Abstract

An autonomous self-sustained ~24 h oscillation of metabolic activity functions as a physiological clock to allow living systems seasonal adaptation of behaviour, like flowering or hibernation. Until recently circadian rhythms were considered to be a characteristic of eukaryotic cells despite a very early report by Halberg on the observation of a circadian rhythm in E. coli. With the detection of circadian oscillations of metabolic activity in Synechococcus, circadian rhythmicity has proven to exist on all levels of biological organisation from prokaryotic cells to unicellular eukaryotes to higher plants, animals and man. Circadian rhythmicity is an in viva feature of living cells and cannot be observed in vitro. Higher frequency oscillations, however, can be found in many biochemical reactions in vitro as well as in viva. This overview on rhythmic organisation of metabolism in living systems will discuss how macro-parameters like pH, ionic balances (osmos), redox state and phosphorylation potential or hydrophobicity control metabolic functions like photosynthesis, respiration, nitrogen fixation. The temporal control of [...]
From cellular micro-compartmentation to inter-organ communication. The kinetic basis for molecular controls in photoperiodism

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Summary

An autonomous self-sustained \textasciitilde{24} h oscillation of metabolic activity functions as a physiological clock to allow living systems seasonal adaptation of behaviour, like flowering or hibernation. Until recently circadian rhythms were considered to be a characteristic of eukaryotic cells despite a very early report by Halberg on the observation of a circadian rhythm in \textit{E. coli}. With the detection of circadian oscillations of metabolic activity in \textit{Synechococcus}, circadian rhythmicity has proven to exist on all levels of biological organisation from prokaryotic cells to unicellular eukaryotes to higher plants, animals and man. Circadian rhythmicity is an in \textit{vivo} feature of living cells and cannot be observed \textit{in vitro}. Higher frequency oscillations, however, can be found in many biochemical reactions \textit{in vitro} as well as \textit{in vivo}.

This overview on rhythmic organisation of metabolism in living systems will discuss how macro-parameters like pH, ionic balances (osmos), redox state and phosphorylation potential or hydrophobicity control metabolic functions like photosynthesis, respiration, nitrogen fixation. The temporal control of metabolic pathways involves oscillatory networks in transcription, translation or post-translational modulation of protein structure and function. Changes in macro-parameter status are due to precise feedback networks in basic metabolism leading to a circadian rhythm in overall energy metabolism and thus in metabolic control of timing.

Multifactorial changes in environmental conditions like light intensity and -quality, temperature, water status, ionic balances, pressure, etc. are transduced into a network of intracellular signal processing leading to a combinatorial interaction of transcription factors and outputs with cell-, tissue- and organ-specific response-patterns. Compartmentation is coupled to vectorial metabolism and electron transport and the involvement of membrane pores and ion channels. The generation of specific changes in membrane potential can be expected. The physical state of membranes is involved in the transduction of temperature signals and the control of expression of nuclear,
mitochondrial and plastid genes which also can be modulated by photoreceptors like the red / far red reversible phytochromes, the blue / UV-A and the UV-B photoreceptors and the feeding of sugars. Membrane-bound processes are controlling and vice versa are controlled by voltage gated ion channels and pores, giving rise to an overall electro-chemical-hydraulic integration of organs and the whole organism on the basis of Mitchell’s chemiosmotic theory of energy transduction. The electrophysiological integration offers the possibility for characterisation of cells, organs and organisms by electrophysiograms as a means for non-invasive continuous in vivo monitoring of physiology and behaviour.

1. Introduction: The chemi-osmotic theory of energy transduction as related to physiological oscillations and circadian rhythm

Rhythmicity is one of the characteristics of life which expresses itself at all levels of organisation from unicellular systems to man. Rhythmic phenomena in physiology, development and behaviour of all living systems show period lengths ranging from fractions of a second to hourly, daily and even annual cycles.

The most conspicuous rhythm is the so-called circadian oscillation. By definition, circadian rhythms have the following basic features: They persist for some time in the absence of diurnal (i.e. daily) fluctuations in temperature and illumination; their phase can be altered by a brief disturbance of the constant regimen; and their period is relatively independent of constant external temperature, at least within the physiological range.

The circadian rhythm is an endogenous oscillation of metabolic activity with a period length of exactly 24 h when the organisms are synchronized by the daily light-dark cycle of the earth. In constant conditions, however, its period length is only approximately 24 h, i.e. circadian. In contrast to biological rhythms showing other frequencies, circadian rhythms are temperature compensated and almost unsusceptible to chemical manipulation. It is this stability or homeostasis of period length which qualifies the circadian rhythm as a precise physiological timer and thus is the essence of Bünning’s [9,10] theory of the physiological clock.

Despite almost 70 years of effort, searching for the mechanism of circadian rhythms [8], the functioning of the physiological clock is not yet understood. However, there are good reasons to believe that circadian rhythmicity is based on the molecular properties of enzymes and multi-enzyme complexes, on allosteric and feedback control and on the regulatory functions of biomembranes. From an evolutionary point of view, circadian rhythmicity has been considered to be an adaptation of pro- and eukaryotic energy conservation and transformation to optimise energy harvesting by photosynthesis in the daily cycle of energy supply from the environment [13,62,63,71]. It was also assumed that this adaptation is dependent on the division of energy transformation within different compartments of the cell, such as chloroplasts, mitochondria and the glycolytic space [11,32] involving redox mediated transcriptional controls [7]. In photosynthetic prokaryotes, lacking cell organelles, a metabolic micro-compartmentation allows for a similarly sophisticated regulatory network as in eukaryotes [52,75]. Assuming that energy metabolism is the basis of physiological rhythmicity, it is appropriate to review briefly the structural and functional features of Mitchell’s [42] chemiosmotic hypothesis of energy transduction,
as it is the most elaborate and best-supported mechanism of energy flow through living systems.

Mitchell’s [41] basic proposition was that there are vectorial transport processes based on metabolic reactions which themselves are organised vectorially. In particular, he suggested that in a membrane a metabolic process catalysed by an anisotropic group-translocating enzyme and carrier system should have not only the scalar property of chemical transformation, but also the vectorial osmotic property of membrane transport. To describe this type of vectorial chemical process he introduced the term ‘chemiosmotic’. It was the application of this chemiosmotic concept to the problem of respiratory and phosphorylation metabolisms that led to the formulation of the chemiosmotic hypothesis of oxidative and photosynthetic phosphorylation in mitochondria, chloroplasts and bacteria.

The chemiosmotic hypothesis is based on four fundamental postulates corresponding to four structural and functional systems [42], which are of basic importance to our concept of circadian rhythmicity in energy transduction and osmoregulation of organelles and cells [26,29,35,70]:

1. The ATP synthase is a membrane-located, reversible, proton-translocating ATPase, having characteristic H⁺/P stoichiometry (i.e. characteristic ratios in the transport of protons and phosphate).

2. Respiratory and photoredox chains are membrane-located, vectorial, metabolic, proton-translocating systems, having characteristic H⁺/2e⁻ stoichiometry.

3. There are proton-linked (or hydroxyl ion-linked) solute transport systems for osmotic stabilisation and metabolite transport.

4. Systems (1)-(3) are plugged through a topologically closed insulating membrane (vesicle), called the coupling membrane.

By translocating protons, the redox or photoredox systems generate a protonic potential difference across the insulating membrane. Mitchell considered it to be similar to an electrical potential difference and derived from this the concept of ‘proticity’, which is like that of electricity, but refers to the flow of protons in place of electrons. The transduction of energy by proticity is simple and effective because all that is required is a lipid membrane vesicle separating two aqueous proton conductor phases.

The molecular mechanisms of the proton-translocating redox (or photoredox) chain systems must involve specific translocational (conformational) mobilities of the polypeptide and lipid complexes that participate in the catalysis of the chemiosmotic reactions within and across the membrane. Thus it was emphasized by Mitchell that chemiosmotic reactions, in general, are expected to involve specific conformational interactions and conformational energy transductions within the translocation system by which they are catalysed.

In his hypothesis, Mitchell postulated the direct involvement of electron transport components in the pumping of protons across the membranes of mitochondria, chloroplasts and chromatophores. The cell apparently uses the electrochemical gradient created by the proton pump for ATP synthesis. Thus in mitochondria the free energy of oxidation is transduced by an ATPase into the free energy of hydrolysis of ATP. In the photosynthetic systems of chloroplasts and bacteria the major part of the energy trapped in the photochemical reactions is conserved as a gradient in the
electrochemical activity of $H^+$ across the coupling membranes, which is generated by
the flow of electrons and $H^+$ through an anisotropically (vectorially) arranged electron
transport chain.

Coupling factor ATPases function to couple electron transport in mitochondria,
chloroplasts and bacteria to ATP formation. These reversible proton-translocating
ATPases are the link between proton pump and ATP level in the cell. The proton-
translocating ATPase is the most widely distributed of the cation-translocating
ATPases, for it is found not only in bacterial plasma membranes, in mitochondria and
in chloroplast thylakoid membranes but also in the plasma membranes of yeasts,
moulds and green plants.

The above mentioned features of the chemiosmotic hypothesis should be kept in
mind when considering circadian metabolic activity as a function of the coupling of
high-frequency oscillations in different energy-transducing sequences to a circadian
rhythm of energy transduction of the entire cell. Circadian rhythmicity probably
depends on the interaction of highly inter-related reaction compartments of energy
metabolism which are coupled through proton-motive redox chains and metabolite
transport systems to produce a circadian rhythm in proton flow (or proticity) and
concomitant ion movements through the system, i.e. a circadian rhythm in energy
transduction.

Concerning lunar rhythms, it may follow from Mitchell's theory that in marine
organisms living in the tidal zone osmoregulation and the mechano-electrochemical
coupling might be the basis for their timing of the tidal pattern by a physiological
clock, for precise adaptation of their behaviour (e.g. Clunio or fiddler crabs) to a very
specific environment.

In searching for the molecular mechanisms underlying physiological rhythms, it
seems appropriate (1) to stress the ubiquity of high-frequency oscillations in
biochemical reaction chains, with certain key enzymes as molecular high-frequency
oscillators; (2) to emphasize the separation of different energy-transducing metabolic
sequences in different organelles, especially in eukaryotes; and (3) to focus attention
on membrane systems as the sites for control and interaction of cellular energy
transduction, with internal and environmental signals [5,26,29,43,58].

High-frequency oscillations in energy-transducing metabolic sequences could give
rise to low-frequency oscillations of energy flow in the metabolic network of the
whole system, providing a basis for the evolution of a temperature-compensated
circadian rhythm [63]. The metabolic network is controlled by redox and

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Figure 1. Metabolic compartmentation and signalling networks in plant cells. 1A: Photoreceptor mediated compartmental interactions between nucleus, chloroplasts and mitochondria as discussed by Anderson, Chow and Goodchild in 1988 [3], and ten years later, 1B: Networking between phytochrome A (phyA) a blue light receptor (cry1) and inhibition of light responsive genes by sucrose (Suc) via SUN proteins (SUN1,6,7). The signal transduction network is highly interactive between specific inputs modulated by feedback/feedforward and threshold functions. This is demonstrated for the gene regulation of chalkone synthetase (ChS), chlorophyll a/b-binding protein (CAB), plastocyanine (Pc) and asparagine synthetase (As1) (modified after Genoud and Métraux [19]).
From cellular micro-compartmentation to inter-organ communication

**A**

**PHYTOCHROME**

Pr $\leftrightarrow$ Pfr

**BLUE LIGHT**

X $\leftrightarrow$ X*

**CHLOROPLAST**

DNA

RNA

proteins

PChl

Chl

**MITOCHONDRION**

DNA

RNA

proteins

**B**

Far-red light

Blue light

phyA

PSII

cGMP

ChS

As1

Pc

CAB

SUN1,6,7

$\text{c}^+$ and CaM

$\text{Ca}^{2+}$ and CaM

$\text{cry}\text{-Modulated blue-light responses}$
phosphorylation potential, or charge [65] and by the modulation of the hydrophobicity of key elements. The circadian system, the clock’s periodicity, is genetically determined and provides the temporal frame for physiological and behavioural patterns that are necessary for adaptation of organisms and populations to environmental constraints. In the photoperiodic acclimation of organisms, photoredox systems most likely function in signal transduction as modulators of vectorial metabolism in general and in feedback control of oscillating transcription rates in cellular and organismic adaptation in particular. From an evolutionary point of view the circadian rhythmic cell is a hydro-electro-chemical oscillator driven or synchronized by the daily dark/light cycle with a temporal compartmentation of metabolism and a network of metabolic sequences to compensate for oxidative stress in adapting to their light environment. This is best shown in the adaptation of photosynthetic machineries from bacteria [7,32,52,75] to higher plants [3,4], which respond to changing light quality and quantity with coordinated changes of pigmentation, electron transport components, membrane composition, - organisation and function [3]. The light environment of plants may vary by three orders of magnitude in irradiance, in addition, the irradiance available for photosynthesis varies seasonally, diurnally and spatially. The acclimation of plants at the cellular level requires interaction between the nucleus, mitochondria and chloroplasts in a regulatory network involving several photoreceptors like phytochromes, blue light receptors and chlorophyll as shown in Figures 1A and B. Figure 1A reflects the state of the art from 1988 [3] while Figure 1B outlines a signal transduction network based on recent molecular analytical data on signal transduction chains and pathways of gene regulation [19].

The plasma membrane is the interface between apoplast (extracellular matrix) and symplast (protoplast) and as such the structure for integration of environmental and endogenous signals in cellular or organismic adaptation.

Oscillatory metabolic activities are a general feature of the dynamic coupling and control which enable biological systems to be stabilized in space and time. Thus rhythmic variations and homeostasis are based possibly on the same system components.

2. Evolution of eukaryotism and the conservation of circadian rhythms in living systems

Homeostasis of living systems can be understood as the constancy of temporal relations among rhythmic biochemical and biophysical processes on all levels of biological organization. The experimental evidence indicates that the spatio-temporal organization of living systems might ultimately depend on the proper functioning of endogenous physiological clocks. Under natural conditions, the physiological clock is entrained by environmental ‘zeitgebers’ or synchronizers such as light-dark and temperature cycles. These environmental signals are perceived by photo- and thermoreceptors and are transduced into the modulation of metabolism, thus allowing adaptation of developing systems to cope with environmental constraints by appropriate photo- and thermoperiodic responses [59-62,64,67].

The eukaryotic cell probably evolved as an anaerobic autotrophic archaeabacterium in symbiotic association with a respiring eubacterium which generated hydrogen as a
waste product [15,39].

Originating from a reducing environment and creating an oxigenic environment with sunlight as the ultimate energy source, the living systems had to develop mechanisms to protect light-sensitive compounds or metabolic processes by developing a temporal compartmentation of reactions which preferentially operate in darkness and metabolic reactions which can or must operate in light (e.g. cyanobacterial nitrogen fixation and photosynthesis [7,21,27,32,52]). To protect living matter from light or light-driven electron transport, living systems had to evolve mechanisms to protect them from oxidative stress. The chloroplasts, responsible for oxygen evolution and energy conversion in higher plant cells, are equipped with a very sophisticated antioxidative system to compensate for light stress from a disturbed or imbalanced electron transport [4]. On the other hand, reactive oxygen species (ROS) have become very important regulators of metabolism [74].

Enzyme regulation due to a reversible oxidation-reduction change seems to be a very common mechanism for metabolic control in all living systems [6,7,75]. In chloroplasts, the light-dark activation and inactivation are mediated by the ferredoxin/thioredoxin system [51]. Thioredoxins are a group of small proteins which, through their ability to undergo reversible SH/SS redox changes, act as hydrogen carriers in a number of different cellular reactions. In chloroplasts, thioredoxins couple ferredoxin with key regulatory enzymes, thereby linking light to the control of fundamental biochemical processes. Besides thioredoxin, specific enzyme effectors like pH, reduction charge, energy charge and ion movements are involved in the light-dark regulation of enzymes, on the transcriptional, translational and post-translational level [33,56,57,65,73].

3. Compartmentation, membrane state and a mechanism of circadian rhythmicity

The existence of circadian rhythmicity is dependent on the living cell and suggests that metabolic compartmentation is of significance for its generation [6,17,53]. Furthermore, circadian rhythms in photosynthesis, respiration and chloroplast shape suggest that investigation of the metabolic controls may be fruitful for the elucidation of the mechanism of biological rhythms. Interrelations between cellular compartments have already been shown. For example, Könitz [34] has shown reciprocal changes in the ultrastructure of chloroplasts and mitochondria in daily light-dark cycles. Murakami and Packer [45] demonstrated that, within the same cell, mitochondria swell and chloroplasts contract upon illumination and the reverse occurs in the dark. These metabolic interactions are mediated through the structurally integrated multi-enzyme systems of the oxidative phosphorylation reaction chains in the inner membranes of mitochondria and the vectorial electron transport chain of both light reactions across the thylakoids of chloroplasts (e.g. [6,19]. The importance of the entire metabolic network for the display of circadian oscillations is underlined by the fact that, in contrast to the temperature-compensated circadian oscillations of the intact system, isolated organelles display high frequency oscillations [22,25] which are temperature dependent. Similarly in photosynthetic bacteria, cellular signal transduction integrates microcompartmentation of photosynthesis, carbon dioxide assimilation and nitrogen
fixation [32]. From a detailed analysis of rhythms in enzyme activities involved in compartmental energy metabolism with and without feeding of sugars (cf. [31,69] and on changes of nucleotide pool size levels in the short-day plant Chenopodium rubrum L. (see Table 1) we compiled evidence in favour of circadian rhythmicity in overall energy transduction. Originally our working hypothesis on the mechanism of circadian rhythmicity was based on observations of a circadian rhythm in betacyanine accumulation and betacyanine leakiness and the effects of glucose and phenylalanine feeding on amplitude and phase of these rhythms [63]. These observations lead us to suggest that circadian rhythmicity, as the timer in photoperiodism, should be based on a circadian rhythm in energy metabolism. This rhythm would be the result of a compensatory control oscillation between glycolysis and oxidative phosphorylation, coupled to photophosphorylation in cyanobacteria [27], photosynthetic bacteria and green plants [59-61,63,67,68]. This mechanism of circadian rhythmicity could involve energy control of ion transport processes at the membranes of cells and organelles. The membrane’s physical state, e.g. modulated by temperature, could control transcription [58] or via frequency coded calcium oscillations leads to differential gene activation and expression [14,36].

Table 1. Rhythmic phenomena in seedlings of Chenopodium rubrum L. (in brackets period lengths of sub-peaks) (Complemented after Bünning [10]).

<table>
<thead>
<tr>
<th>Phenomenon</th>
<th>Period length (h)</th>
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<tbody>
<tr>
<td>Photoperiodic light sensitivity</td>
<td>30</td>
</tr>
<tr>
<td>Betacyanine accumulation</td>
<td>24-30 (15)</td>
</tr>
<tr>
<td>Betacyanine turnover</td>
<td>24-30</td>
</tr>
<tr>
<td>Adenylate kinase activity</td>
<td>30 (15)</td>
</tr>
<tr>
<td>Energy charge (ATP + ½ ADP/ATP + ADP + AMP)</td>
<td>21-24 (11-13)</td>
</tr>
<tr>
<td>NADPH/NADP ratio</td>
<td>21-24</td>
</tr>
<tr>
<td>Dark respiration</td>
<td>21-24</td>
</tr>
<tr>
<td>Chlorophyll accumulation</td>
<td>15</td>
</tr>
<tr>
<td>Net photosynthesis</td>
<td>15</td>
</tr>
<tr>
<td>Triose phosphate dehydrogenase activity (NADH₂; NADPH₂)</td>
<td>15</td>
</tr>
<tr>
<td>Malate dehydrogenase activity</td>
<td>12-15</td>
</tr>
<tr>
<td>Glutamate dehydrogenase activity</td>
<td>12-15</td>
</tr>
<tr>
<td>Glucose-6-phosphate dehydrogenase activity</td>
<td>12-15</td>
</tr>
<tr>
<td>Gluconate-6-phosphate dehydrogenase activity</td>
<td>12-15</td>
</tr>
<tr>
<td>Pyridine nucleotide, pool size [NAD(H₂); NADP(H₂)]</td>
<td>12-15 (6)</td>
</tr>
<tr>
<td>Turgor controlled growth phenomena:</td>
<td></td>
</tr>
<tr>
<td>- stem extension rate</td>
<td>23-27</td>
</tr>
<tr>
<td>- leaf movements</td>
<td>23-27</td>
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</tbody>
</table>

Taking into account the symplastic organisation of higher plants, the concept of compartmental feedback becomes even more attractive in view of bioelectric phenomena. The symplastic organisation of higher plants might be the basis for
translocation of electric, photoperiodic and morphogenetic stimuli [18]. The energetic integration of the entire system could be based on the same symplastic organisation, so that proton translocation or a flow of 'proticity' [42] and concomitant ion movements would give rise to a circadian rhythm in electric potential paralleled by circadian leaf movements and stem extension rates [1,71].

The circadian rhythm in transcellular current of a single cell, as observed by Novak and Sironval [46] in *Acetabularia*, probably arises from the compartmental feedback between mitochondria chloroplasts and glycolysis, as suggested in our concept for a mechanism of circadian rhythmicity. The vacuole could be involved in this control net by acting as a reservoir for metabolites as in the case of oscillations in Crassulacean acid metabolism. A similar concept might hold true for photosynthetic active bacteria in general and their temporal organisation of metabolism, but certainly for circadian rhythmic behaviour of the cyanobacterium *Synechococcus* [27,30].

4. Hydraulic-electrochemical oscillations as integrators of cellular and organismic activity

As mentioned above, the symplast of higher plants is probably not only the network for rapid electrical integration of metabolic activities but also the route for the translocation of sucrose and the transfer of the flowering stimulus. An observation that may have some bearing on the significance of changes in membranes during signal transduction is the detection of alterations in the distribution of the endoplasmic reticulum in cells of the shoot apex of *Chenopodium album* after photoperiodic stimulation [20]. In spinach the plasma membrane of apical cells is modified during flower induction [12,49]. Changes in pH and Ca\(^{2+}\) patterning as measured with fluorescent dyes can be observed as the earliest events of photoperiodically inductive conditions in flower initiation in *Chenopodium rubrum* [2,72]. The endoplasmic reticulum may have particular significance in cell coordination since it is continuous with the nuclear membrane and with the intercellular connections as well and may be the network for protein translocation. In the case of flower induction, the temporal organisation of development at the apical meristem might involve rhythmic symplastic transport of metabolites and the interaction of rhythmic (bioelectric) signals originating in the leaves [46] or a frequency-coded electrochemical communication between leaves and the shoot apex [71].

Communication by surface membrane action- or variation potentials in higher plants has been observed for a series of systemic responses (for references, see Wagner et al[71]). Changes in action potentials triggered by light to dark or dark to light transitions can be related to changes in photosynthetic electron transport [55]. Observations on phytochrome action in the moss *Physcomitrella patens* are indicative of activation of plasma membrane anion channels [16] leading to membrane depolarisation as a very fast first step in signal transduction.

Bearing in mind the circadian rhythm in transcellular current reported in *Acetabularia* [47], it seems possible that a circadian rhythm in proton flow - of action and variation potentials - may be of great significance in the circadian coordination of the whole plant, and the communication between plant organs like the leaves and the shoot apex in photoperiodic flower induction [71].
In 'The cell as a resonating system', B. Goodwin [23] advanced the idea that the living cell is a resonating unit which cycles continuously through a set of states. The oscillating activity should represent a type of biological energy. Environmental signals are perceived and transduced into the modulation of metabolism and, as suggested by Goodwin [24], development and adaptation of living systems should be controlled by physiological oscillations. He postulated that spatio-temporal organisation should be effected by means of periodic intracellular events which propagate from cell to cell in the differentiating tissues.

Following a similar line of thinking, Mager [37,38] emphasized that oscillations seem to be essential for system organisation in biology. The above theoretical considerations are supported by the demonstration of endogenous rhythms in the various sequences of energy metabolism (cf. Table 1; [62]). The display of circadian rhythms in energy charge and reduction charge favour the concept that the many nonlinear oscillators of cell metabolism are coupled such as to evolve the circadian frequency of the system as a whole [65].

The internal coupling of metabolic pathways as well as the transduction of environmental signals is achieved through nucleotide and ionic (e.g., \( \text{Ca}^{2+}/\text{Mg}^{2+} \)) ratios via redox shuttles and transport systems in the different energy transducing biomembranes. Thus nucleotides and ions could function as secondary messengers in transduction and amplification of signals. In this way the network of energy transduction during perception and transmission of signals could involve changes in membrane potential and surface charge densities as general regulators of membrane protein activities. Change in membrane structure could be directly involved in information processing as suggested for signal transduction in cellular functions on cytoskeletal proteins [58]. In plants the microstructural and symplastic organisation could be the basis for integral electrochemical signal transmission.

An interplay between oscillating enzymatic reactions and contractile elements of the structural proteins of the cell has even been used to design a mechanochemical model of the biological clock [54], and most significant, with protoplasts from *Phaseolus* pulvinar motor cells a circadian rhythm in volume oscillations could be shown [40]. This 'electrochemical' view of metabolic control could be very relevant in relation to the mechanism of growth and differentiation. Numerous experimental findings show the involvement of stable electric fields in growing and differentiating cells. The

Figure 2. Flow diagram of postulated regulatory loops involved in circadian regulation of metabolism and behaviour on the cellular level. The basal regulatory loop generates a circadian rhythm in energy transduction which controls circadian rhythmic changes in sensitivity of membrane-bound receptors; in return, membrane-bound photoreceptors or secondary messengers via conformational changes of membranes synchronize the circadian rhythm in energy transduction with the daily light-dark cycle. The integration of metabolism is achieved via circadian rhythmic changes in redox- and phosphorylation potential and hydrophobicity. A circadian rhythm in protein synthesis depends on autoregulatory availability of transcription factors like PER or TIM and their phosphorylation to produce a translocation-competent heterodimer for control of transcription in the nucleus (for details, see text; after Wagner and Cumming [63]; Wagner et al., [71]).
LIVING SYSTEM characterized by spatial and temporal compartmentation and membrane bound activities; displays growth and differentiation within the limits of genetic information.

Environmental factors / signals such as TEMPERATURE, HUMIDITY, SUBSTRATE, PRESSURE, ELECTRIC IMPULSES, HYDRAULIC IMPULSES.

ENERGY TRANSDUCING SYSTEMS showing endogenous circadian rhythmicity determining energy dependent phenomena via phosphorylation- and/or redox potential, ionic ratios, changes of membrane state and the modulation of transport processes.

PHOTORECEPTOR SYSTEMS e.g. Phytochrome (photoisomerization acting through conformational changes of signal transducing components).

membrane bound or -binding PHOTORECEPTOR SYSTEMS.
experimental evidence indicates the possibility that the chemical reaction network is controllable by electric fields. This could open up a way to visualize the synchronisation of circadian rhythms by electric and magnetic fields and thus could allow for synchronizing inputs from so called subtle geophysical factors [48].

The elucidation of the molecular features of circadian time measurement and temporal organisation of adaptation of development has very much profited from studies with rhythm mutants of *Drosophila* and *Neurospora*. Certain gene products are responsible for the synthesis of components of the circadian pacemaker. From the interaction of the proteins coded by the genes *period (per)* and *timeless (tim)* with their own mRNA a rhythm in the content of proteins and transcripts is observed during a day, which continues under constant conditions. Maximum concentrations of PER and TIM proteins are shifted in time in comparison to their mRNA contents.

The basis for this timing is an autoregulatory negative feedback as schematized in Figure 2. At the end of the light phase PER and TIM form a heterodimer which is transported from the cytoplasm into the nucleus, there inhibiting the expression of *per* and *tim*. At the beginning of the light phase no transcription of the genes takes place. During the day the two proteins are degraded and the expression of *per* and *tim* begins again to start a new cycle.

PER, TIM and other 'clock proteins' influence their own production as transcription factors in feedback loops via threshold levels. The interaction of transcription factors is influenced by phosphorylation/dephosphorylation processes and is thus linked to energy metabolism. The interplay of regulatory loops and their connection to environmental 'zeitgebers' like light, temperature etc. is shown in Figure 2.

Both, in higher plants and cyanobacteria [28] there are indications from sequence similarities data, that photoregulated histidine kinases might be involved in sensory networks via phytochrome photoconvertibility [50].

Essential for the functioning of the regulatory loops in oscillatory protein synthesis in eukaryotes are transport processes between cytoplasm and nucleus which control in a circadian pattern the availability of transcription factors in the nucleus. In plants phytochrome and cryptochrome are primarily involved in the modulation of transcription factor dynamics (cf. Fig. 1B). The feedback control of oscillatory transcription seems to be an universal principle of (circadian) rhythms in protein synthesis. The control of development and behaviour necessitates the integration of a whole series of endogenous and exogenous signals which control the complex pattern of gene expression. The adaptation of biological systems thus requires the combinatorial interplay of a limited number of transcription factors as the basis for the wide spectrum of gene expression.

5. Conclusions

It is very likely that physiological rhythms are based on the same structural and functional principles underlying Mitchell's chemiosmotic hypothesis of energy transduction. Allosteric enzymes could function as molecular high frequency oscillators as in glycolysis while compartmental feedback between organelles with vectorially organized metabolic reactions in their enclosing membranes could give rise to a circadian rhythm in energy transduction by proton flow through the entire cell. Thus the coupling between compartmented metabolic sequences is possibly achieved
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through cycles in nucleotide ratios and ionic balances via transport mechanisms and redox shuttles (see Fig. 2). In the different energy-transducing biomembranes, which could act as coupling elements and frequency transformers. The membranes themselves could act as high-frequency oscillators [43,44]. Such high-frequency membrane oscillators could be the basis for perception and transduction of high-frequency signals from the environment [46].

The ratios of coupling nucleotides would be relatively temperature independent and the nucleotides themselves could thus, as rate effectors in compartmental feedback, fulfill the requirements for precise temperature-compensated time keeping. Proton flow and concomitant ion movement through the symplast could be the basis of rhythmic electrochemical integration of the whole plant [65,70].

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