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THERMOSYNTHESIS: WHERE BIOLOGY MEETS THERMODYNAMICS

Dedicated to the memory of Aad Tervoort (1942-1995)

1. THE IMPORTANCE OF ORDER

Hermann Haken has summarized the question of the relation between order and the origin of life very well (the italics are mine)¹:

The spontaneous formation of well organized structures out of germs or even out of chaos is one of the most fascinating phenomena and most challenging problems scientists are confronted with. Such phenomena are an experience of our daily life when we observe the growth of plants and animals. Thinking of much larger time scales, scientists are led into *the problems of evolution and ultimately, of the origin of living matter*. When we try to explain or understand in some sense these extremely complex biological phenomena it is a natural question, whether processes of self-organization may be found in much simpler systems of the unanimated world.

In recent years it has become more and more evident that there exist numerous examples in physical and chemical systems where well organized spatial, temporal, or spatio-temporal structures arise out of chaotic states. Furthermore, as in living organisms, the functioning of these systems can be maintained only by a flux of energy (and matter) through them. In contrast to man-made machines, which are devised to exhibit special structures and functionings, these structures develop spontaneously – they are self-organizing.

So for many physical processes the self-ordering or self-organization is well understood. Not so for biology, and in particular not for the origin of life². The present study gives a solution to this question of the origin of biological order, maybe the most important scientific problem. *The origin of biological order is*

¹ H. Haken, Synergetics, Berlin-Heidelberg 1977 Springer, p. 4.

² Again, Haken: "In view of the physical phenomena and thermodynamic laws we have mentioned above, the possibility of explaining biological phenomena, especially, the creation of order on a macroscopic level out of chaos, seems to look rather hopeless. This has led prominent scientists to believe that such an explanation is impossible", ibidem.

proposed to be convection. The first organisms are presumed to have lived on the thermal cycling experienced while they were carried along by convection currents in volcanic hot springs (Fig. 1).

A heat engine takes up heat at a high temperature, converts part of the heat into work, and rejects the rest of the heat at a low temperature. The steam

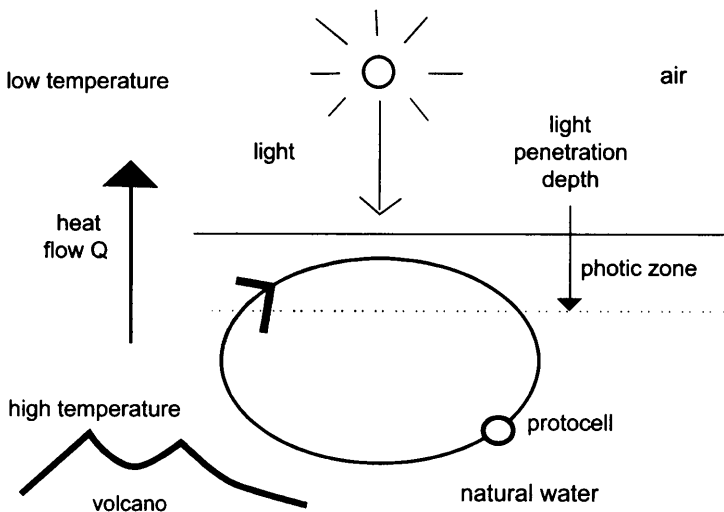


Fig. 1. *Convection cell.* Convection occurs in volcanic hot springs and other natural waters. In a convection cell a fluid is heated from below and cooled at the top. Convection is the best known example of self-organization by a dissipative structure. Free energy is dissipated as heat moves from a high to a low temperature. A protocell suspended in the convection cell will be thermally cycled, which presumably is used to conserve a – very small – part of the free energy available in the convection cell. When the fluid is illuminated from above, the protocells are also subject to cyclic illumination.

engine is an example: the boiler is the high-temperature heat source and external cooling water the low-temperature heat sink (Fig. 2). The pressure of steam moves the piston against a large force. Upon cooling of the steam its pressure collapses; the piston is then reset with little effort. The enclosed area in the pressure vs volume plot indicates the external work done in one cycle. The water within the engine is thermally cycled: it boils, condenses, boils again, etc.

Slight modification, if at all, of present day enzymes may give them the capability to function as heat engines during thermal cycling as well. This process is named *thermosynthesis* (TS). Applied to the origin of life, thermosynthesis permits biological use of convection, a process that is self-organizing.

In addition, thermosynthesis starts the path of the evolution of biological energy conversion that leads to photosynthesis (PS).

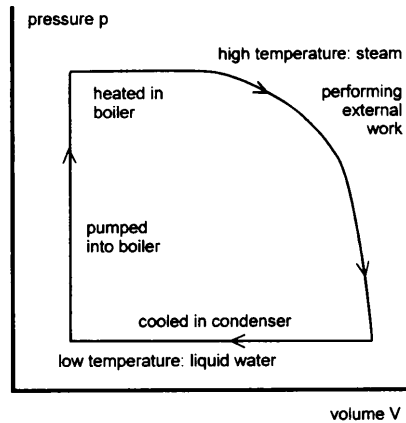


Fig. 2: Pressure-volume diagram of water circulating in a steam engine. The water is heated in the boiler and becomes steam. The pressure increases. The steam moves the piston; as the steam expands it performs external work, and still takes up heat from the boiler. Thereafter the steam condenses in the condenser and its volume decreases. Finally the resulting water is pumped again into the boiler. Heat moves from a high to a low temperature; free energy is conserved in external work.

Two types of TS are distinguished: protein-associated (PTS) and membrane-associated thermosynthesis (MTS). In PTS – a process similar to the mechanism of the F_1 part of the enzyme ATPsynthase – thermal cycling causes cyclic folding and unfolding of an enzyme, in synchrony with its catalytic cycle. Upon folding, substrates find themselves in a dehydrated cavity. In the local absence of water the substrates condense, which does not cost free energy. The high free energy of the products in the solvated state results in these products being locked in by the enzyme, remaining tightly bound. They are released only upon thermal unfolding of the enzyme. Pertinent products are peptides and polyphosphates.

MTS uses temperature dependence of the electrical polarization of a membrane, as occurs during a thermotropic phase transition of the membrane lipids. The enzyme ATPsynthase charges the membrane at one temperature, and discharges it at another. A higher charge/ATP ratio during charging results in a net gain of ATP. Photosystem 0 (PS0) is an intermediate mechanism in the path towards contemporary bacterial photosynthesis; it uses the same ATPsynthase as MTS and works on cyclic changes in light intensity. Upon illumination dipoles are formed that increase the membrane potential. In the dark the dipoles vanish. During evolution the light-induced dipoles length-

ened until they spanned the membrane. Acquisition of quinones then permitted standard bacterial photosynthesis: discharge of the dipole by means of the quinones, accompanied by proton translocation, completed the light-driven proton pump that operates in bacterial photosynthesis.

The ramifications of TS and PS0 are numerous. A model for the evolution of biological energy conversion (Ch. 3) is of interest on its own. As a bonus, it permits a model for the origin of life (Ch. 4). TS may still occur at present in those organisms or biomembranes that move in thermal gradients (Ch. 5), for instance through thermoclines, and it may play a role in the many contemporary biological phenomena associated with thermal cycling such as the induction of germination, budding and flowering (Ch. 6). In the chloroplast only a few, small changes suffice to enable PS0 activity. The peculiar properties of its ATPsynthase are consistent with PS0, the occurring oscillations resemble PS0 oscillations, and many dynamic phenomena such as State 1-2 transitions and temperature effects may be vestiges from MTS/PS0. An appropriate introduction to thermodynamics is given in Chapter 2.

Many references are given in my previous papers³; the present study gives only a few key references.

2. STARTING POINTS

2.1. THERMODYNAMICS – AN INTRODUCTION

The life scientist may doubt that thermodynamics can yield the key for understanding the origin of life, and its present functioning⁴.

In contrast, the present study claims that knowledge of thermodynamics is *essential* for an understanding of the origin of life and how organisms work.

