of the constant illumination (38). Increasing the intensity of short wavelength light ($<$ 400 nm) shortens τ, while the period lengthens in higher intensities of long wavelength light (Fig. 1). Thus, the circadian system of *Gonyaulax* perceives light via at least two receptors and/or input pathways. Their effects appear to be additive because spectrally balanced white light does not alter τ significantly when the intensity is changed.

The fact that creatine shortens the period of the *Gonyaulax* (39) bioluminescence rhythm also indicates that there are two different light input mechanisms. Creatine shortens τ in short wavelength light but is almost ineffective in long wavelength light (41) (Fig. 2). Thus creatine appears to act on the light transduction mechanism responsible for the circadian responses to short wavelength light. The involvement of light transduction in the effects of creatine is suppor-