LIGHT, TIME AND MICRO-ORGANISMS

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TIME DOMAINS IN BIOLOGY

Direct, individual human experience of the natural world can be measured in milliseconds or in decades. Our evolving picture of the world depends upon comparison of our individual experience with those of other people, which extends the time-scale, upwards, by perhaps a factor of a thousand. Thus rare astronomical and geophysical events, which most people never witness, are important ingredients of an overall picture, and now influence even our understanding of living organisms and their evolution.

Of course, experimental science incorporates indirect experience, too. This enormously extends, in both directions, the time domains with which we must feel comfortable in order to describe important events. A device for presenting time on an intelligible, logarithmic scale, and designed to be relevant to early events in photosynthesis, was introduced many years ago by the physicist Martin Kamen (Kamen, 1963). This device has been widely adopted (Gregory, 1989; Whitmarsh & Govindjee, 1995). By analogy with p*H* (the negative logarithm, to the base ten, of hydrogen ion concentration), Kamen introduced the pt_s scale, where the lifetime, t, of a process produces a value for pt_s for that process given by

$$pt_s = -\log_{10} t(s)$$

where t(s) denotes time, measured in seconds.

In an attempt to provide an overview of microbial responses to light and time, I have extended Kamen's pt_s scale into time domains of which a photophysicist might be expected to disapprove, but with the same objective of making a reference axis upon which mutually related, if temporally distant, events may be described and compared (Fig. 1).

Fig. 1 is arranged in four columns. The left-hand column is the pt_s scale itself, with t (s) values also given. t (s) will be more familiar to, for example, certain kinds of photosynthesis researcher, for whom millisecond events are at the fuzzy, qualitative, and descriptive end of the scale, and there seen in rather the same way that a molecular microbiologist tends to view topics such epidemiology.

The second column of Fig. 1 places important processes along the pt_s scale at positions corresponding to their lifetimes, where these are known, or can be guessed with reasonable confidence. The third column is a similar arrangement of the events to be discussed in this presentation. Columns two ("Lifetimes") and three ("Events") are central to the topics discussed in this overview, and encompass the events to which reference will inevitably be made elsewhere in this symposium.

The right-hand column of Fig. 1, "Data", is intended to provide points of reference. At positive values of pt_s , these are given as the distance travelled by light during the corresponding time interval. For example, during the initial charge separation of the primary photochemistry of purple bacterial photosynthesis, light travels approximately 1.2 mm. The velocity of light is 3×10^8 m s^{-1}, and is not entirely remote from direct experience, at least for those who may initially be puzzled by the delay of 245 ms between terrestrial and satellite reception of a "live" BBC radio broadcast. At larger, negative values of pt_s, the "Data" column includes selected historical events whose names are placed on the scale next to the time that has elapsed since they took place, and "pt_s" then, strictly speaking, means "Δpt_s". In these time domains the

An article with the same title and author may be cited and is published in *Microbial Responses to Light and Time, eds* M.X. Caddick, S.Baumberg, D.A. Hodgson & M.K. Phillips-Jones, pp. 1-31. Cambridge: Cambridge University Press, 1998.

distance travelled by light is normally expressed in light-years (that is, in multiples of 3 x 10^{11} km).

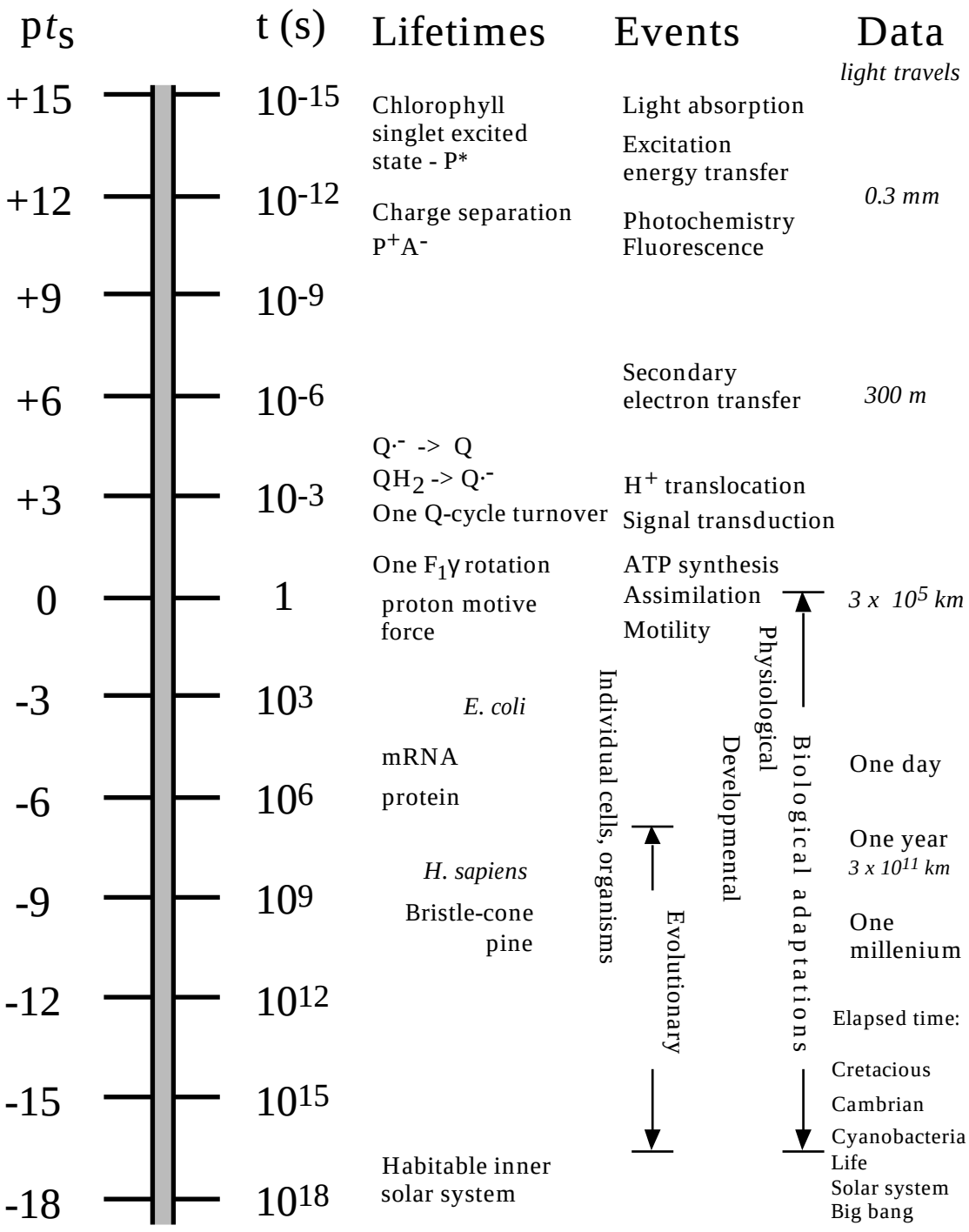


Fig. 1. The p t_s scale. pt_s = -$\log_{10}$t(s) where t(s) is time, measured in seconds. As a supplement to this overview, the reader may find it useful to consult the internet site http://plantcell.lu.se/ltm/ where the pt_s scale and some of the structures and events included here are presented dynamically or interactively, and include hypertext links to primary or related sources of further information.

One curious feature of Fig. 1, to which I wish to draw attention but shall not discuss further, is that the seconds, minutes, and hours of direct human experience,

experimental biology, and so on, stand midway between the fastest and slowest events of which we can conceive, in a manner analogous to the "anthropic principle" where the log scale is not one of time, but of distance (Barrow & Tipler, 1986).

My overview is divided into the present introduction, a concluding section, and, in between, a brief description of selected events occurring within each of the five time domains that each have $\Delta pt_s = 6$ and which, together, cover almost the whole pt_s scale of Fig. 1. Actually, the whole scale has the value $\Delta pt_s = 33$; the last 3 units encompass events that will not be discussed in any sort of detail, and these will be subsumed into the fifth domain. No overview from this distance could be generally accepted as balanced, still less complete: the selection of events reflects the theme of the symposium, recent progress, and the author's current interests.

$15 > pts > 9$

FEMTOSECONDS TO NANOSECONDS. THE FIRST TIME DOMAIN

Light absorption

The use of pumped lasers and synchrotron radiation pulses as actinic light sources has now extended studies of the interaction of light with matter ("spectroscopy") into the sub-nanosecond ("ultrafast") time domain. The term "femtochemistry" describes the study of the fastest of these events. Photosynthesis is well-known as the process by which light is absorbed and converted into a useful form in the biosphere, and is the source of the global redox disequilibrium that appears to be the earth's unique planetary signature of life. Besides this primary and, for us, indispensable interaction of light with living matter, light as an environmental signal must also depend upon principles governing its absorption and the chemical changes that may then take place.

Fig. 2 shows a simplified "Jablonski" diagram, in which the energy of an electron in an atom or molecule can be described from quantum mechanics as possessing one of a relatively small number of discrete values. Absorption of a single quantum of light depends on the availability of an electron whose permitted energy change falls within the range determined by the energy of the light quantum that induces it. The energy of the quantum is proportional to the frequency of the radiation, and inversely proportional to its wavelength - a Jablonski diagram turned through 90 ° yields an absorption spectrum.

Light absorption and the movement of the electron between energy levels occur on a femtosecond time scale. Internal conversion may occur between one energy level and a lower one, and is accompanied by release of energy as heat. There are then four processes that may then take place. The first is thermal, or non-radiative, de-excitation. This is the usual route for atoms and molecules absorbing specific energies without further events of direct biological relevance. The remaining three processes that follow absorption can occur at different rates, and all are important properties of photosynthetic systems. Fluorescence is important for what it tells us. Fluorescence is the re-emission of a quantum of light, and occurs in picoseconds to seconds. Slow fluorescence, or "luminescence" may be induced by changes in the physical or chemical state of the molecule containing the electron. Chlorophyll and bacteriochlorophyll have an inherently high, and variable, yield of fluorescence. Fluorescence emission from chlorophyll occurs with a lifetime typically measured in picoseconds, and the fluorescence lifetime of chlorophyll depends on its physical environment as well as on the rate of the competing processes of energy transfer and photochemistry. The next route for the falling electron (Fig.2) is energy transfer, which is a sub-picosecond phenomenon. This is the fate of excitation of most chlorophyll molecules bound to protein in photosynthetic membranes as well as that of the water-soluble phycobilins that perform a similar, light-harvesting, function in cyanobacteria and red algae.

Light harvesting

The function of light-harvesting pigments is to collect light energy over a far larger area than would be possible if each molecule were required itself to participate in storage of

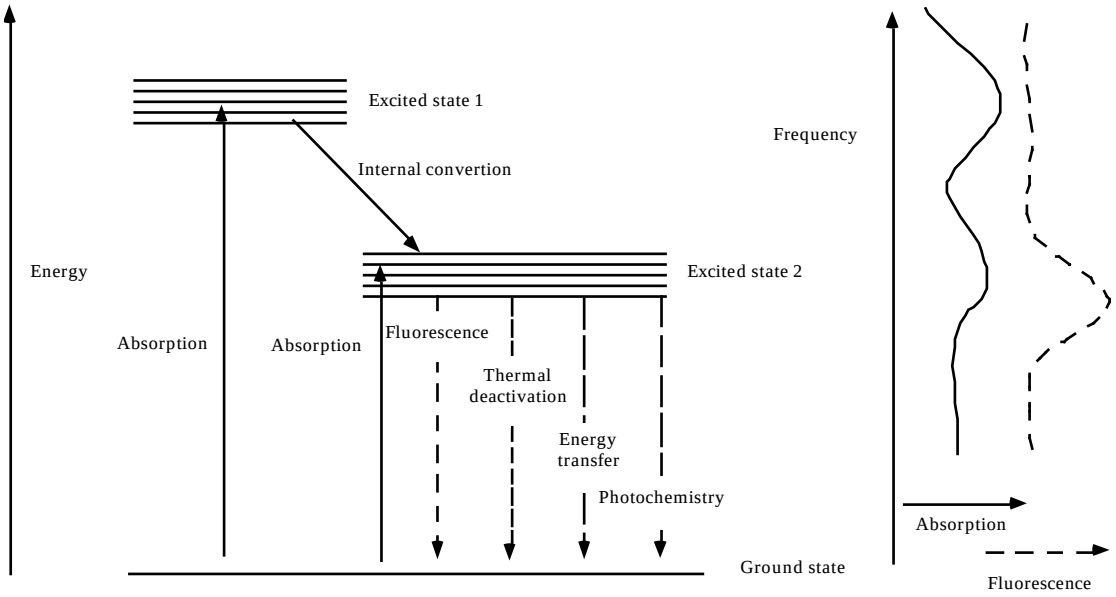


Fig. 2. Energy states of an electron, excitation, and the routes of de-excitation.

that energy in chemical form. The reason for this constraint is the relatively long time required for regeneration of the ground state by the final route for de-excitation, namely photochemistry. Although the chemical structure of the molecules involved may be the identical, the division of function between light-harvesting pigments (carrying out energy transfer) and reaction centre pigments (carrying out photochemical charge separation) is a fundamental feature of all photosynthetic systems. This division is imposed by the physical nature of light and the disparity between the time taken for its absorption and for its conversion into chemical form.

Our understanding of the early events in the biosphere's harnessing of light energy has increased dramatically in the last two decades, and it happens that the progress has been made almost entirely with photosynthetic bacteria. Some events have certainly been clarified for the chloroplasts of multicellular, green plants, but a rigorous description of the fundamental events of photosynthesis depends absolutely on an atomic resolution of the distances involved in energy and electron transfer. These were first secured for a membrane-intrinsic light-harvesting complex by Cogdell and co-workers with their structure of the LH II of the purple, non-sulphur photosynthetic bacterium *Rhodopseudomonas acidophila* (Freer et al., 1996). For the photochemical reaction centre, the equivalent step was made a decade earlier by Michel and co-workers with their extraordinary structure for the reaction centre of *Rhodopseudomonas viridis* (Deisenhofer et al., 1985) (Fig. 3a). The broadly confirmatory structures of light-harvesting complexes (see Freer et al., 1996) and reaction centres (Yeates et al., 1986) from other species were also obtained with purple bacteria. There is also the high-resolution structures of Huber and associates of the more specialised light-harvesting structures of phycobiliproteins (Duerring et al., 1991), and the partial structure of the chloroplast light-harvesting complex II, at slightly lower resolution, of Kühlbrandt and co-workers (Kühlbrandt et al., 1994).

The structure and function of the intrinsic membrane pigment-protein complexes that function in light-harvesting in purple, non-sulphur photosynthetic bacteria (the

Rhodospirillaceae) are discussed by Cogdell (this volume), and any further description of this breakthrough is, at this point, superfluous.

Reaction centres

In reaction centres, the chlorophyll or bacteriochlorophyll molecules (P) involved in the primary photochemical reaction of photosynthesis receive excitation energy from their chemically identical light-harvesting antenna pigment molecules, but the reaction centre pigments themselves are held by histidine ligands in an environment close to an electron donor (D) and acceptor (A), such that the excited state (P*) returns to the ground state (P) via an oxidised species (P^+), the electron being lost to the acceptor, thus:

$$DPA \rightarrow DP^*A \rightarrow DP^+A^-$$

In the third state (DP^+A^-) the excitation energy is said to have been "trapped" by photochemistry. The rise-time of the absorption change (a photochemical bleaching) that reports on the generation of P^+ has been timed at 4 ps ($pt_s = 11.4$) for reaction centres of purple bacteria, and this is synchronous with the reduction of the acceptor, bacteriophaeophytin (Youvan & Marrs, 1986). Subsequent events are again determined by the kinetics of a number of competing reactions, but the "useful" reaction is forward electron transfer from A^- to a secondary electron acceptor, a quinone, which takes 200 ps ($pt_s = 9.7$). Re-reduction of P^+ to restore the ground state by the donor (in purple bacteria, a *c*-type cytochrome), together with movement of the electron from the first to a second quinone, takes about 200 μs ($pt_s = 6.7$). The second quinone accepts a second electron by the same route, and moves on to provide electrons to the Q-cycle, discussed in time domain two. The sequence of events is shown in Fig. 3b.

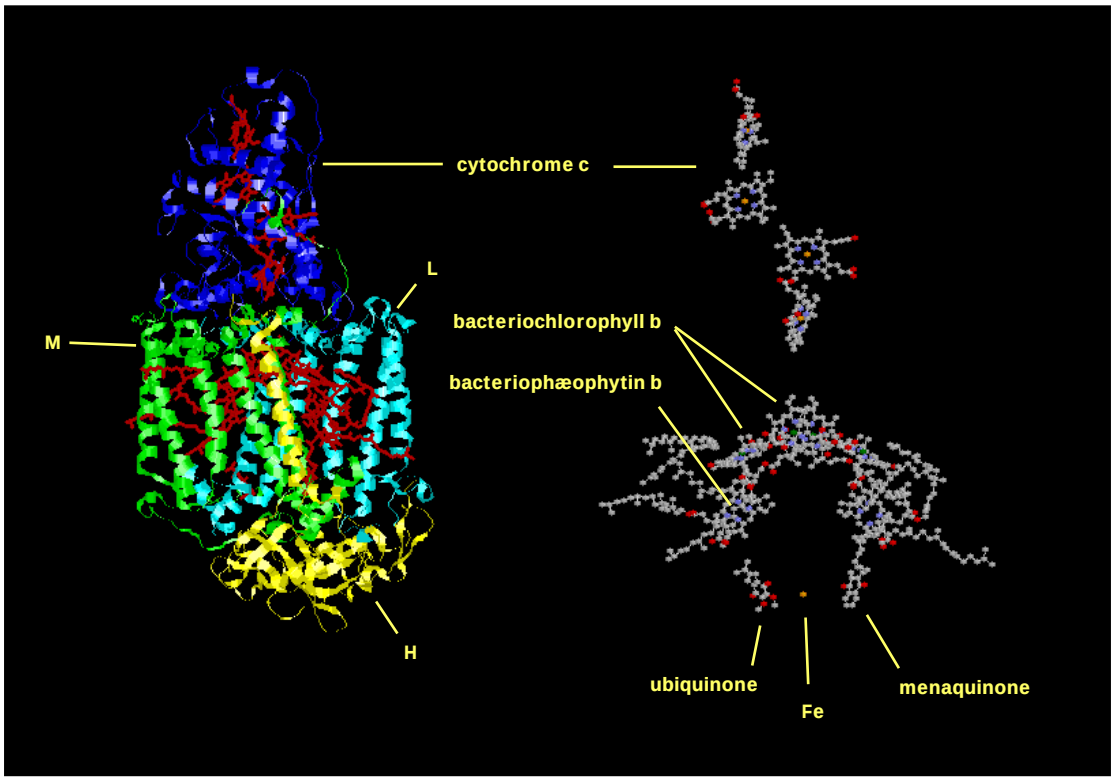


Fig 3A. Primary photochemistry of photosynthesis. Structure of the reaction centre *of Rhodopseudomonas viridis*. On the left is the whole protein, with individual chains represented as individually-coloured ribbons and the heterogeneous atoms of the cofactors as red sticks. On the right the cofactors alone are shown as ball-and stick models with cpk colours.

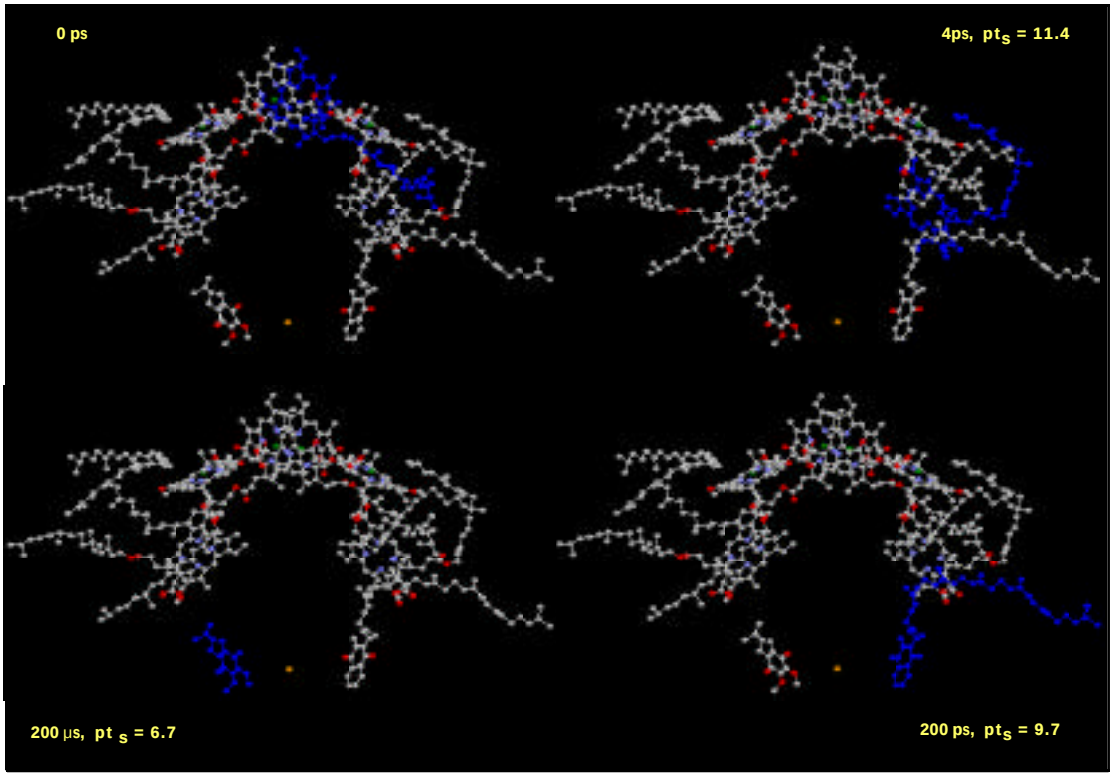


Fig 3B. The sequence of primary electron transfer events. This presentation is based on one originally published, but using the *Rhodobacter sphaeroides* structure (Yeates et al., 1986), by Youvan and Marrs (1987). A and B constructed from Brookhaven protein databank coordinate file 1prc using the program RASMOL (Sayle & Milner-White, 1995).

Around 2 x 10^9 years ago (Δpt_s = -16.8) some photosynthetic bacteria appear now to have developed the singularly useful trick of supplying the electron to P^+ from a tyrosine side chain, generating tyrosine cation radicals that are capable of sequential abstraction of hydrogen atoms from water, producing molecular oxygen. The oxygen-evolving donor side of photosystem II in modern cyanobacteria and green plants is thus now viewed as a member of class of free-radical proteins, along with ribonucleotide reductase and galactose oxidase (Hoganson & Babcock, 1992). There are strong arguments, supported by conservation of the histidine chlorophyll ligands, that the reaction centres of oxygenic photosystem II are homologous with those of anoxygenic purple bacteria (Diesenhofer et al., 1985). The best photosystem II structures to date, for example that of Rhee et al. (1997), support this idea. An intriguing evolutionary consequence of this view is that oxygenic photosynthesis, which requires the coupling, in series, of two distinct types of reaction centre (photosytems I and II), must have depended upon lateral transfer of genes between the evolutionary precursors of the modern green, sulphur bacteria (whose single reaction centre resembles photosystem I) and those of the purple bacteria (Blankenship, 1994).

$$9 > pts > 3$$

NANOSECONDS TO MILLISECONDS. THE SECOND TIME DOMAIN

The second time domain is, for the purposes of this overview, one of secondary electron transfer, photoisomerisation, ligand binding, catalysis, and protein structural change. Fig. 3B already extends the primary events of photosynthesis well into this region. To this can be added the photocycle of bacteriorhodopsin (Subramaniam et al., 1993).

In reality the early events of signal transduction also must fall into this domain, but most lie outside the range of conventional biochemistry - even the best stopped-flow apparatus has a mixing time of a few milliseconds. Light signal transduction (for example, Hoff, Jung & Spudich, 1997; Spudich, this volume), like photosynthesis, is an exception, since the substrate can be added in pulses. Long before the use of lasers, conventional flashlamps with μs flashes took photobiology and photochemistry well into this time domain. In photosynthesis, light itself has already done its job within the first time domain, and the "light reactions" of photosynthesis are largely passive, thermodynamically "downhill" electron transfers that are initiated by the much faster events of primary photochemistry.

Nevertheless, there is a crucial set of secondary electron and proton transfers that occur in bioenergetic membranes, including photosynthetic membranes if the donor happens to be a reaction centre. One intermediate in these reactions is particularly short-lived, but may provide a key to understanding quite distant biological responses to light and time.

The Q-cycle

The Q-cycle (Mitchell, 1976) is an important component of energy coupling in most photosynthetic and respiratory systems, and appears to be the primary function of cytochrome bc_1 (or "*b-f*") complexes. The Q-cycle takes two electrons from the bulk quinone pool in the membrane, passes one on to an iron-sulphur protein and hence to cytochrome *c*, and recycles the other back into the pool by means of its transfer between two cytochrome *b* haems, arranged across the membrane. At this "Q_o" site ("o" for "outer") each of the two electron transfers releases one proton. At the "Q_i" ("inner") site a proton is bound when quinone is re-reduced by the electron from cytochrome *b* , and another when an electron is supplied from the original donor to the pool. The overall result is that movement of one electron from the donor, via the quinone pool, to cytochrome c drives translocation of two protons from the inner to the outer aqueous phase. This resolves a stoichiometric anomaly apparent in earlier formulations of the chemiosmotic hypothesis. The overall process belongs in the third time domain, since, in general, one complete turnover of the Q-cycle is complete in about 60 ms: $pt_s = 1.2$. However, a recent proposal for the component reactions, which lie in second time domain and, I shall argue, are necessary for understanding events in subsequent time domains, is now outlined.

One inexplicable feature of the Q-cycle has, until recently, been the requirement for bifurcation of the electron transport chain at the Q_o site: by what means may the quinol be forbidden from donating both of the electrons it carries in the thermodynamically-favoured direction, that is, to the Fe-S centre? Even after a single electron transfer, the intermediate semiquinone should be a good donor to the iron-sulphur protein. In other words, why does recycling through cytochrome *b* occur at all?

Recent structures from X-ray crystallography for the iron-sulphur and for the intrinsic membrane domain of the bc_1 complex have provided Crofts et al. (1997) with the structural basis for an ingenious solution to this problem. Fig. 4 presents the fundamentals of Crofts's idea. The quinol in its Q_o site is initially much closer in space to the to the iron-sulphur centre than it is to the low-potential *b*-haem, and electron transfer to the iron-sulphur centre is then kinetically favoured. After the first electron transfer, the semiquinone centre moves closer to the low-potential *b*-haem, permitting the second electron transfer to occur in a different direction. After its reduction, the iron-sulphur centre moves away from the Q_o site and towards cytochrome c as a result of rotation of the mobile head-group of the iron-sulphur protein.

The kinetics of the component reactions are known in some detail, and are consistent with Crofts's proposal (1997). I suggest here that one way of considering the Q_o site mechanism in functional terms is to view the semiquinone anion radical as indispensable but dangerous. This dilemma that may provide an insight into the persistence, in evolution, of extra-nuclear genetic systems in eukaryotes, as discussed in time domain five. Thus the steady-state concentration of the semiquinone is

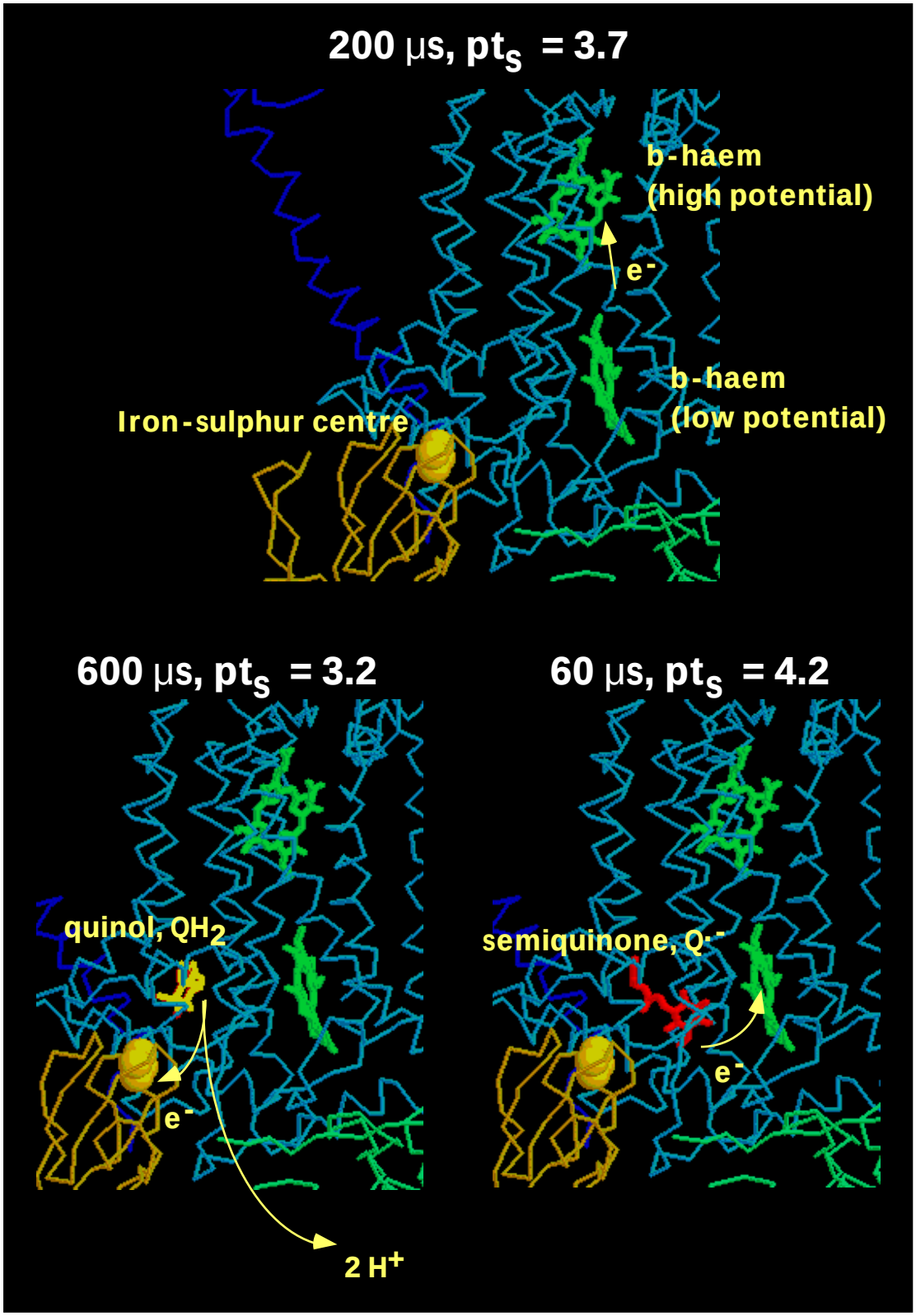


Fig 4. The Crofts model for quinol oxidation at the Q_o site of the cytochrome bc_1 complex: bifurcated electron transfer. The cytochome bc_1 complex contains an iron-sulphur protein, a cytochrome *b*, and a cytochrome c_1 (the latter not shown). At the Q_o site of the complex is the iron-sulphur centre itself, and the two haems of the *b*-type cytochrome. On entering it's binding site, the quinol passes an electron to the iron-sulphur centre and releases a proton. The semiquinone then moves in space, from the iron-sulphur protein to the low potential *b*-heme, passing an electron to the haem and releasing a second proton. Electron transfer occurs from the low-potential *b*-heam to the high potential b-haem, and the

quinone leaves its binding site. Subequently, the mobile head of iron-sulphur protein rotates, carrying the iron-sulphur centre away from the Q_o site and towards the haem of cytochrome c_1, to which it donates the electron. The overall result is that single electron has moved from the quinone pool to the cytochrome c_1, but two protons have been released into the outer, aqueous phase. The figure is adapted from graphics kindly provided by A. R. Crofts (Crofts et al. 1998).

maintained at the lowest possible value by the first electron transfer, from the quinol to the iron-sulphur centre, being around ten times slower than the second electron transfer, from the semiquinone to the low-potential *b*-haem. Quinol oxidation to semiquinone occurs in 600 μs: pt_s = 3.2. the semiquinone is oxidised to quinol in 60 μs: pt_s = 4.2. The electron transfer between the low-potential and high-potential *b*-haems takes around 200 μs: pt_s = -6.7.

$$3 > pts > -3$$

MILLISECONDS TO KILOSECONDS. THE THIRD TIME DOMAIN

ATP synthesis

Mitchell's "chemiosmotic" mechanism for the coupling of electron transport to ATP synthesis (Mitchell, 1961) falls clearly into the early part of this domain. Recent progress in understanding the elements of chemiosmosis has been remarkable, and has again come from structural biology, as illustrated by the primary route for generation of the proton motive force that was discussed in the previous section.

F-ATPases

The proton motive force, a transmembrane electrochemical gradient of hydrogen ion concentration, may drive any one of a number of endergonic reactions, chief among which is synthesis of ATP. The means by which ATP synthesis results from movement of protons back across the coupling membrane can be inferred clearly from the structure of the extrinsic F_1-ATPase of bovine heart mitochondria. The structure, from Walker and co-workers (Abrahams et al. 1994), shows a radial, 3-fold symmetry, with each 120 ° sector, although composed of an common α−β heterodimer, containing a different ligand binding site associated with the catalytic subunit, β. The shape of each ligand binding site appears to be determined by the asymmetry of the single γ subunit, which forms a spindle-like structure inserted through the central core of the roughly cylindrical $\alpha_3\beta_3$ domain, where the points of interaction between γ and $\alpha_3\beta_3$ are large, hydrophobic amino acid side chains. From the structure alone (Fig. 5) the irresistible conclusion is that F_1 is a bearing. Rotation of γ within the central axis of F_1 would obviously induce sequential changes in the conformation of each α−β heterodimer, a structural basis for Boyer's binding-change mechanism. Each heterodimer binds ADP and phosphate loosely; then ADP and phosphate tightly; and, finally, ATP, which is seen *in situ* in the crystal structure in the form of a non-hydrolysable ATP analogue.

The idea that the γ subunit acts as a camshaft is currently supported by two quite independent lines of evidence. A direct and visually compelling demonstration has been provided by Yoshida and co-workers (Noyi et al., 1997), who successfully tethered the γ subunit to an actin filament and the $\alpha_3\beta_3$ headgroup to an inert, metal surface: upon addition of ATP, some actin filaments were observed, in a light microscope, to rotate. During ATP hydrolysis, the angular velocity of the actin filament depended on its length, but all rotations were anticlockwise. Junge and co-workers (Sabbert et al., 1996) used a fluorescence tag (eosin) on the γ subunit of an immobilised chloroplast (C)F_1 in order to study movement of the tag by polarised absorption relaxation after photobleaching. The conclusion is that ATP induces rotation

of γ relative to the hexagonal $\alpha_3\beta_3$ array. One complete ATP-induced rotation takes 100 ms: $pt_s = 1.0$.

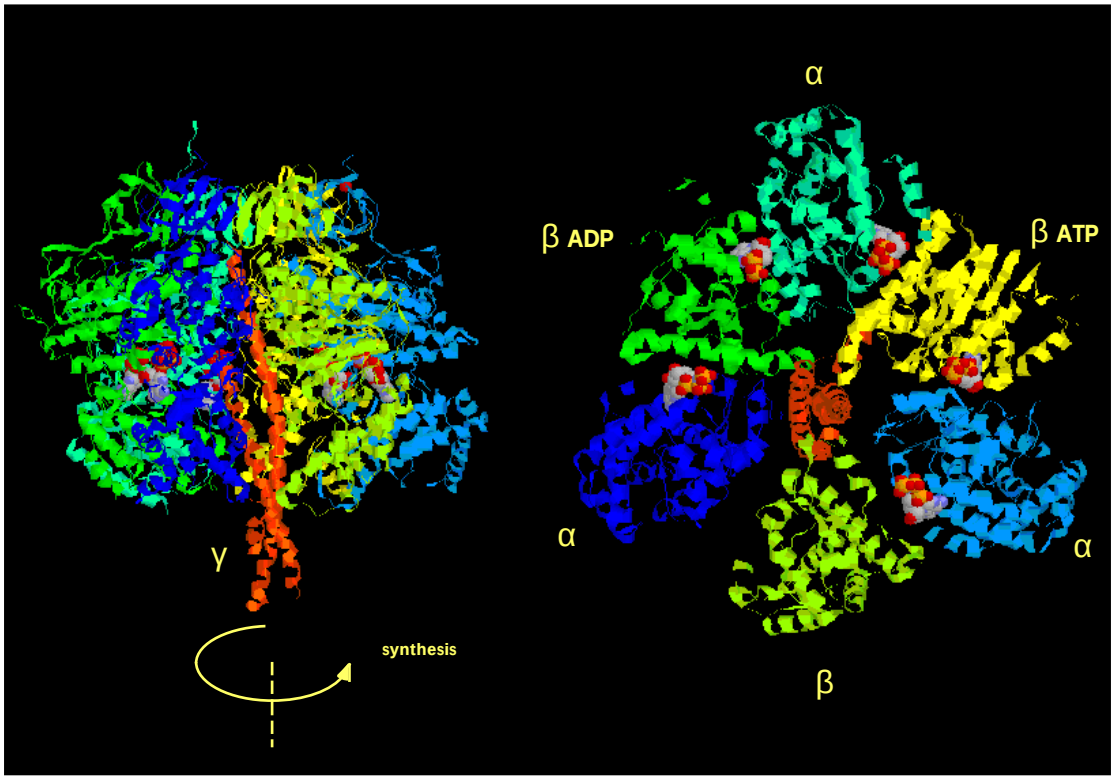


Fig 5. Structure of the F_1-ATPase. Left, the complete hexagonal array, viewed parallel to the membrane plane, with polypeptide chain as individually-coloured ribbons and the adenine nucleotides as space-filling models. Right, a view normal to the membrane plane (as if from the inner, cytoplasmic aqueous phase) with a 57 % Z-slab in order to reveal the binding sites for the ATP analogue and ADP. The rotation of the γ-subunit, which is mechanically coupled to F_o (Fig. 6), is predicted to be anticlockwise during ATP synthesis, and one complete rotation will release three molecules of ATP. Constructed from Brookhaven protein databank coordinate file 1bmf using the program RASMOL (Sayle & Milner-White, 1995).

In the reverse process, ATP synthesis, what causes the rotation of the γ subunit of F-ATPase? Here Junge et al. (1997) have made the ingenious suggestion that the membrane-intrinsic F_o is linked mechanically to the γ subunit, and that rotation of the core of F_o is driven by the inward movement of protons. According to this hypothesis, protonation of regularly-spaced, acidic amino-acid side chains occurs from the outer, aqueous phase. Provided these side chains are in their protonated, uncharged, form, they are able to enter the hydrophobic environment of the membrane. Their deprotonation occurs into the inner aqueous phase, as favoured by the electrochemical gradient of proton concentration, but is sterically possible only after rotation of the core of F_o. This proton-driven stepping motor (Fig. 6) may have been adapted, in evolution, not only to use monovalent cations other than H^+, but to provide different ring sizes of F_o, in effect giving different gear ratios, that is, H^+/rotation and therefore H^+/ATP stoichiometries, according to the free energy available from the respiratory or photosynthetic chain that the cell is able to deploy. This mechanism also suggests the tantalising possibility that the gear ratio may be selected according to physiological circumstances. In my own laboratory it has recently been found that plant mitochondrial F_1-ε and Fo-b subunits are phosphorylated under specific conditions (Struglics et al., submitted). Fo-b is proposed by Junge et al. (1997) as the stator that prevents rotation of the hexagonal $\alpha_3\beta_3$ array whilst the γ subunit rotates within it. If not a gear lever,

phosphorylation of subunits of ATPase may plausibly suggest the existence of a molecular clutch. If the automotive analogy can be extended, a clutch, or coupling control, may be a necessary pre-requisite for any physiological changes in H^+/ATP stoichiometry that maintain an optimal balance of effort and load without energy transfer inhibition, or stalling.

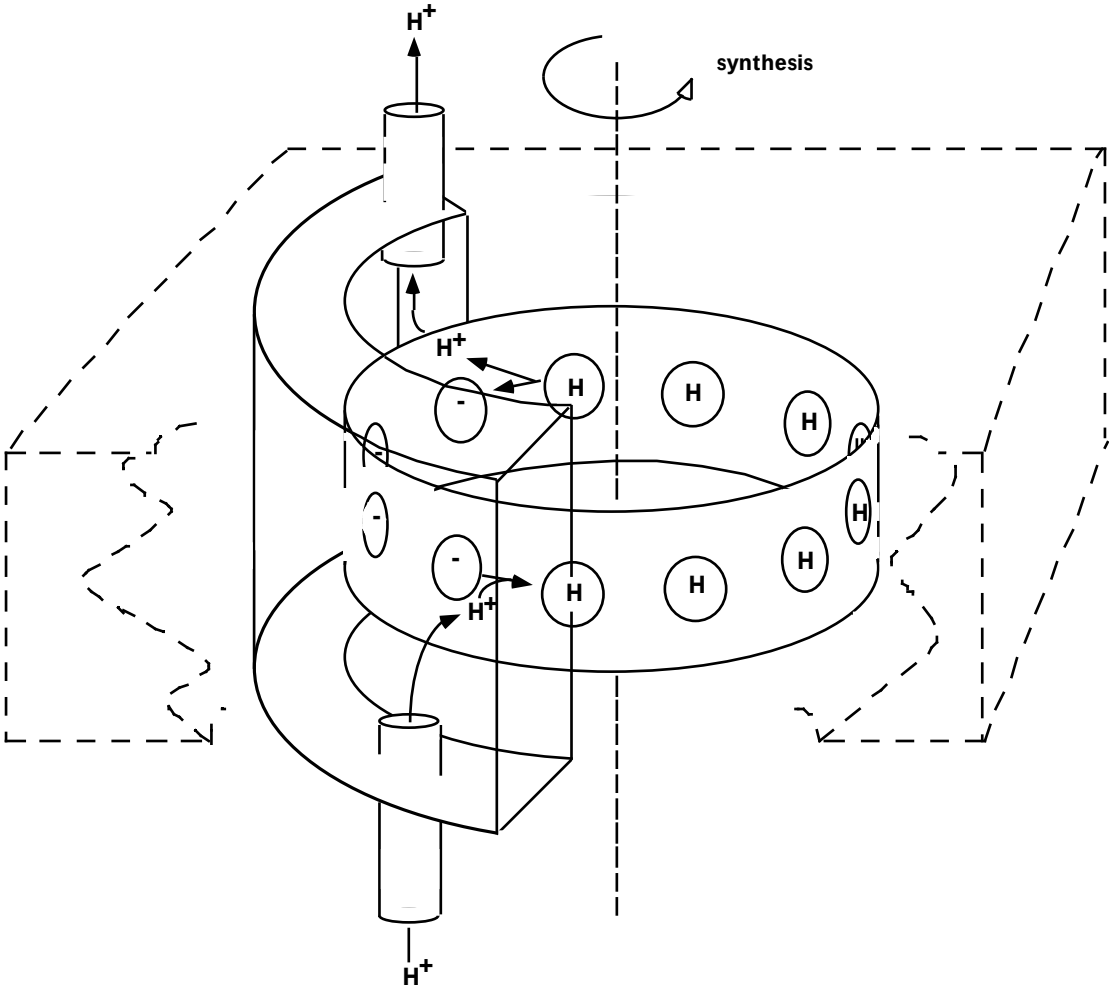


Fig 6. F_o-ATPase as a proton-driven, rotary stepping motor, as proposed by Junge (1997). The inner ring, with twelve proton or hydrogen atom binding sites, is coupled mechanically to F1-γ (Fig. 5). The sense of rotation of the inner ring of F_o, viewed from the top, is anticlockwise during ATP synthesis (as shown), when protons move inwards, down the gradient of electrochemical potential. If F_o remains coupled to F1-γ during ATP hydrolysis, outward proton translocation will be driven by clockwise rotation of the inner ring. Drawing after Junge (1997).

Adaptation

In Fig. 1 it is seen that adaptations begin in third time domain. This is true even if one includes responses such as nerve signal transmission and the perception of environmental signals, though for vision the initial events probably occur in domain two. Fig. 7 magnifies the part of the pt_s scale of Fig. 1 corresponding to the boundary between domains three and four. Fig. 7 adds the reference points of light-distance and more familiar units of time above the scale, and presents a correlation of different categories of adaptations with an extended "central dogma" diagram denoting various stages of gene expression. Although it takes part of the section devoted to events in time

domain three, there must logically now follow a discussion of biological adaptations generally.

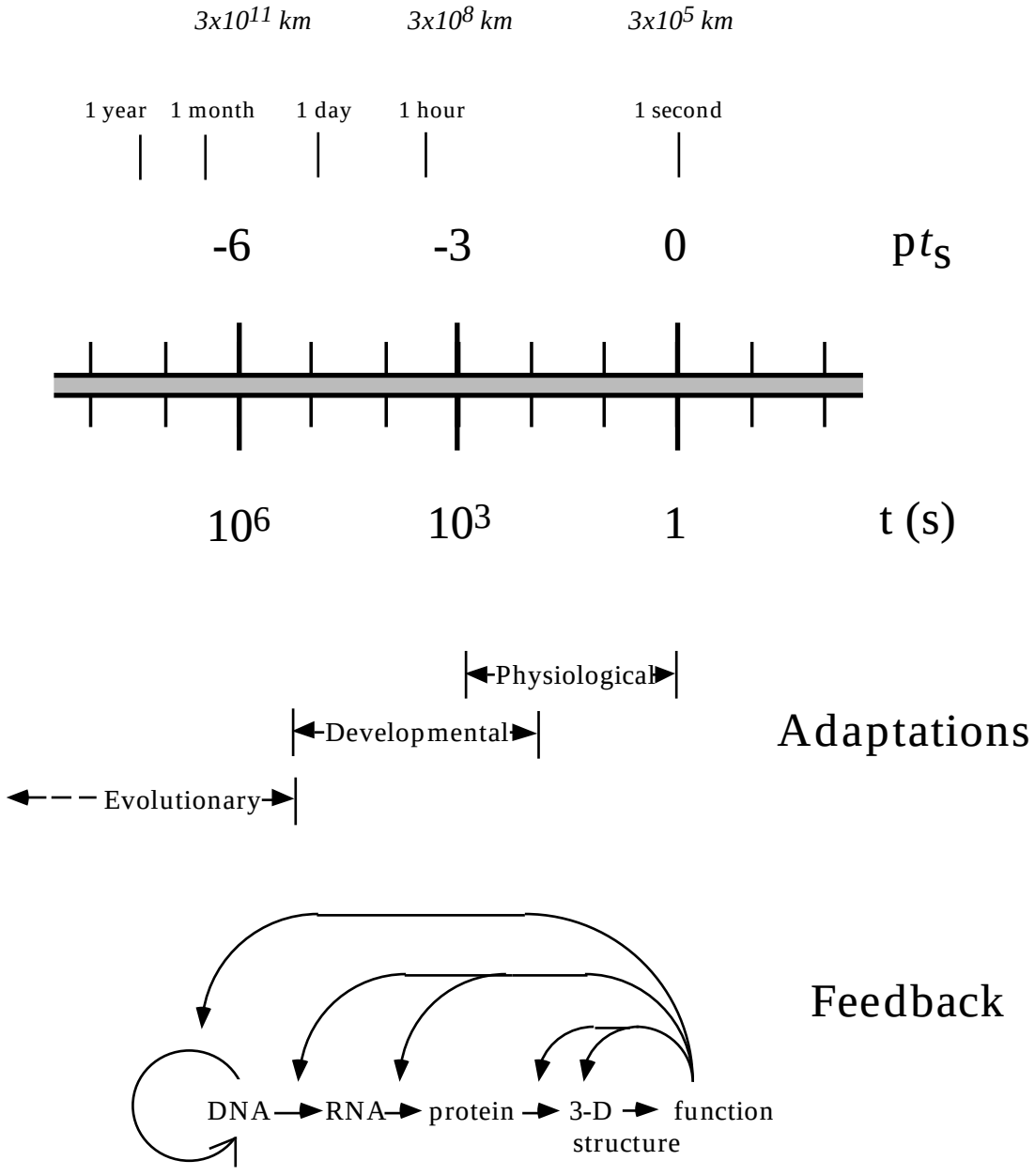


Fig 7. The time domains of biological adaptations and gene expression. This diagram represents an expanded and supplemented part of the scale in Fig. 1, but with adaptations more specifically defined as having $p(t)_s$ ranges appropriate for prokaryotes.

Homeostasis, the maintenance of a constant internal environment in the face of external changes, is a necessary and fundamental feature of all living systems. I propose to divide the adaptations involved into three categories, here termed "physiological"; "developmental"; and "evolutionary". Other authors have wished to reserve the term "adaptation" for only one of these three kinds of response to the environment. Maynard Smith (1966), for example, reserves "adapted" for "genetically adapted", which is equivalent to "evolutionarily adapted", and, by way of distinction, uses the terms "physiologically versatile or tolerant" and "developmentally flexible". I suggest that the last three adjectives suggest passive properties of systems, and

"adaptation", which suggests an active process, is preferable. Furthermore, in plant physiology, a careful distinction is sometimes made between "adaptation", which may be either physiological or evolutionary, and "acclimation", which refers to what a microbiologist would perhaps recognise as a developmental process.

Apart from the idealised arrangement of the three sorts of adaptation along the pt_s axis, there are further, non-trivial distinctions to be made between them. Firstly, only physiological and developmental adaptations occur within the life-time of an individual organism. The latter depends, of course, very much on the sort of organism being considered. For most microorganisms (especially when grown in a laboratory culture) both physiology and development occur well within the time domain $9 > pts > 3$. For all living things it is also possible to arrange the three sorts of adaptation according to the level of gene expression at which feedback is exerted as a result of any given environmental change (Fig. 7).

Thus physiology concerns largely a feedback control of structure, function, and molecular recognition of macromolecules - for the large part, proteins - that are already in place. Physiological adaptation is a fine-tuning of the machinery you already have. Where such processes as motility are involved, physiological adaptation takes the form of behavioural adaptation, for example, phototaxis (for example, Sprenger et al., 1993).

Development then becomes a matter of influencing transcription, mRNA processing and stability, and translation, in such a way that the environmental signal affects the final composition of the system that interacts with the new environment. Developmental adaptation is usually a matter of choosing a new set of components with which to work, and sometimes one of assembling new components from the old set in a different way. The borderline between physiology and development is perhaps less clear in microbiology than in other fields, but here I wish to propose a line of demarcation, for convenience: any adaptation that occurs within the lifetime of an individual cell or organism can be described as "developmental" if it requires protein synthesis *de novo*, and "physiological" if it does not. The question of whether protein assembly counts as physiology or development then depends on one's definition of a protein, and becomes semantics.

Evolutionary adaptation, which comes into time domains four and five, arises from the question of whether the new environment can be used at all to make a second set of instructions, however these may be interpreted by development and physiology. The feedback loop from function to DNA replication in Fig. 7 is intended to represent this crude, qualitative line of information flow, and not the more subtle, developmental effects seen, for example, in the cell cycle. Evolution is the gradual departure of each succeeding set of instructions from an arbitrarily-chosen original. Clear thinkers long ago abandoned teleology in evolution (Dawkins, 1986; Dawkins, 1996). Attempts to rescue it now seem largely incoherent (Teilhard de Chardin, 1966). Nevertheless, the full implications of the view that evolution proceeds by a blind mechanism have perhaps not been thoroughly assimilated by our culture and society. Fig. 7 presents what some may regard as an equally bleak overview of physiology and development, in which the wonders of development might be thought to be reduced to the operation of a few hierarchically-arranged feedback loops. In the final section I shall return to this question, and suggest that it is not quite so simple. To forestall concern, I must add that teleology does not come into it, and that my intention is, in fact, to provide an explanation of what might otherwise seem to be purposeful behaviour in physiology and development. Moreover, the supporting evidence is recent, central to this symposium, and comes first from microbiology; the area of the life sciences where one is, perhaps, least likely to become emotionally involved with the subject matter.

Protein phosphorylation.

If physiology is fine-tuning the structure, function, and interactions of pre-existing proteins, a clear example is post-translational, covalent modification. The most versatile and widely-deployed case appears to be protein phosphorylation. In chloroplasts, protein phosphorylation has an accepted, central role in the mechanism by which the

light-harvesting complex II becomes redistributed between photosystem I and photosystem II (Allen, 1992). Protein phosphorylation is implicated in physiological control of excitation energy transfer in cyanobacteria and purple photosynthetic bacteria, too, despite the absence, in the latter, of the problem of redistribution of light-harvesting function between reaction centres of two different kinds. In chloroplasts, it is now known that phosphorylation of a sub-population of light-harvesting complex II polypeptides at the periphery of photosystem II induces a structural change at their amino-terminus (Nilsson et al., 1997). The structural change initiates a series of events, most likely involving dissociation of LHC II trimers, that lead to the eventual association of phospho-LHC II as a functional part of the light-harvesting antenna of photosystem I. (Fig. 8).

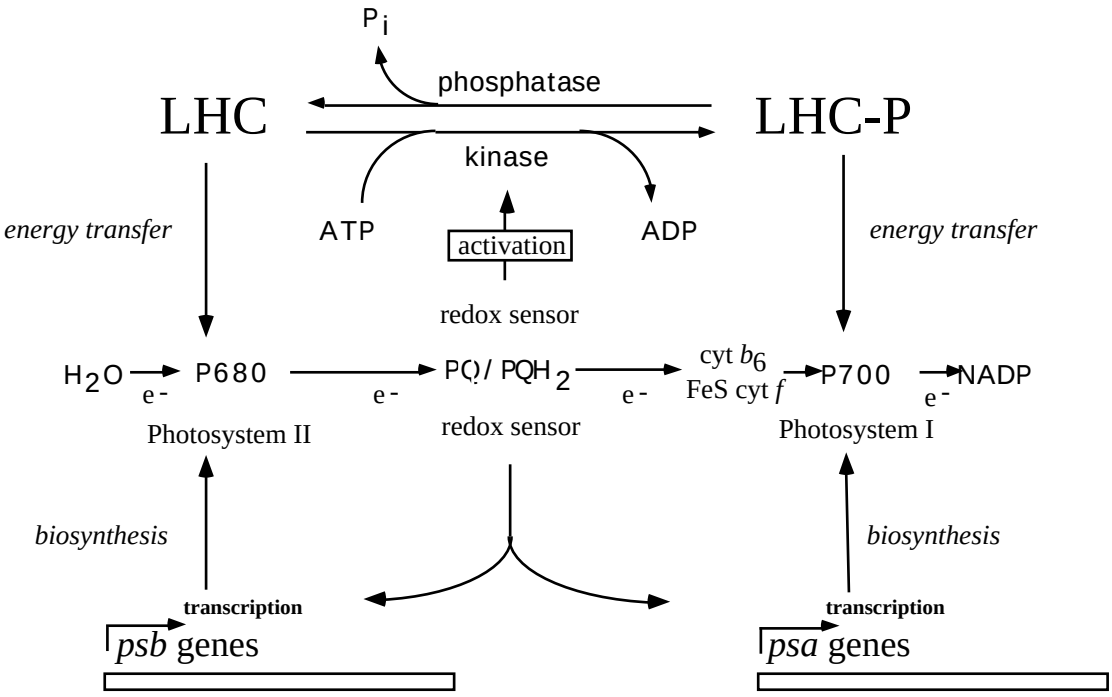


Fig 8. Redox control of phosphorylation of chloroplast light-harvesting complex II and of reaction centre gene expression. Control of photosystem stoichiometry is assumed to result from redox effects on expression of genes for components of the two photosystems. In this scheme, photosystem stoichiometry adjustment and fine-tuning of light-harvesting by protein phosphorylation are complementary adjustments to the same redox signal. "LHC" is LHC II in chloroplasts, and a putative phycobilisome component in cyanobacteria and red algal plastids.

In an attempt to isolate components of the protein kinase-phosphatase system that must be involved in this process, a technique of time-resolved fluorescence imaging has been developed. This technique that allows the screening of a population of cells, colonies, or small plants for mutants whose phenotype is an inability to carry out redistribution of excitation energy between photosystem I and photosystem II in synchrony with the control, or wild-type (Allen, Dubé and Davison, 1995). Whatever the enzymology behind this physiological adaptation, it is clear that light controls the ability of the photosynthetic apparatus to collect light energy efficiently by means of its effect on photosynthetic electron transport: the LHC II kinase is under redox control (Allen, 1992; Allen et al., 1981) (Fig. 8). The light-induced phosphorylation of LHC II results from activation of its kinase by either chemical or photochemical reduction of the quinone pool (plastoquinone in chloroplasts and cyanobacteria) to which reference was made in the context of the Q-cycle. The half-time of light-induced LHC II

phosphorylation in isolated chloroplasts is four minutes (Telfer et al., 1983): $pt_s = -2.4$. In cyanobacteria, the phenomenology is essentially the same, but the lateral movement of LHC II is replaced by movement of its analogue, the extrinsic phycobilisome, between photosystem I and II (Allen, 1992; Allen, Sanders & Holmes, 1985). Although protein phosphorylation has still not been directly demonstrated to be involved, there is good circumstantial evidence that this is the case, and the half-time of the movement of the phycobilisome to photosystem I from photosystem II is forty-five seconds (Mullineaux & Allen, 1988): $pt_s = -1.7$.

$$-3 > pts > -9$$

KILOSECONDS TO GIGASECONDS. THE FOURTH TIME DOMAIN

Although the border of the fourth time domain corresponds fairly closely with the doubling time of *Escherichia coli* in log phase, the fastest biological events to be discussed in this context are predominantly developmental. The first example can be considered as an extension of the physiological adaptation described at the end of the previous section.

Developmental adaptations: photosystem stoichiometry adjustment and complementary chromatic adaptation.

The imbalance in distribution of absorbed excitation energy that initiates the physiological, and relatively rapid redox response of LHC II phosphorylation has a long-term, developmental counterpart in adjustment of photosystem stoichiometry. The response is to the same signal, and the adjustment in both cases is one of greater balance in light capture by the two photosystems in order that their rates of electron transfer may remain equal, a requirement of their being coupled electrochemically in series (Hill & Bendall, 1960). Photosystem stoichiometry adjustment (Allen, 1995) differs from LHC II phosphorylation in the time-scale over which the response occurs. In the cyanobacterium *Synechococcus* 6301 in one laboratory culture (Allen et al., 1989), and for the higher plants mustard and pea (T. Pfannschmidt et al., unpublished), the half-time of the increase in the ratio of photosystem I to photosystem II after transfer to photosystem II-specific light is approximately sixteen hours: $pt_s = -4.5$.

Recent results from my laboratory support the idea that redox signals from the plastoquinone pool of chloroplasts initiate changes in photosystem stoichiometry in parallel to their effects in regulation of phosphorylation of LHC II pea (T. Pfannschmidt et al., unpublished). For cyanobacteria, Fujita and co-workers (1994) have provided a number of independent lines of evidence that the redox state of plastoquinone is, again, the predominant factor determining the relative rates of assembly of the two photosystems. Our own results tend to suggest that redox control of transcription of chloroplast genes is the primary response, at least in higher plants. The direction of the control is such that reduction of the plastoquinone pool by surplus photosystem II light or by chemical inhibition of the electron transport chain increases transcription of genes encoding subunits of photosystem I. Conversely, oxidation of plastoquinone by photosystem I light or by the action of inhibitors decreases photosystem I gene transcription and increases that of photosystem II. The conclusion is that expected, both teleologically and through the precedent of the scheme in Fig. 8: any imbalance in excitation energy distribution causes changes in gene expression, through redox control, that tend to correct the imbalance itself. Redox homeostasis is clearly important enough to require multiple pathways of feedback control, acting at different levels of gene expression (Fig. 7).

My aim in stressing the action of light on gene expression through photosynthesis and redox control is not to detract from the importance of light itself as an environmental signal, which it obviously is, even in animals, in non-photosynthetic developmental stages in plants, and in some chemotrophic bacteria. Progress has been made recently in understanding the photoreceptors and the associated signal transduction pathways of complementary chromatic adaptation. Complementary

chromatic adaptation is long-established phenomenon by which some cyanobacteria switch on transcription of light-harvesting phycoerythrin genes in green light, and of phycocyanin genes in red light. The cells thereby assume a colour that is complementary to that of the light in which they grow. The signal transduction pathway is seen, from genetic transformation that gives complementation of mutant phenotypes, to contain a two-component system (Kehoe & Grossman, 1996), and the likely sensor shows some sequence similarities to phytochrome (reviewed by Allen & Matthijs, 1997), a major photoreceptor in plants.

Is a decision made about the appropriate level of adaptational response?

If physiological and developmental responses that achieve broadly the same effect can be distinguished and, at the same time, are initiated by a single environmental signal, how is the appropriate level of response selected? To take a homely analogy, one does not install new central heating every time the weather becomes overcast, nor does an overcoat provide a permanent solution to the discomfort of life in the sub-arctic. I suggest that measurement of the duration of the stimulus may play some part in the cell's decision. This is not to argue that cells possess foresight, though something analogous will be considered in the final section of this overview. Equally, one could argue that developmental events come into play only when physiological responses that may restore the *status quo* have failed. As regards operation, in parallel, of control of protein phosphorylation (physiology) and of gene expression (development), a possible scenario is outlined in Fig. 9.

Two-component systems contain two components: a sensor and a response regulator. Response regulators are proteins that become phosphorylated on an aspartate on a surface-exposed loop. The phosphate group is transferred to the aspartate from a phosphohistidine side chain of the response regulator's cognate sensor (Stock, Ninfa & Stock, 1989). The histidine of the sensor becomes phosphorylated in response to the relevant environmental signal. The sensor is thus a response regulator kinase, mediating transfer of phosphate from ATP to the response regulator if, and only if, environmental conditions are right. Certain sensors, notably ArcB of *E. coli* (Iuchi and Lin 1993), contain both sensor and response regulator domains, and the assumption can be made that a sensor's response regulator domain serves to damp the signal passed to the authentic, discrete response regulator. The suggestion outlined in Fig. 9 is that the branch-point of a bifurcated signal transduction pathway that leads either to a physiological or to a developmental response may be the transfer of the phosphate group of the sensor domain to an aspartate, either of its own response regulator domain, or to that of a separate response regulator.

The basis of the "decision" taken by a system about the appropriate level of response to an environmental signal, would, according to this proposal (Fig. 9), be a simple consequence of success or failure of the most rapid response in restoration of the preferred internal environment. If the sensor contains both sensor and response regulator domains, and if the kinetically favoured pathway for phosphoryl transfer is intramolecular, then phosphorylation of the sensor's own aspartate (k_1) would produce a relatively rapid, physiological response, which, in this example, may be activation of a protein kinase. If full activation of this kinase then fails to restore homeostasis, it will also fail to switch off the stimulus initiating phosphorylation of the sensor's histidine. At the same time, the kinetically preferred intramolecular phosphoryl transfer from histidine is prohibited by the acceptor (aspartate) being already in its phosphorylated form. The kinetically less favoured, intermolecular phosphoryl transfer (k_2) may then predominate. This second phosphoryl transfer is to the aspartate of the response regulator that controls gene expression. Fig. 9 provides an outline of one mechanism by which developmental adaptation may be initiated when the stimulus caused by disparity in external and internal conditions persists even after physiological adaptation has reached the limit of its response. This general mechanism (Fig. 9) is built on a specific proposal for redox signalling in photosynthesis, where the sensor is a redox sensor and the two responses are phosphorylation of light-harvesting proteins and adjustment of photosystem stoichiometry (Allen and Nilsson, 1997).

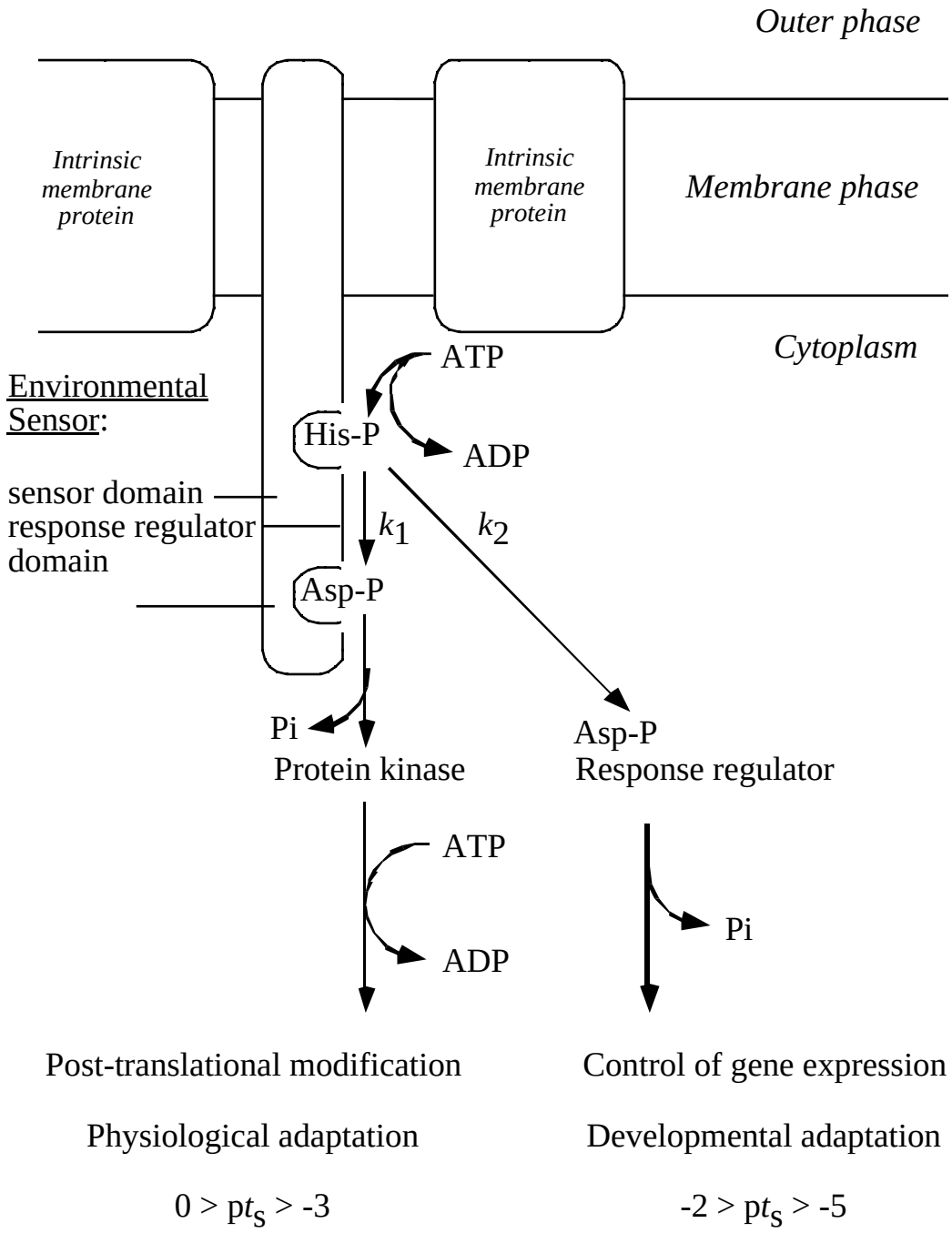


Fig 9. A proposal for control of post-translational covalent modification by phosphorylation and, in parallel, of gene expression: bifurcated signal transduction. If the putative redox sensor contains both sensor and response regulator domains, and if the kinetically favoured pathway for phosphoryl transfer (k_1) is intramolecular, then phosphorylation of the sensor's own aspartate would initiate a physiological response, for example by activation of a protein kinase. If full activation of the kinase then still fails to restore redox poise, then phosphoryl transfer (k_2) may occur to the aspartate of the response regulator that regulates gene expression. An assumption of the proposed mechanism is that intramolecular phosphoryl group transfer is kinetically favoured over intermolecular transfer: $k_1 > k_2$. In Fig. 11, an additional possibility is suggested, which is that biological clocks provide an input that arbitrates beween physiological and developmental responses. The input could achieve this effect by acting on the sensor and response regulators in such a way as to alter the ratio k_1:k_2.

-9 > pts > -15

GIGASECONDS TO KILOTERASECONDS. THE FIFTH TIME DOMAIN

The major transitions in evolution.

Living organisms evolve, and their evolution has clearly been accompanied, in many cases, by increasing complexity. It is nevertheless a simple fallacy to conclude that a more complex organism is in some sense more highly evolved, or "higher" than a simpler one. Parasites are usually simpler that the free-living species from which they evolved. Parasites aside, many people still cling, intuitively, a crude, anthropocentric idea that all other living things are failed attempts along the path to the eventual emergence of human beings. The terms "lower eukaryotes" and "higher eukaryotes" are nevertheless still in general currency. A cynical view is that these are just fancy ways of saying "yeast" and "myself", respectively. A good way of exposing the contradictions inherent in these terms is to ask their user where on the scale one should place an oak tree, or an octopus. One then often finds that the progressivist fallacy attaches great significance to such characteristics as being a chemoheterotroph, living on the land, and to having a nervous system or an immune response. An articulate cyanobacterium might regard each of these steps as retrograde, limiting future evolutionary possibilities to narrow and highly specialised physical environments. Microbiologists are mostly free of progressivist, anthropocentric tendencies, and will recognise that their favourite species, far from being "primitive", has probably had longer to evolve in something like its present form. One might then wonder why vertebrates, for example, are thought to be more highly evolved than pseudomonads or purple photosynthetic bacteria. The latter, in particular, have retained the ability to do almost anything.

Maynard Smith and Szathmáry (1995) have considered the major transitions in evolution, and propose a solution to the apparent paradox of increasing complexity without there being any rational basis for the idea of evolutionary progress. The solution is the existence of transitions in levels of organisation, particularly where these involve information transfer. Thus the largest step, second to the origin of life itself, was probably the separation of information coding from its translation - the emergence of the specialised roles of DNA, RNA and protein from the "RNA world" in which chemically similar macromolecules performed both catalysis and replication. The general theme of such major transitions seems to be division of labour, in which each new level of organisation incorporates specialised components which, on their own, become less versatile, but which, acting in a way that complements other specialised components, create new possibilities for the environments that can successfully be exploited by the whole. According to this view, the evolution of human language ($\Delta pt_s \sim -13$) is then the most recent major evolutionary transition.

The persistence of extra-nuclear genetic systems in eukaryotes.

Another clear example of a major evolutionary transition, and one that intuitively fits with the general description, is the appearance of eukaryotes from prokaryotes. The idea that eukaryotes arose by endosymbiosis (championed, for example, by Margulis 1981) has now become orthodox. Among the divisions of labour that endosymbiosis allowed was the separation of energy-coupling membranes from those that formed the interface of the cell with the extracellular milieu. Following such specialisation into subcellular compartments, or organelles, a large-scale copying of genetic information from the endosymbiont to the nucleus of the host cell must have occurred.

I have made a suggestion about why this copying of information has been incomplete in the case of chloroplasts and mitochondria. The argument depends on the interaction of some of the events discussed in this overview, and, at least for chloroplasts, upon the adaptations to light proposed for time domain four. A key feature of adaptations to changing light quality and quantity is that the primary influence of such changes on photosynthesis is felt through changes in the redox state of components of electron transport chains. In time domain three we considered the

precedent of redox-controlled protein phosphorylation in chloroplasts, and it was pointed out that a parallel, developmental redox response also occurs, in the form of photosystem stoichiometry adjustment. The selective value of maintenance of redox homeostasis may be two-fold. First, in order to function efficiently, redox poise must be maintained so that the input of light energy matches the capacity of the reaction centres to utilise it. Second, the redox chemistry initiated at photosynthetic reaction centres is an inherently hazardous process, producing the most indiscriminately reactive chemical species that occur in living cells. Single electron transfers at very high and low redox potential have a high probability of generating free radicals, especially of oxygen, such as superoxide, singlet oxygen, and the hydroxyl radical. Even at the moderate potentials of the proton-motive Q-cycle, the semiquinone anion radical has a crucial role (Fig. 4), and yet it is an effective generator of superoxide. It follows that adaptations that compensate for light-induced redox changes within photosynthetic systems may be essential in order to safeguard the cell from self-destruction. The assumption of the evolutionary proposal is that chloroplasts today encode those proteins whose redox functions require that their genes be retained within the same cellular compartment as that where the redox signals originate (Allen, 1993).

A complementary proposal, that of Raven et al. (1994), is that the selective pressure tending to move genes from the endosymbiont to the nucleus is a decreased mutation frequency. The reason for the inherently high mutation frequency in organelles may be precisely the same generation of oxygen free radicals that, according to my own proposal (Allen 1993), is minimised, though never eliminated, by redox control of gene expression. Genes are safer, and perhaps capable of being more capable of being appropriately deployed, in the nucleus. The chloroplast or mitochondrion is the wrong place to keep a genetic system, and, according to this synthesis of independently-derived ideas (Allen & Raven, 1996), the proximity of genes for the key redox components of photosynthesis and respiration to the electron transport chains in which their gene products participate is a necessary price to be paid, and serves the same end as removal of other genes to the nucleus.

Thus there are powerful selective forces operating selectively on different endosymbiont, and, today, organellar, genes, which have thus segregated rather strictly between the nucleus, the chloroplast, and the mitochondrion. This view reinforces the idea that there must be an over-riding reason for the evolutionary retention of extra-nuclear genetic systems. The transition envisaged (Allen, 1993; Allen & Raven, 1996) for the evolution of chloroplast and mitochondrial genomes is depicted in Fig. 10. With some variations between major eukaryotic kingdoms and phyla, the present-day list of chloroplast and mitochondrial "structural" genes is precisely one of the components of photosynthesis and respiration that would seem to be most crucial for the regulation of organelle redox homeostasis (Allen, 1993). The chloroplast genome always encodes reaction centre proteins (time domain 2), for example, and most mitochondria likewise retain genes for components operating at the highest and lowest redox potentials of the respiratory electron transport chain. In addition, perhaps because of the involvement of the semiquinone anion radical in the Q-cycle (time domain 2; Fig. 4), cytochrome *b* is encoded in both chloroplasts and mitochondria. To this list of essential structural genes must then be added only genes for components of the minimal organellar genetic system that is required for the structural genes to be expressed. The components of the redox regulatory system itself (as depicted, for example, in Fig. 8, and in more general terms in Fig. 9) do not belong either to the category of key redox elements of electron transport chains or to the category of genetic system genes, and are therefore predicted to be nuclearly-encoded (Allen 1993).

Other evolutionary effects of light.

Microorganisms may carry out photosynthesis, and may exhibit phototaxis, phototropism, and photonasty (photocontrol of development in which the change induced is not influenced by the direction of the source of light). True vision involves production of a focused image of the external world, and the optical requirements for an eye probably cannot be satisfied by microorganisms, requiring true multicellularity with

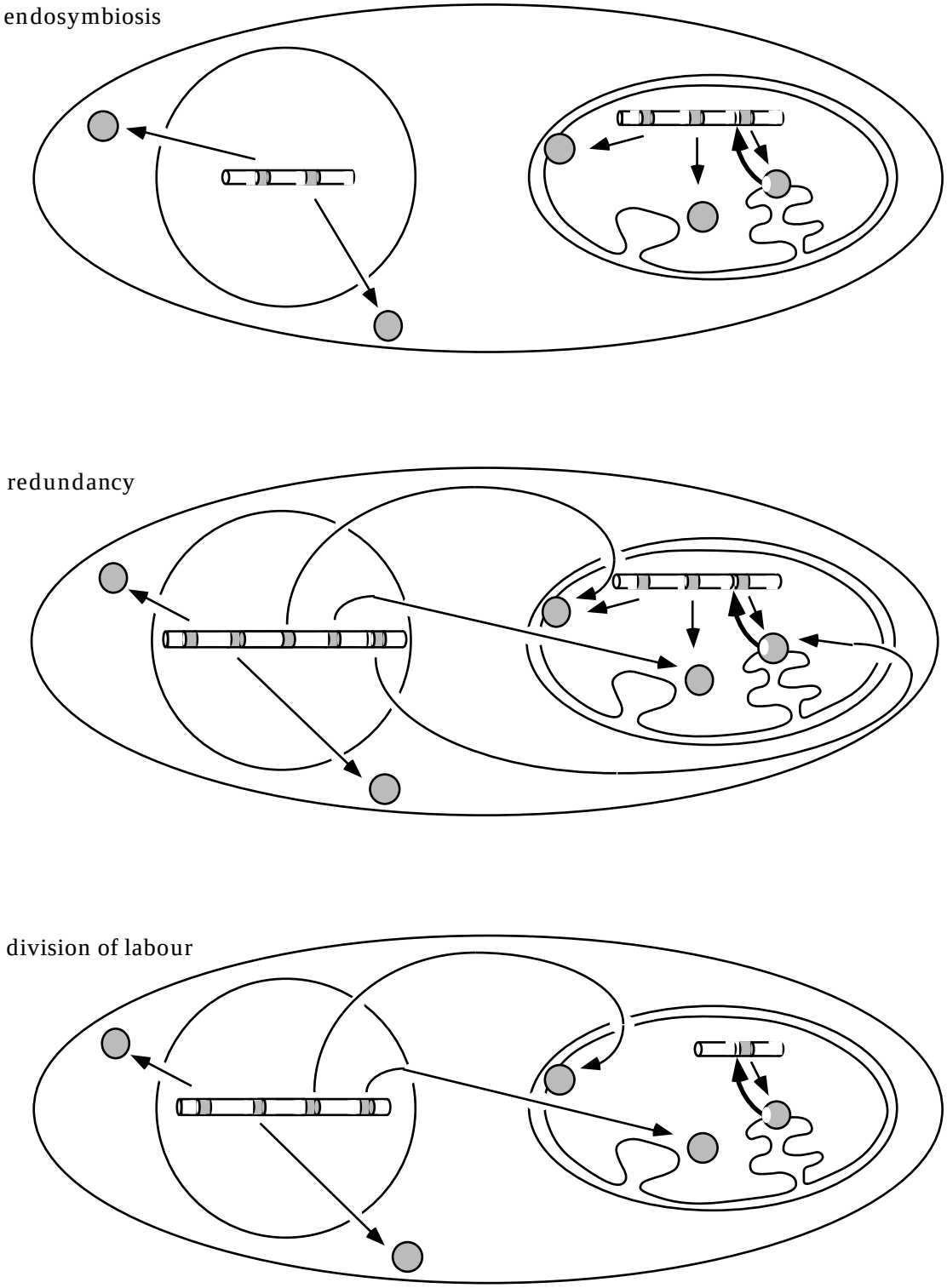


Fig 10. Possible stages in the evolution of division of labour between nuclear and cytoplasmic (chloroplast and mitochondrial) genetic systems. It is assumed that any gene may be copied between the endosymbiont (to the right of the schematic cell) and the host cell nucleus (to the left). Following endosymbiosis, most endosymbiont genes are subject to free radical mutagenesis, which they evade upon transfer to the nucleus: the endosymbiont copy is lost. In contrast, genes for certain key redox components of bioenergetic organelles remain *in situ*, since their expression must be subject to redox regulation in order to respond directly to environmentally-induced changes in electron transport. Loss of the symbiont or organellar copy of such genes would uncouple redox control, and hence increase free radical production within the organelle and the cell as a whole. The nuclear copy of each of this subset of genes is therefore redundant, and is lost. Adapted from Allen (1993) and Allen & Raven (1996).

cell specialisation and division of labour. Nevertheless, the evolutionary origin of vision (Nilsson, 199x) in the late pre-Cambrian ($\Delta p t_s$ = -16.5) is only half as distant as the origin ($\Delta p t_s$ = -16.8) of oxygenic photosynthesis (discussed in time domain 2) and is likely to have depended on the free energy made available by increasing atmospheric concentrations of oxygen. Vision requires, and then places an extra selective value, on both size and motility, since it permits visually-guided predation. This premium on size and motility may be expected to have enforced the dilemma of the mitochondrion: increased oxidative phosphorylation for motility must increase free radical production, and thereby make mitochondria less able accurately to replicate their own DNA. Mitochondrial replication is therefore in competition with mitochondrial function. Elsewhere (Allen 1996) I have suggested (Allen, 1996) a solution to the mitochondrial dilemma, which is entirely consistent with global view of evolutionary transitions described earlier (Maynard Smith and Szathmáry, 1995). The proposal is that mitochondria replicate and synthesise ATP only because of a division of labour, and that an individual mitochondrion may carry out either ATP synthesis or replication, but not both - not even at different times. The division of labour is thus a specialisation between one sex (female) that carries a subset of mitochondria which never function in ATP synthesis but instead act as a template for all other mitochondria, and another (male) in which mitochondrial replication, and thus mitochondrial inheritance, is abandoned altogether. Oxygenic photosynthesis was, according to this viewpoint, a necessary precursor of both vision and heterogametic sex, whose origins must therefore have been roughly contemporaneous. Furthermore, the adaptive mechanisms that may have evolved to allow prokaryotes to respond to changing light environments were the same as those that trapped genetic information in cytoplasmic, bioenergetic compartments, as a compromise in what might otherwise have been a complete separation of bioenergetic and genetic functions within the eukaryotic cell.

The pre-Cambrian explosion of varieties of multicellular animals (Gould 1991), a few of which laid the foundations of all modern animal phyla, obviously produced immense evolutionary possibilities. In the context of this overview, it may be enough to point out that the acquisition, by the first cyanobacteria, of the ability to use the well-developed photochemistry of photosynthetic reaction centres as a thermodynamic sink for water oxidation was a revolution for more than geochemistry: it provided the conditions for an evolutionary route out of a purely microbial world. As I hope is already clear, I do not argue that this was progress. I do argue that this was a major transition in evolution, and subsequent transitions in levels of organisation, including the one in which we now participate, would have been impossible without it.

CLOCKS AND THE EXPECTATION OF ENVIRONMENTAL CHANGE

Of the five familiar time intervals used for reference in Fig. 7, three correspond to the periods of a single astronomical rotation: the earth about its axis; the moon around the earth; and the earth around the sun. All three are used by at least some living organisms as environmental cues for developmental adaptation, crossing time domains three and four, and usually signalling the moment for progression from one developmental stage to another. It would be interesting to know if the lunar cycle, for example, is used as a cue only in multicellular animals that possess true vision. Although an eye may, to us, be helpful in distinguishing the phases of the moon, the accompanying changes in the intensity of the reflected sunlight are quite within the dynamic range to which living cells can and do respond. Circadian clocks in cyanobacteria should perhaps alert us to the additional possibility of twenty-eight day cycles in plants and microorganisms.

The discovery by Golden and co-workers (Kondo et al., 1994; Golden, this volume) of a cyanobacterial circadian rhythm has far-reaching consequences. Firstly, cloning techniques have allowed the comparison of “clock” genes from widely differing types of organism and reveal clear indications of homology (Kay , 1997). The occurrence of a biological clock in a prokaryotes gets completely away from the idea that such devices require a nervous system. The sequence similarities of clock components with those of known photoreceptors is interesting and exciting: it suggests that the photocontrol of development in processes such as complementary chromatic

adaptation may have been the starting point for the elaboration, perhaps initially by autofeedback, of a developmental control that ran freely in the absence of light signals, but which retained the ability respond to light in order to be regulated, or synchronised.

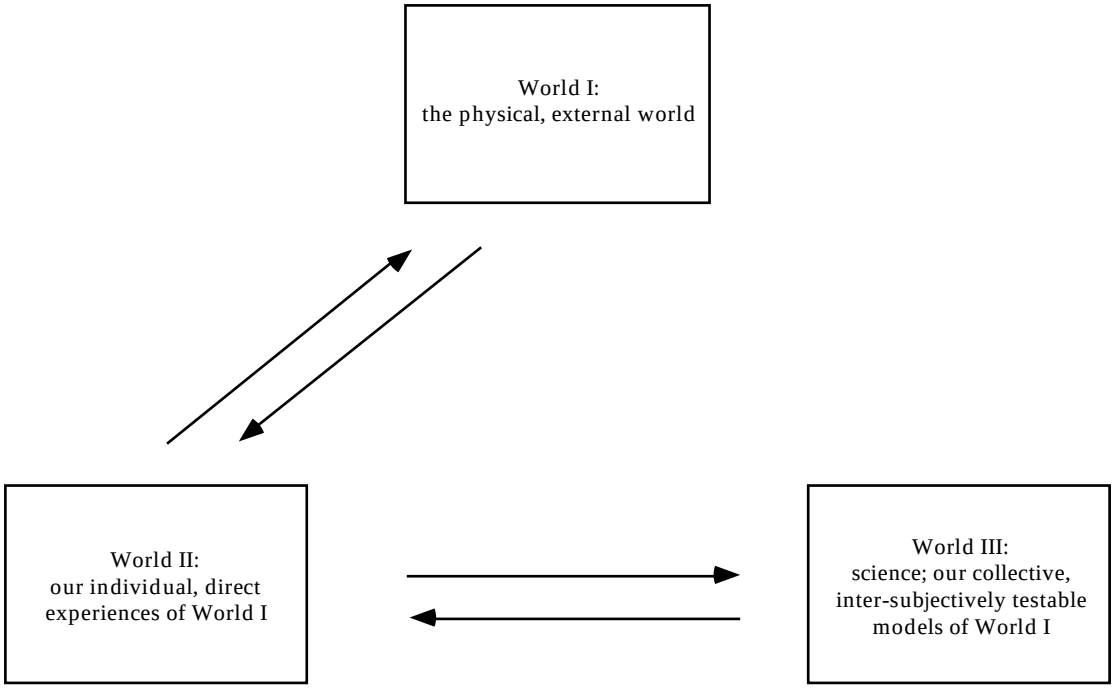
What is currently known of the molecular mechanisms underlying circadian rhythms will be described in a number of contributions to this symposium. At the time of writing this overview, I hope to learn, among other things, the frequency of the oscillator, which ought to be in place in Fig. 1 and Fig. 7 as a fundamental feature of living cells, and one that quite clearly must integrate cellular responses to light through time. In this attempted synopsis, I should like, finally, to make an observation about the potential philosophical impact of any molecular or biochemical mechanism for measurement of time that can be demonstrated to operate even in single cells - and "even" prokaryotes.

In the discussion of the interrelation of kinds of adaptation in time domain three, I suggested that the "blind" operation of negative feedback loops such as those depicted in Fig. 7 might be insufficient to account for adaptations, particularly developmental ones. In time domain four, I also raised the question of whether it is possible to imagine that cells can make a decision about the appropriate level of adaptive response, without invoking an intelligent or purposeful guiding hand. I suggest that the existence of molecular clocks in living cells may provide the basis for answering both these points.

The upper half of Fig. 11 shows a model of Sir Karl Popper (Popper, 1972). The arrows between boxes are intended to indicate the direction of information transfer. Popper was concerned with providing a conceptual and logical framework for scientific knowledge, without the "progressivist" assumption that the development of science can be measured as the extent to which it approaches "the truth" - whatever that may be. Thus, in Fig. 11, there is no direct connection between human knowledge ("World III") and the real world ("World I"). Nevertheless, World III may be modified by means of comparison of experience ("World II") with the expectations, derived from World III, that determine our perceptions. Conversely, there is a weaker sense in which the external world may be modified as a result of our perception of it: the arrow from World II to World I may indicate the selection of particular experiences, since all observation is guided expectation, though it could also be interpreted as changes that we make to our environment as a result of our knowledge of it.

The lower half of Fig. 11 show a simple translation of Popper's model into terms more specifically relevant to the topic of this symposium. Again, the arrows indicate direction of information transfer. In place of World I, the lower diagram has changes in the light environment. Adaptations then consist not merely of "blind" responses to external changes, but of responses that arise in some way from the outcome of a comparison of the external signal with the internal reference provided by the oscillator or clock. Transient shading might thus be distinguishable from sunset, for example, and progressive changes in daylength might be identifiable as such, and correlated with seasonal changes in, for example, temperature. In the lower diagram, the arrow rising from left to right indicates the cell's or organism's ability to carry out changes - adaptations - that tend to restore its immediate physical environment and eliminate the need for further adaptation. If a physiological adaptation fails to do this, the internal time reference might arbitrate in the deployment of an equivalent developmental adaptation: trees do not shed their leaves at dusk. In a general sense, the box that replaces World III in the lower diagram could be labelled the sum of the cell's inherited predispositions to act in certain ways, as outlined by Popper himself (1972). The major evolutionary transition of human language then fits, again, into the definition of Maynard Smith and Szathmáry (1995): only in the upper diagram does the lower, right-hand box become separated from the continued existence of the individual organism that carries the reference: we may, if we choose, change our minds. In Popper's (1972) words; "The difference between the amoeba and Einstein is that the amoeba must die with its theories".

In conclusion, I should like to propose that a major theme in biological evolution has been light, time, and the interplay between external signals that cells select as significant by means of reference to their internal clocks. Light is, and always has been, at least as important as any other factor in the physical environment. Because of



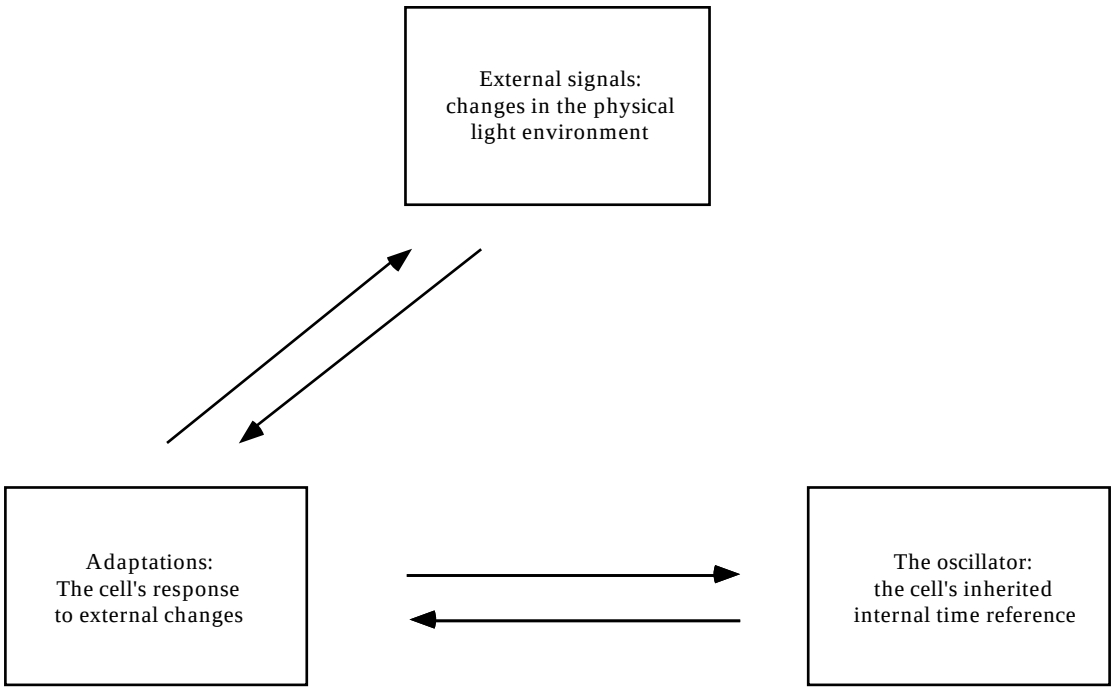


Fig 11. An analogy between Popper's hypothesis of "World III" (Popper, 1972) and biological responses to light that may be selected with reference to time. The oscillator or internal time reference arbitrates between levels of response to changes in the physical light environment. A possible mechanism for such arbitration is outlined in Fig. 9.

their reliance on vision, this is still true in organisms that have long abandoned phototrophy. The existence of a circadian oscillator in prokaryotes, particularly, is delightful, and underlies the central importance of life's responses to light and time. The possible origin of molecular clocks from photoreceptors, and their subsequent evolution and adaptive radiation can, I suggest, be seen as a new paradigm in biology. Biological clocks enlarge the meaning carried by Dawkins's (Dawkins, 1986) striking metaphor for evolution - "the blind watchmaker". Besides producing intricate structures that have function without purpose, evolution has itself come up with time-reference, and one that uses light as a mechanism of calibration. Evolution is thus, in a literal sense, a watchmaker: only in the figurative sense is it blind.

ACKNOWLEDGEMENTS

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