³ A.W.J. Muller, Thermoelectric energy conversion could be an energy source of living organisms, "Physics Letters" 1983, v. A 96, p. 319-321; A.W.J. Muller, Thermosynthesis by biomembranes: energy gain from cyclic temperature changes, "Journal of Theoretical Biology" 1985, v. 115, p. 429-453; A.W.J. Muller, A mechanism for thermosynthesis based on a thermotropic phase transition in an asymmetric biomembrane, "Physiological Chemistry and Physics & Medical NMR" 1993, v. 25, p. 95-111; A.W.J. Muller, A model for biogenesis based on thermosynthesis, "Origin of Life and Evolution of the Biosphere" 1994, v. 24, p. 253-254; A.J.W. Muller, Were the first organisms heat engines? A new model for biogenesis and the early evolution of biological energy conversion, "Progress in Biophysics and Molecular Biology" 1995, v. 63, p. 193-231; A.J.W. Muller, Hypothesis: the thermosynthesis model for the origin of life and the emergence of regulation by Ca²⁺, "Essays in Biochemistry" 1996, v. 31, p. 103-119.

⁴ "Thermodynamics with his emphasis on heat engines and abstract energy concepts, has often seemed irrelevant to biochemists. Indeed, a conventional introduction to the subject is almost certain to convince the student that much of thermodynamics is sheer sophistry and unrelated to the real business of biochemistry, which is discovering how molecules make organisms work", K.E. Van Holde, Physical biochemistry, New York 1971 Prentice Hall, p. 3.

Knowledge of its history, in turn, helps in understanding thermodynamics. A sketch based on Cardwell's study⁵ of its main concepts, their development and its practical use is given here; for exactness and detail the reader is referred to the extensive literature.

Energy. The concept of energy emerged in the seventeenth century during the study of the dynamics of collisions where $\frac{1}{2}mv^2$ was called the *vis viva*. The physicist's *vis viva* was related to the engineer's measure of "duty", or mechanical work, by Hachette in 1811.

Work. The qualitative concept of work is obvious from everyday life. Quantitatively, work is defined as the product of force and distance. Work conservation explains the lever and the pulley, for which it is known since Archimedes that "what is gained in force is lost in distance." Man developed machinery to use the work done by animals, the wind and falling water. Within this machinery work decreases monotonously – because of friction – as one moves away from the application point of the external force.

Friction. In the world around us movement tends to vanish. Beeckman (≈ 1630) knew that only in the absence of friction – an object gliding on ice – movement would continue indefinitely. Newton generalized this idea to his Second Law of Motion: force causes acceleration.

Efficiency and reversibility. Water mills can be used for pumping water. Engineers calculated their efficiency as the ratio of the energies of the water pumped and the water fallen. The importance of a low turbulence of the falling water for a high efficiency was recognized. According to Déparcieux (1752), a water mill would have an efficiency of 1 in the absence of friction, which would permit reversibility.

Heat. The distinction between heat and temperature became possible upon the development of the thermometer in the early 17th century. A century later different substances were shown to have different heat capacities. At that time the law of conservation of mass was discovered, and Lavoisier and Laplace similarly proposed the conservation of heat, an idea opposed by Rumford (1800), who showed that heat can be generated *de novo* by friction. Incorrectly generalizing calorimetry experiments, most physicists continued however to assume the conservation of heat.

Heat engines. The steam engine was the first heat engine. Newcomen's steam engine (1712) was considerably improved by Watt (1765), who realised that heating and cooling the steam at different places increases efficiency, defined by Ewart in 1813 as the ratio of work produced and heat input, W/Q . Engineers noticed that the efficiency increased with the steam pressure

⁵ D.S.L. Cardwell, *From Watt to Clausius*, Iowa City 1989 Iowa State University Press.

and/or temperature. Carnot (1824) observed that a temperature difference, ΔT , was essential for a heat engine: *Carnot's principle*. He showed that W/Q was proportional to ΔT , and that heat conduction decreases the efficiency. His ideas were however ignored. Writings found after his death (1832) state that the work done by a heat engine originates from part of the incoming heat – heat is *not* conserved.

The First Law of Thermodynamics. Mayer (1842) stated first publicly that (1) heat and work can be converted to one another, and (2) their sum, the energy, is conserved. In formula: $U = Q + W$ (U is the energy, Q the heat, W is work done *on* the system). The germ of Mayer's insight was the observation that in the tropics the blood in the veins is as red as in the arteries. Physicians explained this by a lessened oxygen consumption, less food being burned for maintaining the body temperature. Mayer deduced that a certain amount of food can result in variable amounts of work and heat – but that their sum is constant. From there to the First Law is only a small step. He calculated the heat released upon the impact of a falling object. Quickly, the experimental basis of the First Law was improved by Joule (1845, 1849), the mathematical basis by Helmholtz (1847).

2.2. ENTROPY, A GENERALIZED MEASURE OF FRICTION

Are there natural limits to the amount of heat that a heat engine can convert into work? This question can be answered by considering heat pumps such as the refrigerator, in which heat is brought from a low to a high temperature. By considering a reversible heat engine driving a reversible heat pump, Clausius (1850) and Thomson (1852) derived the – deceptively simple – formula:

$W = Q \Delta T/T$ for the work generated by a reversible heat engine and the work consumed by a reversible heat pump (T equals the temperature in K). The fraction $\Delta T/T$ is called the *Carnot ratio*.

For many mechanical processes that involve rigid bodies or moving fluids generated heat is a suitable measure of friction and inefficiency. Gases yield however a clear counterexample for such a measure. A gas that expands may cool, the explanation for the haze of condensed water formed as one opens a can of beer. Expanding steam that performs work takes up heat. Can heat due to friction be distinguished from the heat involved in other processes?

For the just mentioned reversible heat engines and heat pumps introducing friction causes $W < W_{rev}$ for the heat engine (here work is done *by* the system), while for the heat pump $W > W_{rev}$ (here work is done *on* the system). For both machines the difference is converted into heat, causing irreversibility. Conversely, irreversibility is however not always associated with heat generation. Examples are (1) spontaneous cooling, heat conduction from a high to

a low temperature, and (2) pressured gas that escapes in a vacuum. The concept of entropy includes such irreversible processes in a generalization of friction.

Consider a *reversible* increase of the temperature of a system (state l, low temp. $T_l \rightarrow$ state h, high temp. T_h) by means of two different reversible heat pumps 1 and 2, both pumps obtaining the heat from a reservoir at a temperature T_r . The heat dQ_i added during an intermediate step i at a temperature T_i relates to the heat taken from the reservoir, dQ_r , by $dQ_i = dQ_r + dW_i = (T_i/T_r) dQ_r$, where dW_i is the work done on the pump, equal to $dQ_r \Delta T/T_r$. Then: $dQ_r = T_r \{dQ_i/T_i\}$. Integration yields: $Q_r = T_r (\int_1^h dQ_i/T_i)$. For the other pump $Q_r' = T_r' (\int_2^h dQ_i'/T_i')$. Suppose that Q_r and Q_r' are *not* equal, with $Q_r > Q_r'$. It follows from the First Law that the second pump must have done more work W' than the first pump (W). Let the first pump run the described process, followed by a backwards run of the second pump: the reversibility of the second pump then results in a net gain of work $W' - W$ by conversion from a heat $Q_r - Q_r'$ from a single source. This contrasts with Carnot's principle (and Thomson's principle, mentioned hereafter). It follows that Q_r must be equal to Q_r' , and that $\int_1^h dQ/T$ must be the same for all reversible heat engines and heat pumps. This integral is the *entropy*.

Follow the entropy in a reversible heat engine, the ideal steam engine. As the boiler heats the steam the boiler's entropy decreases and the steam's entropy increases (and external work is done). During condensation the steam's entropy decreases, but the entropy of the cooling water increases. At the end of the cycle the entropy of the water/steam has not changed, but the entropy of the cooling water has increased with the same amount as the entropy of the boiler has decreased. For a reversible cyclic process the entropy of a system plus surroundings remains constant, although the entropy of the partial systems can decrease, increase or vary.

The Second Law of Thermodynamics. During friction work is converted into heat Q_f . The entropy then increases – without a corresponding decrease in entropy somewhere else – with Q_f/T , where T is the temperature of the point of friction. During heat conduction there is no loss of work, but the entropy increases as well: as the heat Q moves from the high to the low temperature the entropy increases with $Q (1/T_l - 1/T_h)$. One can prove that for an irreversible decrease in gas pressure the entropy also increases. Friction and irreversibility are therefore associated with an increase in the sum of the entropies of a system and its surroundings. This leads to Clausius famous formulation (1865) of the Second Law of Thermodynamics: "The entropy of the universe tends to a maximum". Equivalent formulations of the Second Law are *Thomson's principle*: "It is impossible by a cyclic process to take heat from

a reservoir and convert it into work without transferring heat from a hot to a cold reservoir” and, Clausius, again: “Heat cannot move on its own from a low to a high temperature.” Clearly, entropy is a suitable generalized measure of friction.

The importance of entropy naturally leads to the questions of its interpretation on the molecular level and to the cause of its monotonic increase. Statistical mechanics studies have given as answer that the entropy of a macroscopic (macro) state is associated with the number of different ways the present particles can be distributed. Consider the distribution in space, in a given volume, of the molecules of a gas. Divide this volume in parts V_i , and notice the number of particles n_i in these parts V_i ; then at a certain time one microscopic (micro) state (denoted by the numbers of particles in all volumina V_i) out of many (number n_A) possible different micro states (set A) is assumed. If the pressure decreases, and the volume increases, both the entropy and the number n_B of possible different micro states (set B) increase: the two go hand in hand. Set A is a subset of B, but the chance that its micro states are assumed is negligible, as $n_A \ll n_B$. The natural increase in entropy corresponds to the natural tendency of systems to move towards a macro state with a larger disorder, a larger number of different micro states.

For an expanding gas the entropy increase can thus be explained in terms of statistical mechanics. For other systems entropy changes are often *not so easily explained*, and may therefore be somewhat mysterious; they are however *easily measured*, and thus *well defined*. Since the entropy change of a compound equals $dS = dQ/T$, and $dQ = C dT$, where C is the heat capacity, one can write $dS = \int_1^h C/T dT$: the entropy derives from the heat capacity. The amount of work that a system can do equals $F = U - T S$, where F is the Helmholtz free energy. F and U are suited to treat systems with a constant volume. It is more practical to consider systems at constant pressure. This is easily done by adding a term p V to F and U, yielding the free enthalpy G and the enthalpy H, with $G = H - T S$. The difference between G and F and between H and U is often small. G is also called the Gibbs (free) energy, or the chemical potential, because, just as an object tends to fall to its lowest potential, a system, by increasing the entropy S, tends to ‘fall’ to the lowest G.

In chemistry entropy yields the explanation of *endothermic* chemical reactions, reactions in which heat is *absorbed*. As an example we treat the simple chemical reaction: $A \rightleftharpoons B$. The increase in free energy as the reaction goes to the right equals:

$$\Delta\Delta G = \Delta G_B - \Delta G_A = (\Delta H_B - T\Delta S_B) - (\Delta H_A - T\Delta S_A) = (\Delta H_B - \Delta H_A) - T(\Delta S_B - \Delta S_A).$$

For the reaction to go to the right, $\Delta\Delta G$ has to be smaller than zero. The equilibrium constant $K = [B]/[A]$ equals $\exp(-\Delta\Delta G/RT)$. Assume that at

a certain temperature T_i $\Delta\Delta G = 0$, with $\Delta H_A = \Delta H_B$, $\Delta S_A = \Delta S_B$. At a higher temperature T_h then

$$\Delta\Delta G(T_h) = (\Delta H_B(T_h) - \Delta H_A(T_h)) - T_h (\Delta S_B(T_h) - \Delta S_A(T_h)) = \Delta\Delta H(T_h) - T_h \Delta\Delta S(T_h),$$

with $\Delta\Delta H(T_h) = \int_{T_i}^{T_h} (C_B - C_A) dT$, $\Delta\Delta S(T_h) = \int_{T_i}^{T_h} (C_B - C_A)/T dT$.

Assume $C_B > C_A$ just above T_i , and $C_B < C_A$ just below T_h , with $\int_{T_i}^{T_h} (C_B - C_A) dT = 0$. This might occur when B does, and A does not, undergo a kind of phase transition just above T_i . Then $\Delta\Delta H(T_h) = 0$, but, because of the weighing factor $1/T$, $\Delta\Delta S(T_h) = \int_{T_i}^{T_h} (C_B - C_A) dT/T > 0$, and thus $\Delta\Delta G(T_h) < 0$. At the high temperature the direction of the reaction is $A \Rightarrow B$, without heat absorption or rejection, but with an increase in entropy. More generally, it shall be clear that with proper values of $C_A(T)$ and $C_B(T)$ both $\Delta\Delta H(T_h)$ and $\Delta\Delta S(T_h)$ can be larger than zero, with $\Delta\Delta G(T_h) < 0$. In that case heat is absorbed during the reaction, as indeed is found in an endothermic reaction. As late as 1878 some chemists considered the heat rejected during a chemical reaction to be a good measure of chemical affinity⁶; the inappropriateness of this idea should now be clear!

Entropy is very important in biochemistry, in particular in the *hydrophobic effect*, which stabilizes proteins and membranes. Its gist is the observation that 'greasy-like' substances that contain many hydrocarbon groups (e.g., lipids, certain amino acid residues) often hardly dissolve in water as spatially separated molecules. Although the ΔH term may be negative, the $T\Delta S$ term is even more negative, resulting in a positive ΔG . The decrease in entropy upon solvation is attributed to a structure change in the water surrounding the molecule; this change has been compared to the formation of small local "icebergs." When two hydrophobic molecules become neighbours, the amount of structured water around the molecules decreases, resulting in an increase in entropy: as a result the molecules are driven towards each other, forming micelles (in soap solutions), biomembranes and stabilizing protein structures. Within these structures little water may be present⁷.

During dry conditions synthesis of peptides and high-energy polyphosphates does not cost free energy. Solvated in water they have a high energy and are unstable with respect to hydrolysis⁸. The formation of high-energy polyphosphate compounds typically occurs within proteins in the local absence of water⁹.

⁶ W.J. Moore, Physical chemistry, London 1962 Longmans. p. 63.

⁷ The hydrophobic effect is still the subject of intensive research and much debate, see v. 259 (1995) of "Methods in Enzymology".

⁸ A.B. Meggy, Glycine peptides. Part II. The heat and entropy of formation of the peptide bond in polyglycine, "Journal of the Chemical Society" 1956, v. B 1956, p. 1444-1454.

⁹ L. DeMeis, Role of water in the energy of hydrolysis of phosphate compounds. Energy

The concept of entropy is used in calculating reversible isothermal heat uptake or rejection, such as for instance during the isothermal expansion of steam in the steam engine. A small amount of such volume work is given by $dW = + p dV$ (dW is done by the system). Work can be done in many other ways, and is typically the product of two parameters: a wire is stretched:

$dW = J dL$ (J tension, L the length); a surface film is stretched: $dW = \gamma dA$ (γ surface tension, A the surface area); a battery is charged: $dW = E dq$ (E the potential difference, dq the amount of charge); a capacitor is charged: $dW = E dq$, with polarization of the dielectric, etc. When one of these parameters (e.g. γ) depends on the temperature then the heat uptake/rejection upon an isothermal change of the other parameter (e.g. ΔA) is easily calculated. The entropy change is proportional to the first derivative of that parameter to the temperature (e.g. $\Delta S \propto d\gamma/dT \Delta A$; for the steam engine: $dP/dT \Delta V$). In particular the heat uptake/rejection during the charging of a capacitor containing a dielectric with a temperature-dependent polarization can be derived in this manner.

2.3. SELF-ORGANIZATION AND CONVECTION

Self-organization. The Second Law states that nature tends to disorder. Nevertheless order is continuously created around us. In addition to man-made machines, order arises in many systems, during chemical oscillations, during chemical reactions, during the formation of the electromagnetic field of a laser, etc. A close look shows that in such systems either the internal entropy increases or the entropy of the surroundings of the systems increases, compensating for the internal entropy decrease. In the latter case the system maintains its order at the cost of an external dissipation, and is therefore called a *dissipative system*. Many dissipative systems such as the steam engine are man-made. Living organisms are also dissipative systems, with at present a complex structure. They probably evolved from simpler dissipative systems. What was the first one?

Convection. The simplest and most frequent observed self-organizing dissipative system in nature is the convection cell: it only requires a fluid with a sufficient high temperature gradient. The external entropy increase during convection is due to the transport of heat from a high to a low temperature. Temperature differences are therefore important, the hot sun and the cold universe constituting a major difference. Obviously, the sun causes a high temperature during the day and summer, and in the tropics: the cold universe causes a low temperature at night and in the winter, and in the polar areas. The resulting air

transduction in biological membranes, "Biochimica et Biophysica Acta" 1989, v. 973, p. 333-349.

movements, due to convection, drive the wind in the earth's atmosphere, causing the rainfall which in turn causes rivers to flow. Both wind mills and water mills are therefore ultimately powered by the heat transfer from the sun to the universe. (Another heat source is the decay of radioactive elements inside the earth.) In the atmosphere convection causes winds and clouds, in the ocean currents and thermoclines, in the earth's mantle continental drift, and on a smaller scale, in meteoric waters in volcanic areas it causes ore deposition, an ordering of chemicals. Most order in the inanimate world is the – direct or indirect – result of convection.

2.4. GELS

Gels can be considered as polymers that strongly interact with a solvent, and in that manner immobilize it. For the present purpose it is of interest that the interaction with the solvent can strongly depend on the temperature. A small temperature change can cause the polymer to collapse, and presumably form a local environment in which the solvent is absent. In the case of a gel in water, some collapsed polymers are assumed to be the site of dehydration reactions that result in peptide and polyphosphate formation as occur in present day ribosomes and ATPsynthases.

2.5. CAPACITOR-BASED ENERGY CONVERTERS

Figure 3 gives the electronic scheme of a capacitor-based energy converter, which generates electrical power from cyclic changes in the temperature of light intensity. Its core can be a dielectric with a temperature-dependent dielectric constant, or, even better, a *pyroelectric*, a substance that contains an electric dipole that depends on the temperature. As already mentioned, a temperature dependent polarization leads to heat uptake or rejection during electric potential changes – this heat is the origin of the electrical work gained. Pyroelectricity is widespread, occurring in crystals, minerals, polymers, including biominerals and biopolymers. In the early 60s the thermodielectric converter was considered for power generation in satellites; the dielectric at the surface of a rotating satellite would be thermally cycled in the sunlight¹⁰.

A similar device has been proposed that works on fluctuating light. In the photodielectric effect light changes the polarization of a dielectric¹¹. Placing the light sensitive dielectric on top of an *electret*, a material with a constant electric polarization, yields a system in which the potential *increases* upon il-

¹⁰ P.M. Margosian, Parametric study of a thermoelectrostatic generator for space applications, 1965 NASA Techn. Note TN D-2763.

¹¹ R.W. Glazebrook, A. Thomas, Solar energy conversion via a photodielectric effect, "Journal of the Chemical Society. Faraday Transactions II" 1982, v. 78, p. 2053-2065.

lumination. When the electret is a pyroelectric, energy can be won from both thermal cycling and fluctuating light.

A combined MTS/PSO system is obtained by the substitutions capacitor → biomembrane, battery/load → ATPase/synthase with a variable stoichiometry, light-sensitive dielectric → photosynthetic reaction centers (RCs), and electret → molecular dipole layer. This dipole layer is best analyzed in terms of the dipole potential: The potential across a layer of dipoles with dipole moment μ (μ equals the product of the separated charge and their distance) normal to the surface, placed at a surface density N in a medium with dielectric constant ϵ , equals $\Delta\Psi_{\text{dipole}} = \Delta\Psi_D = N \mu/\epsilon$. For monolayers at the air-water interface this potential is easily measured and can be as high as several hundreds of millivolts (Fig. 4). It is temperature sensitive, especially during thermotropic phase transitions: for a dimyristoyl phosphatidylcholine monolayer $\Delta\Psi_D$ decreases 85 mV going from 22 to 26°C. In an asymmetric membrane consisting of two different monolayers the net dipole potential is called the *Membrane Dipole Potential* (MDP or $\Delta\Psi_D$). It is normally screened by counter ions. In PSO the *Photosynthetic Dipole Potential* (PDP or $\Delta\Psi_{\text{PDP}}$) is caused by dipoles induced by light within Rcs.

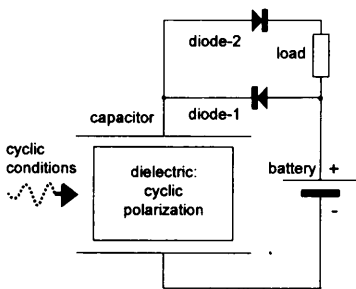


Fig. 3. *Capacitor-based energy converter.* The converter consist of a battery, a capacitor that contains a dielectric, two diodes, and a load, which takes up the gained free energy. The polarization of the dielectric varies cyclically due to cyclic changes in the temperature or light intensity. Let a polarization change of the dielectric first cause a drop of the voltage across the capacitor. The capacitor is then charged by the battery through diode-1. Next, reversal of the polarization change causes the voltage across the capacitor to rise again. The capacitor then can only discharge through diode-2, and the load; upon the latter then electrical work is done.

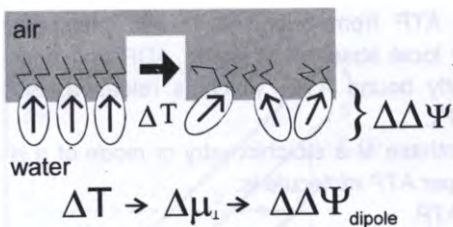


Fig. 4. The dipole potential across a monolayer of lipids at the water-air interface changes as the lipids undergo a thermotropic phase transition. The phase transition decreases the average dipole moment normal to the interface, causing the dipole potential to decrease.

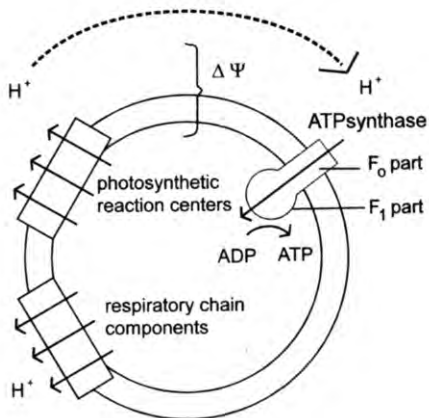


Fig. 5. The chemiosmotic mechanism. During respiration and photosynthesis, chains of enzymes pump protons across a membrane, causing the build-up of a potential and pH difference across the membrane. The protons return through the ATP synthase enzyme, which utilizes the difference in membrane potential and pH to synthesize ATP. The enzyme consists of two parts: a proton translocating moiety within the membrane, the F_0 part, and the ATP synthesizing moiety outside the membrane, the F_1 part.

3. THE EVOLUTION OF BACTERIAL PHOTOSYNTHESIS FROM THERMOSYNTHESIS

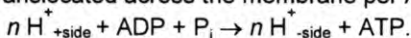
3.1. MITCHELL'S CHEMIOSMOTIC MECHANISM AND BACTERIAL PHOTOSYNTHESIS

In 1960 Peter Mitchell gave a model for how mitochondria make ATP during respiration: the free energy from the electrons involved in oxidation/reduction is transduced by protons that are pumped across the inner mitochondrial membrane. The free energy is stored in a difference in electrical potential ($\Delta\Psi$) and ΔpH across this membrane. The protons fall back through the membrane, through the F_0 part of the enzyme ATP synthase, which transduces their free energy into the free energy of ATP (Fig. 5). This *chemiosmotic mechanism* was shown to be applicable to photosynthesis as well. F_0F_1 -ATP synthase (F_0 and F_1 are the two parts of the enzyme) is present in bacteria, mitochondria and chloroplasts. F_1 's structure is known¹². Here the *binding*

¹² J.P. Abrahams, A.G.W. Leslie, R. Lutter, J.E. Walker, Structure at 2.8 Å resolution of

change mechanism¹³ synthesizes ATP from bound ADP and phosphate (Fig. 6). When tightly bound in the local absence of water, ADP and phosphate spontaneously react to tightly bound ATP, which is released upon a conformational energy input from F_0 .

The reaction catalyzed by ATPsynthase at a stoichiometry or *mode* of $n H^+$ translocated across the membrane per ATP molecule is:



The reaction is in equilibrium for $\Delta\Psi = V_n = V_1 / n$, where V_1 follows from the ΔG for ATP hydrolysis, ΔG_p : $V_1 = \Delta G_p / F \approx 470 \text{ mV}$ (F is Faraday's constant). In the presence of a ΔpH across the membrane, a term involving ΔpH must be added to $\Delta\Psi$, yielding the $\Delta\mu_H^+$ or *electrochemical proton gradient* across the membrane; for the present purpose this ΔpH term in the $\Delta\mu_H^+$ can be ignored. ATP is synthesized in a mode n for $\Delta\Psi > V_n$ and is hydrolyzed, i.e., protons are pumped, for $\Delta\Psi < V_n$. Whether ATP is synthesized or hydrolyzed at given $\Delta\mu_H^+$ thus depends on n . For different enzymes n differs. Values from 1 to 9 have been reported.

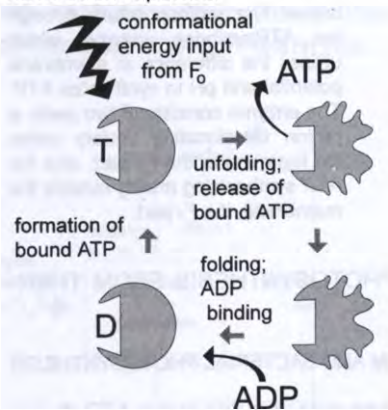


Fig. 6. The binding change mechanism of F_1 ATPsynthase. F_1 can change between a state with tightly bound ATP (T), indicated by a trapezium, and a state with loosely bound ADP (D) and phosphate; looseness is indicated by a rectangle. Tightly bound ATP can be released by a conformational energy transfer from another moiety: in the chemiosmotic mechanism (fig. 5) this free energy originates from the F_0 moiety which lets proton pass through the membrane.

MTS/PS0 postulates variability of n within a single enzyme. There is evidence supporting such variations. A simple scheme is variation – with the temperature, membrane voltage, membrane fluidity, etc. – between two values, say n and m ($n < m$). Each mode then has its own equilibrium membrane potential, V_n and V_m . Mechanistically, a mode variation can be caused by variation in the number of active proton carriers, i.e., subunits c (its *Asp/Glu-61* residue is the proton binding site) within F_0 .

F_1 -ATPase from bovine heart mitochondria, "Nature" 1994, v. 370, p. 621-628.

¹³ P.D. Boyer, The binding change mechanism for ATP synthase. Some probabilities and possibilities, "Biochimica et Biophysica Acta" 1993, v. 1140, p. 215-250.

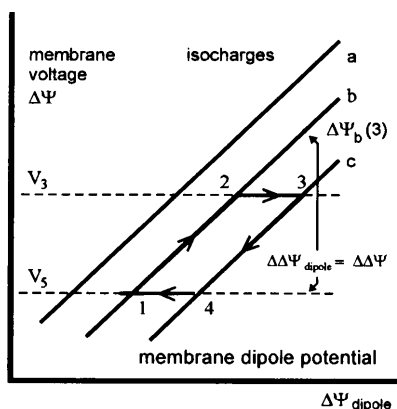


Fig. 7. The MTS /PS0 cycle: How ATP can be won from cyclic changes of the membrane dipole potential by an ATPase/synthase with a variable stoichiometry. The graph of the cycle consists of isocharges and isopotentials. Isocharges (a, b and c) result from temperature/light-intensity dependency of the dipole potential in the absence of charge translocation across the membrane. V_3 and V_5 are the equilibrium potential for mode 3 ($3H^+/ATP$) and mode 5 ($5H^+/ATP$). Isopotentials result from an ATPase/synthase activity switched-on/off by $\Delta\Psi$: ATPsynthase is active either for $\Delta\Psi > V_3$ in mode 3, or for $\Delta\Psi < V_5$ in mode 5. The cycle starts in 1, with $\Delta\Psi = V_5$, where mode 5 is inactivated because $\Delta\Psi$ has increased by

a $\Delta\Psi_{dipole}$ increase (1). Isocharge b is followed until V_3 is reached and mode 3 is activated (2). $\Delta\Psi_{dipole}$ continues to rise, but a discharge by ATPsynthase action keeps $\Delta\Psi$ at V_3 (2 \rightarrow 3). The free energy obtained as ATP equals $dQ V_3$, in which $dQ = C (\Delta\Psi_b(3) - V_3)$, with C equal to the electrical capacity of the membrane. A subsequent decrease of $\Delta\Psi_{dipole}$ decreases $\Delta\Psi$, which inactivates mode 3 (3). Isocharge c is followed until $\Delta\Psi$ drops below V_5 , and mode 5 is activated-ATPase action (4). Along isopotential V_5 the membrane is recharged, at a cost of $dQ V_5$, until point 1 is reached again (4 \rightarrow 1). The net performed electrical work is $dQ (V_3 - V_5)$; this work is converted into the free chemical energy of ATP.

In MTS/PS0 the membrane is charged by ATPsynthase (acting as a proton pumping ATPase) in a high mode and is discharged in a low mode (Fig. 7): the enzyme is presumed to be active in mode n only for $\Delta\Psi > V_n$ and active in mode m only for $\Delta\Psi < V_m$. Both modes are inactive for $V_m < \Delta\Psi < V_n$. As a result free energy is won as ATP from membrane potential fluctuations. Net ATP is gained, since a certain number of transported protons yields more ATP during forwards mode n activity than is consumed during their backwards return by mode m . Although there are major differences as well, the postulated enzyme with its low and high modes can be associated with the chloroplast CF_0F_1 with its oxidized and reduced states, and their distinct threshold potentials for ATP synthesis, which suggest mode changes¹⁴.

¹⁴ V. Junesch, P. Gräber, Influence of the redox state and the activation of the chloroplast ATP synthase on proton-transport-coupled ATP synthesis/hydrolysis, "Biochimica et Biophysica Acta" 1987, v. 893, p. 275-288.

3.2. PROTEIN-ASSOCIATED THERMOSYNTHESIS

The chemiosmotic mechanism is complicated, and its components are mutually dependent. In what sequence were its components acquired? TS permits a sequence starting with a progenitor of the F_1 subunit of ATPsynthase, which is proposed to be the pertinent primordial heat engine. During thermal cycling it synthesized ATP similarly to the binding change mechanism using a temperature-induced binding change (Fig. 8). pF_1 folds at a low temperature, binding ADP and P_i , rejecting heat and forming tightly bound ATP that is released upon unfolding at a higher temperature, with uptake of heat. The cycle is closed after a temperature decrease.

Upon nucleotide binding to its non-catalytic site, the unfolding temperature ($\approx 60^\circ\text{C}$) of the chloroplast CF_1 increases $\approx 7,5^\circ\text{C}$. Unfolding has a ΔH of $\approx 4000 \text{ kJ/mol}^{15}$. Applying the same value to the unfolding of pF_1 in an P TS-cycle, the Carnot ratio $7,5/333$ permits a work W on bound substrates of $W \leq (\Delta T/T) \Delta H = 90 \text{ kJ/mol}$; i.e., $\approx 15 \text{ kJ/mol}$ per α and β unit, assuming three of each. The value of the ΔG of ATP synthesis is somewhat higher ($\approx 45 \text{ kJ/mol}$ in chloroplasts), but could become equal to W at lower phosphorylation potentials, or at a higher ΔT . For the next chapter it is of interest that W has the same magnitude as the free energy cost for peptide formation: $8\text{-}16 \text{ kJ/mol}^{16}$.

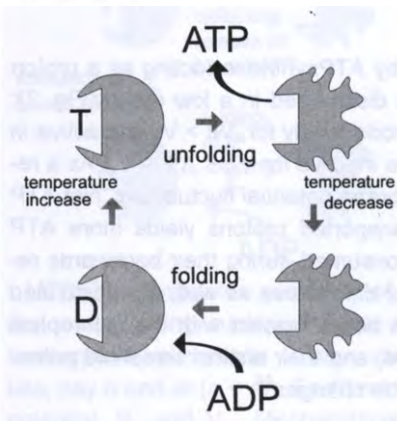


Fig. 8. The postulated thermally-induced binding change mechanism for pF_1 . During a thermal cycle the enzyme loosely binds ADP and phosphate at one temperature, folds, converts these bound substrates to tightly bound ATP, and releases this tightly bound ATP at a high temperature by unfolding.

¹⁵ Z.-Y. Wang, E. Freire, R.E. McCarthy, Influence of nucleotide binding site occupancy on the thermal stability of the F_1 portion of the chloroplast ATPsynthase, "Journal of Biological Chemistry" 1993, v. 268, p. 20785-20790.

¹⁶ M.G. Schwendinger, B.M. Rode, Investigations on the mechanism of the salt-induced peptide formation, "Origins of Life and Evolution of the Biosphere" 1992, v. 22, p. 349-359.

3.3. THE EVOLUTION OF ATPSYNTHASE

Contemporary F_1 consists of the α , β , γ , δ , and ϵ subunits at a stoichiometry of $\alpha_3\beta_3\gamma\delta\epsilon$. α and β are homologous and can both bind $ADP + P_i$ or ATP . The β -subunit is the site of ATP synthesis.

$\alpha\beta$. We begin with a $\alpha\beta$ (a progenitor of the α and β subunits) that is a membrane-associated pF_1 in which a thermotropic membrane phase transition assists in the thermally induced ATP release. During part of the thermal cycle $\alpha\beta$ sinks into the hydrophobic core of the membrane. $\alpha\beta$ does not translocate protons across the membrane.

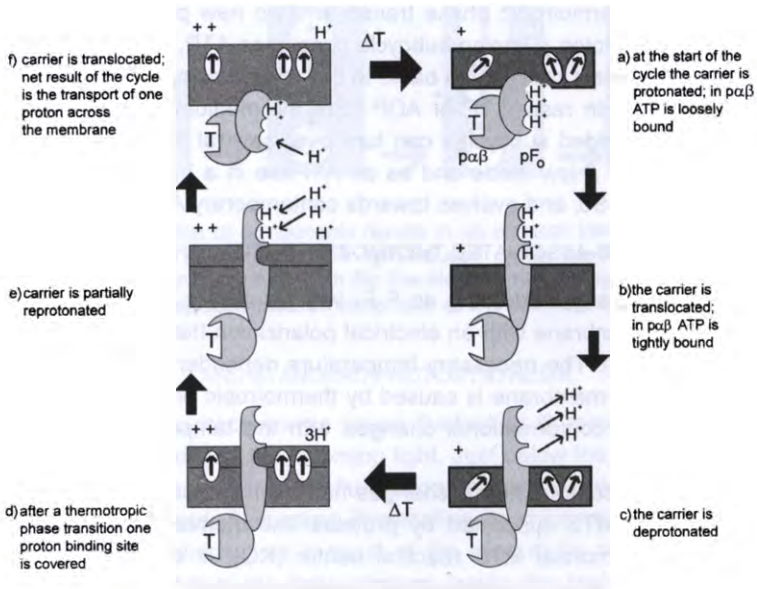


Fig. 9. *Proton pumping thermosynthesizer*. $pF_0\alpha\beta$ consists of $\alpha\beta$, a progenitor of the α and β subunits of modern F_1 , and pF_0 , a proton carrying progenitor of F_0 . In the cycle the pF_0 is first translocated across the membrane (a → b); the required energy originates from tight binding of ATP . After translocation pF_0 is deprotonated (c). A thermotropic phase transition thickens the membrane and increases the membrane potential (d). The thickening lessens the number of accessible protonation sites on the carrier (e). After return of pF_0 one proton remains behind (f). After the reverse phase transition state a is reached again (f → a): the net effect of the cycle is that one proton has been transferred to a higher potential.

$pF_o\rho\alpha\beta$. Addition of a proton carrier, pF_o , yields a proton pumping thermosynthesizer (Fig. 9). During a thermal cycle the carrier moves back and forth across the membrane. The number of protons bound to pF_o varies; as a result it functions as a proton pump. Tighter binding of the $\rho\alpha\beta$ -bound ATP molecule yields the energy for carrier translocation. This binding is loosened again upon return of pF_o . No ATP is produced or consumed; ATP binding energy is only lent for translocation of the carrier across the membrane.

$pF_o\rho\alpha\rho\beta$. Duplication and diversification of $\rho\alpha\beta$ yields two subunits, $\rho\alpha$ and $\rho\beta$. $\rho\alpha$ reversibly transfers pF_o across the membrane when pF_o is not protonated, but when it is protonated, $\rho\alpha$ is supported by $\rho\beta$. The result is an ATP synthesizing complex that is driven by the increase in membrane potential caused by a thermotropic phase transition. Two new partial cycles are now operative: the proton pumping subcycle consumes ATP, and the ATP synthesizing subcycle lets protons fall back. In the subcycles $\rho\beta$ -bound ADP or ATP is exchanged with resp. ATP or ADP from the medium. During one thermal cycle the two added subcycles can turn over several times, i.e., both as an ATPsynthase in a low mode and as an ATPase in a high mode. This pF_oF_1 supports MTS/PS0, and evolves towards contemporary ATPsynthase.

3.4. MEMBRANE-ASSOCIATED THERMOSYNTHESIS

In MTS ATP is gained using an F_oF_1 with a variable stoichiometry and an asymmetric membrane with an electrical polarization that varies with the temperature (Fig. 7). The necessary temperature dependence of the electric polarization of the membrane is caused by thermotropic phase transitions of lipids (Fig. 4), or conformational changes with the temperature of membrane proteins.

MTS by lipid conformational changes has been discussed previously¹⁷. We consider here MTS supported by proteins: the membranous TS-supporting protein, the primordial MTS reaction centre (RC), is called *RC0*. Compare *RC0* with the bacterial photosynthetic reaction centre (BRC) of *Rhodospseudomonas viridis*. This BRC contains 8 negative charges on one side and 6 positive charges on the other side of the membrane, and thus constitutes a large dipole¹⁸. A change in thickness of this BRC must change the MDP. No data is available on the temperature dependency of their thickness but conformational transitions of BRCs upon illumination have been described. Like any heat engine, *RC0* would have to undergo entropy changes during ther-

¹⁷ A.W.J. Muller, A mechanism for thermosynthesis..., op. cit.

¹⁸ J. Deisenhofer, H. Michel, The photosynthetic reaction center from the purple bacterium *Rhodospseudomonas viridis*, "Science" 1989, v. 245, p. 1463-1473.

mosynthesis. Interestingly, during photosynthesis large entropy changes occur in some contemporary BRCs: upon formation of an intermediate dipole (cyt $C_{555}^+ - Q_A^-$) all free energy remaining from the excitation is stored as an entropy decrease¹⁹. Upon electron transfer to Q_B the entropy, and the enthalpy, increase again just as during the high-temperature expansion in a steam engine. Some BRCs mimic therefore during photosynthesis the heat uptake during the power stroke of the postulated RC0.

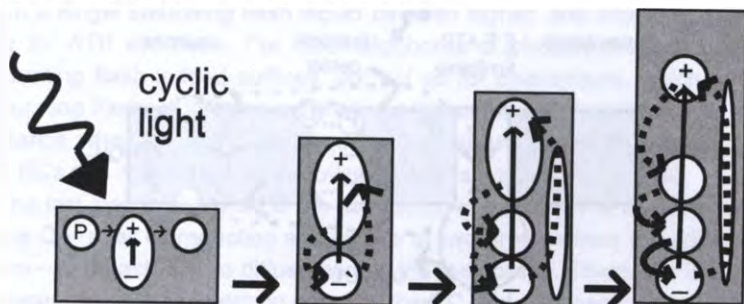


Fig. 10. Stepwise addition of components results in an electron transfer chain in the PS0 reaction centre. The length of the light-induced dipole increases. The chain evolves into a membrane-spanning path for the electron excited by light absorption. A return path for the displaced electron may have evolved simultaneously (elongated ellipse).

3.5. PHOTOSYSTEM 0 AND STANDARD PHOTOSYNTHESIS

In natural waters, surface waves cause fluctuating illumination by variable focusing and defocusing of the incoming light. Just below the sea surface, the main power of the spectrum of the fluctuations has cycle times (t_{cyc}) between 0,1 and 10 s. On land, fluctuating illumination of chloroplasts in leaves is caused by variable shading as a result of the wind or by movement by the protoplasm stream within the light gradient inside the leaf. The cycle-time range of these fluctuations is $\sim 0,1-100$ s. Metastability of excited states at the same timescale is commonly observed in RCs.

In Photosystem 0 ATP is gained from fluctuating light. Light induces metastable dipoles which in turn generate a membrane potential, and a fluctuating membrane potential permits ATP gain by the same ATPsynthase as used in MTS (Fig. 7). In chloroplasts the pertinent dipoles could consist of reaction centers containing oxidized pigments and reduced quinones such as *inactive PSII* RCs, in which the qui-

¹⁹ G.D. Case, W.W. Parson, Thermodynamics of the primary and secondary photochemical reactions in *Chromatium*, "Biochimica et Biophysica Acta" 1971, v. 253, p. 187-202.

none Q_A cannot reduce the quinone Q_B ²⁰. The inactive PSII RCs contribute to the $\Delta\Psi$ across the membrane²¹, and can comprise 75-80% of all PSII RCs.

In the model for the early evolution of photosynthesis PS0 activity gradually increased in MTS/PS0 systems, systems having both thermosynthesis and PS0 capabilities that gained energy from the combined thermal and light-

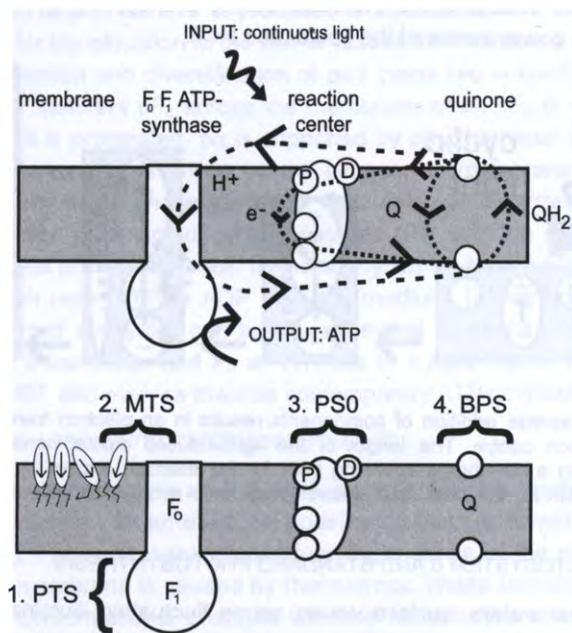


Fig 11. Bacterial photosynthesis (BPS) and its proposed biogenesis. In BPS incoming light excites an electron in the reaction centre. The electron crosses the membrane while moving along a line of stepping stones. Next it reduces a quinone, which also picks up two protons from the medium. The resulting quinol diffuses across the membrane. Upon quinol oxidation, the electron returns to the reaction centre, closing an electron-transport cycle. In addition the protons are released, which thus have been pumped across the membrane. The protons return through ATPsynthase, with simultaneous synthesis of ATP, closing the proton cycle and yielding the end-product of BPS. The components of the machinery of BPS are proposed to have been acquired while photosynthesis evolved from thermosynthesis. The proposed acquisition sequence is (1) the F_1 part of ATPsynthase during the emergence of PTS; (2) the F_0 part of ATPsynthase and the lipids of an asymmetric membrane during the emergence of MTS; (3) the reaction centre acquired the stepping stones for the excited electron one after the other during the emergence of PS0; (4) quinones were added during the emergence of BPS.

²⁰ J. Lavergne, E. Leci, Properties of inactive photosystem II centers, "Photosynthesis Research" 1993, v. 35, p. 323-343.

²¹ R.A. Chylla, J. Whitmarsh, Light saturation response of inactive photosystem II reaction centers in spinach, "Photosynthesis Research" 1990, v. 25, p. 39-48.

intensity cycling occurring during circulation in a convection cell (Fig. 1). Intermembrane charge carriers were stepwise added, increasing the distance between the excited electron and the oxidized pigment until it equalled the membrane thickness (Fig. 10).

In PS0 the RC density in the membrane must be high to generate a sufficient high $\Delta\Psi_{PDP}$; ATP is synthesized upon a single RC saturating flash. If the excited electron could move across the membrane, then the potential resulting from a single saturating flash would be even higher, and should certainly suffice for ATP synthesis. For chromatophores of photosynthetic bacteria one saturating flash indeed suffices, but not so for chloroplasts, which need two saturating flashes. In standard photosynthesis the RC density can be low, for instance when ATPsynthase requires a minimum threshold potential. In PS the RCs can make multiple turnovers, which is not possible in PS0.

The last step in evolution is the acquisition of the ability of an RC-bound quinone Q – after its reduction and uptake of two protons from the adjacent medium – to detach and to diffuse across the membrane. Thereafter the quinone reduces the primary electron donor in the RC and releases the two protons at this other side of the membrane. The proton pump constituted by the RC/Q combination makes ATPsynthase mode switching redundant. The obtained system functions in continuous light, i.e., it performs standard bacterial photosynthesis (Fig. 11).

3.6. INCREASING POWER, THE DRIVING FORCE OF THE EVOLUTION OF BIOENERGETICS

The power of MTS/PS0 is easily calculated in terms of observable quantities. The power of MTS is very low. Nowadays some bacteria however still live in habitats where energy must be scarce: in deep seawater, in soil, in meteoric waters²². For dormant microorganisms maintenance energy may approach zero. Higher organisms are continuously metabolically active, even when dormant, but lower organisms can temporarily be completely inactive. The first organisms may have gained energy only intermittently, during irregular environmental changes.

An increasing power by decreasing cycle times is the driving force for the process MTS → PS0 → PS. The cycle time of fluctuating light (~1 s) can be ~10 times shorter than the cycle time of thermal cycling; a commonly used turnover time for photosynthesis is 10 ms²³.

²² T.O. Stevens, J.P. McKinley, Lithoautotrophic microbial ecosystems in deep basalt aquifers, "Science" 1995, v. 270, p. 450-454.

²³ B. Kok, G.M. Cheniae, Kinetics and intermediates of the oxygen evolution step in photosynthesis "Current Topics in Bioenergetics" 1966, v. 1, p. 1-47.

The much smaller power of TS/PS0 in comparison to photosynthesis, respiration or fermentation makes avoiding interference of the latter in an experimental demonstration of TS/PS0 difficult.

4. THE ORIGIN OF LIFE

4.1. THE PROTEINOID WORLD

The pF_1 concept not only yields a starting point for the stepwise evolution of bioenergetics, but it also permits a model for the origin of life. Cells are assumed to have preceded enzymes, and enzymes genes²⁴. Miller²⁵ has shown how amino acids could plausibly be formed under prebiological conditions. Fox²⁶ has shown that mild heating (> 100 °C) of these amino acids – for instance in tidal pools – results in their condensation to small polypeptides called *thermal proteinoids* or *proteinoids*. Dissolved in hot water, proteinoids form bacteria-shaped microspheres or protocells that have been called *proteinoid microspheres*. Protocells can grow by uptake of proteinoids and then divide.

4.2. THERMOZYMES

The first "living entity" must have contained an energy-converter²⁷, identified with a heat engine²⁸, more specifically with pF_1 . The first organisms behaved as heat engines while being thermally cycled during convection in Darwin's "warm little pond". It follows from rRNA studies that the last common ancestor of all living organisms indeed lived at the boiling point of water²⁹, a condition favouring convection. The early ocean may have boiled. Curiously, Archelaus of Athens already implicated convection in the origin of life in 450 BC: "when the earth was first being warmed, in the lower part where the warm and cold were mingled together, many living creatures appeared".

In the model, some of the proteinoids in the first cells acted during thermal cycling as pF_1 heat engines and condensed substrates during thermal cycling. Enzymes that work on a thermal cycle have been named *thermozymes*. By using temperature-induced conformation changes, thermozymes avoid the

²⁴ F. Dyson, *Origins of life*, Cambridge 1985 Cambridge University Press.

²⁵ S.L. Miller, A production of amino acids under possible primitive Earth conditions, "Science" 1953, v. 117, p. 528-529.

²⁶ S.W. Fox, K. Dose, *Molecular evolution and the origin of life*, San Francisco 1972 Freeman.

²⁷ S. Granick, Speculations on the origins and evolution of photosynthesis, "Annals of the New York Academy of Sciences" 1957, v. 69, p. 292-308.

²⁸ K.E. Van Holde, The origin of life. A thermodynamic critique, in: H.O. Halvorson, K.E. Van Holde (eds.), *The origins of life and evolution*, New York 1980 Liss, p. 31-46.

²⁹ C.R. Woese, Bacterial evolution, "Microbiological Review" 1987, v. 51, p. 221-271.

problem of regular enzymes that binding of substrates lowers the free energy, while the activation energy for the reaction step requires an increase in free energy. Phosphorylation of membrane components such as lipids and proteinoids by thermozymes yielded amphipathic molecules that stabilized the protocells by preventing leakage of membrane components and protected against dissolving at high temperatures; the protocell protected the phosphorylating proteinoid, and the phosphorylation of its components protected the protocell.

Many biochemicals are condensation (dehydration) products. The assumed substrate ambiguity³⁰ of the first pF₁ led to many condensation products during thermal cycling: proteins, RNA or its progenitor pRNA, and condensing agents, such as ATP or its progenitor, pATP, for which there are several candidates.

A protocell that contains one pF₁ can grow during thermal cycling. After division one daughter cell was endowed with the pF₁, the fertile daughter. The line of fertile cells synthesized a library of new randomly constituted proteins. After many attempts another pF₁ was synthesized, leading to the rare event of two fertile daughter cells. In this manner a population of PTS utilizing protocells slowly grew. The daughter pF₁s performed similar reactions but were probably not identical, they were *inexactly replicated*³¹.

4.3. THE GENETIC APPARATUS

It is proposed that pF₁ condensed information-carrying macromolecules, progenitors of RNA, and that it permitted replication of this progenitor RNA. The originally random information present was selected according to the criterion of support of pF₁ propagation. In a proposed RNA substitute, ribose-phosphate has been replaced by *N*-(2-aminoethyl)glycine, leading to a *peptide nucleic acid* that contains no phosphate. These nucleic acid analogs hybridize with DNA and can even form a double helix³². The first translated proteins may therefore have been translated from a template of an RNA progenitor. When the RNA progenitor was replaced cannot be indicated.

At present, tRNA activates amino acids, rRNA (together with the ribosome protein rS1) concatenates the activated amino acids, and mRNA yields the information for protein synthesis. RNA molecules that contain only 16 nucleotides can already function as tRNA. In the past, some RNA presumably increased the synthesis rate by pF₁ of pF₁ by increasing the precision of the di-

³⁰ S. Black, Pre-cell evolution and the origin of enzymes, "Nature" 1970, v. 226, p. 754-755.

³¹ F. Dyson, op. cit.

³² P. Wittung, P. Nielsen, O. Burchardt, M. Egholm, B. Norden, DNA-like double helix formed by peptide nucleic acid, "Nature" 1994, v. 368, p. 561-563.

rection of the amino acid concatenation process; this RNA evolved into an mRNA coding for a pF₁. pF₁ and rRNA collected charged tRNAs and mRNA according to the evolving genetic code and synthesized a protein library.

At first the genetic apparatus coded for only one amino acid, glycine³³ (F₁ uses a glycine-rich loop), and used only one base, guanine. Other bases may have been absent, or may have had a random composition. When a triplet did not code for an amino acid, the apparatus inserted amino acids randomly. Such a genetic apparatus is an improvement over random protein synthesis. The number of functional triplets increased step-by-step, enhancing precision resulting in a genetic apparatus that is able to translate a certain mRNA to yield a pF₁. This apparatus thus comprises a pF₁, an mRNA coding for pF₁ and additional rRNA and tRNA. Upon acquiring an accurate translation machinery gene duplication and diversification rapidly resulted in enzymes adapted to individual substrates.

F₁ is indeed old: ATPsynthase was already present in the last common ancestor of the archaebacteria, eukaryotes, and eubacteria. Many proteins are related to F₁, for instance the enzymes with chemomechanical coupling ability and the nucleotide binding proteins. pF₁ thus is the last common ancestor of all enzymes or, "the mother of all enzymes", and also "the mother of all organisms".

The wasting process of the synthesis of a library of randomly constituted proteins is only possible in the presence of an energy source. Researchers who let the origin of life start with replicating nucleic acids may do so because of a perceived need to minimize free energy loss. Dissipation is however inherent to all self-ordering. It follows from the Second Law that waste cannot be avoided and should be accepted as a fact of nature, and also as a fact of life, especially for the first organisms. Later in evolution metabolic efficiency becomes an important selection mechanism, of course.

5. CANDIDATES FOR CONTEMPORARY THERMOSYNTHESIS AND PHOTOSYSTEM 0

The TS/PS0 machinery is simple. Given the diversity of life and of natural environments, it is to be expected that some organisms still use TS/PS0. The presence of organisms (or membranes) is conspicuous in many environments favourable for TS/PS0. Some examples:

Thermosynthesis by passive movement. Candidates for TS are microorganisms present in hot springs or convecting natural waters, Langmuir circula-

³³ H. Hartman, Speculations on the origin of the genetic code, "Origins of Life" 1975, v. 6, p. 423-427.

tions, the earth's crust, especially in convecting meteoric waters, industrial cooling water systems, hot water tanks (*Legionella*) and air-conditioning systems.

Thermosynthesis by active movement. Many natural waters contain thermoclines: the ocean (especially oceanic fronts), lakes, saline ponds, hot springs. Organisms are known to migrate vertically in these thermoclines, which will cause thermal cycling. It is suggestive that thermoclines have a high primary productivity.

Within cells, the protoplasm stream that circulates membranous celorganelles must cause thermal cycling therein when a thermal gradient is present across the cell. Such a thermal gradient is plausible when the organism lives in an interface. Examples are algae at snow/air, sea/air, sea/sea-ice interfaces and surfaces in industrial heat transfer systems. The palisade cell in a leaf also often contains a thermal gradient, as it is heated on the sun side and is cooled by transpiration at the shade side.

Photosystem 0. Good PS0 candidates are the thylakoids in plants restricted to habitats with intermittent illumination, the plants with thylakoids that contain RC crystals (because of the high RC density in the membrane), and of course the aspen. Just under the surface of natural waters strong fluctuations in light intensity occur because of variable focusing and defocusing of sunlight by waves.

Extraterrestrial environments. The plausibility of TS enhances the chance that life has arisen somewhere else in the solar system. Convection occurs in the atmospheres of many planets. Convection may also occur in the ocean below the surface ice on Jupiter's satellite Europa, which may be subject to tidal heating. Thermal springs and meteoric waters on Mars are also plausible niches for thermosynthesizers. Living on TS is possible on every planet that is internally sufficient hot to permit convection of natural or meteoric waters; this possible independence of sun or starlight, makes extraterrestrial life much more plausible.

6. RELATIONS TO CONTEMPORARY PHYSIOLOGY

Although the power of TS/PS0 is much less than the power of PS, TS/PS0 may have remained important for metabolism if it accomplishes chemical reactions that cannot, or only with difficulty, be attained during the static conditions that characterize PS.

6.1. CHLOROPLAST DYNAMICS

In chloroplasts and in bacterial chromatophores photophosphorylation in flashing light and continuous light differs: in the former it is driven by the mem-

brane potential ($\Delta\Psi\approx 100$ mV), in the latter by the difference in pH ($\Delta\Psi\approx 30$ mV). The flashing light response is compatible with the PS0 mechanism.

PS0 and PS can share the same ATPsynthase, and combined PS0/PS systems are therefore possible. A light pulse therein will increase $\Delta\Psi$ both by causing a PDP and by charge transport across the membrane. Since inactive PSII could be PS0-active, and DCMU blocks electron transport between Q_A and Q_B , a combined PS0/PS system can be obtained by poisoning part of the RCIIIs with DCMU. Such DCMU addition – or heat inactivation – does indeed not inhibit the conversion of light into free chemical energy during photoacoustic experiments with flashing light (1-1000 Hz) completely³⁴.

Combined MTS/PS0/PS activity during combined light/thermal cycling is easily imagined. Dipole potentials are difficult to distinguish from surface potentials³⁵. The surface potential changes at 18°C. The zeta potential of a membrane – determined by electrophoresis – may be proportional to the sum of the surface and surface dipole potential³⁶. The light-induced zeta potential³⁷ also changes at 18°C.

Strong temperature dependency has been observed for numerous photosynthetic partial processes. Hysteresis³⁸ of the chloroplast absorption spectrum during one thermal cycle (10-25°C) may be due to hysteresis in the membrane potential as in MTS. