Hypothesis

A MODEL FOR THE MOLECULAR BASIS OF CIRCADIAN RHYTHMS INVOLVING MONOVALENT ION-MEDIATED TRANSLATIONAL CONTROL

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1. Introduction

Circadian rhythms with periods of about 24 h are ubiquitous amongst both unicellular and higher organisms and are manifest in a number of biochemical, physiological and behavioural parameters [1,2]. It is usually assumed that these rhythms have an underlying biochemical basis which is common to them all and several models have been proposed in attempts to explain this biochemical basis. These models have not been wholly successful in accounting for the experimental evidence relating to circadian rhythms and for that reason I wish to propose an alternative molecular model. This model is an attempt to explain the circadian rhythmicity shown in various biochemical and physiological parameters by unicellular organisms. It may well be improper to impose such molecular models on the physiological and behavioural circadian rhythms of higher organisms since these are manifestations of a higher level of organisation consisting of a multiple of oscillators (since each cell in a multicellular organism can be regarded as a circadian oscillator) capable of interacting with one another in order to bring about concerted action. Any proposed model for the molecular basis of circadian rhythms must be able to explain the following characteristic properties of and experimental facts concerning circadian rhythms (detailed in [3]):

1. They are endogenous in that they persist even under conditions of constant light and temperature.

2. The period of the oscillation is largely independent of temperature provided that it is relatively constant; this is known as temperature-compensation.

3. The phase of the oscillation may be altered by pulses of light, darkness or temperature and the time at which the pulse is given during the cycle determines the size and direction of the phase shift.

4. Ion transport and continued protein synthesis have both been implicated in the maintenance of circadian oscillations. Phase shifts have been demonstrated in rythms of *Phaseolus* [4] and *Gonyaulax* [5] due to the action of the K^+ ionophore valinomycin and K^+ itself has been shown to cause a phase shift in *Aplysia* [6]. Experimental evidence in support of a role for continued translation in circadian oscillation has come from work on the effects of protein synthesis inhibitors on the phase of rhythms in *Acetabularia* [7], *Aplysia* [8,9], *Euglena* [10] and *Gonyaulax* [11]. In a systematic study of the effects of cycloheximide, cycloheximide-sensitive and cycloheximide-insensitive phases could be distinguished within a circadian cycle in *Acetubularia* [7].

2. Models of the molecular mechanism of circadian rhythms

Four basic models of the molecular mechanism of circadian rhythms in unicellular organisms have been proposed which are as follows:

A. Several workers [12–14] have suggested that the circadian clock is a complex feedback system

combining a number of the biochemical pathways of the cell; this is known as the network hypothesis.

B. The 'chronon model' [15] is based on the sequential transcription, one gene at a time at a fixed rate. Transcription of the whole genome is supposed in this model to take around 24 h.

C. A membrane model has been proposed [16] in which a feedback system operates whereby a membrane protein involved in the transport of K^+ can be changed from an active into an inactive configuration and vice versa at critical intracellular K^+ concentrations leading to a periodic variation in ion transport. Temperature compensation can be explained in this model (and indeed in any other model involving membranes) by changes in the lipid composition of the membrane in order to maintain a constant membrane fluidity following alterations in temperature. These changes would affect the activity of the ion-transport protein.

D. A more recent model is the 'coupled translation–membrane model' [3]. In this model the synthesis, transport and membrane insertion of a membrane protein is regulated by a feedback mechanism. It is suggested that the degree of insertion of the protein into the membrane and the resulting change in the functional state of the membrane is coupled to its insertion by a feedback process such that insertion of the protein into the membrane reduces the rate of its synthesis, transport or insertion.

Models A and B have been shown to be inadequate in accounting for many of the experimental facts [3]. Model C while capable of explaining much of the data cannot account for the long time constant of the circadian rhythm or the experimentally demonstrable requirement [7–11] for protein synthesis in maintaining the phase of the rhythm. Model D while also capable of explaining much of the experimental data does not account for the effects of altered levels of monovalent ions, particularly K^+, on circadian rhythms [4–6].

3. Monovalent ions and translational control

Recent work has demonstrated the influence of the monovalent ion concentration on translation of mRNA. Thus the formation of cataracts in the embryonic chick eye has been associated with changes in the Na^+/K^+ ratio which differentially affects the translation of the mRNAs coding for the two polypeptide components of δ-crystallin the principle protein of the embryonic chick lens [17]. Differential effects of Na^+ concentration on the in vitro translation of host cell and animal virus mRNA [18] has led to the suggestion that alteration of intracellular monovalent ion concentration may be a general mechanism employed by animal viruses to shut-off host cell protein synthesis while allowing viral protein synthesis to occur [19]. Thus in two situations the rate of translation of mRNAs has been shown to be altered by changes in the monovalent ion concentration and indeed the overall rate of protein synthesis in cells had previously been shown to be dependent on the intracellular K^+ concentration [20].

4. Monovalent ion-mediated translational control in circadian rhythms

In the light of the above evidence concerning the effect of monovalent ion concentration on translation, I wish to propose an alternative model for the molecular basis of circadian rhythms which is basically a synthesis of models C and D above and which I believe is capable of accounting for all the characteristic properties of circadian rhythms. In this model the rate of synthesis of a membrane protein involved in ion transport (possibly the Na^+/K^+ ATPase or a modulator of this activity) is regulated in a feedback fashion by the intracellular monovalent ion concentration. As the membrane protein is synthesized and inserted into the membrane its activity leads to a change in the monovalent ion concentration which would inhibit its own synthesis. This situation would last until, due to the normal turnover of the membrane protein and a resulting change in the monovalent ion concentration in the opposite direction, a critical point is reached at which synthesis of the membrane protein is switched on again. The long time constant of the circadian rhythm can be accounted for by the long half-life of

the membrane protein and the time lag between its synthesis and its subsequent transport to and insertion into the plasma membrane. This model will account for the features of circadian rhythms accounted for by models C and D above and also the importance of both continued protein synthesis and intracellular K^+ levels. The alteration in protein synthetic activity brought about by changes in the intracellular ionic environment would not only change the rate of synthesis of the membrane protein but also the rate of synthesis of other proteins. Thus it is possible to explain how the functioning of one biochemical system with an in-built circadian rhythmicity could lead to the appearance of circadian rhythmicity in a number of biochemical processes within the cell. Attunement of the circadian rhythm to light–dark cycles within the environment would be brought about by the action of photo-sensitive ion gates in the plasma membrane.

This model makes the following predictions:

(i) There is a circadian variation in the amount in the membrane of a protein involved in ion transport.

(ii) There is a circadian variation in the rate of synthesis of this membrane protein.

(iii) There is a circadian variation in the levels of monovalent ions within a cell.

(iv) The translation of the mRNA coding for the membrane protein is sensitive to the above changes in the monovalent ion concentration during the circadian cycle.

If this model is to be validated then all of these predictions must be experimentally tested (which is possible) and confirmed. The critical prediction which is not made by other models and which would allow discrimination between models is (iv). This prediction can be tested when and if the postulated membrane protein is identified.

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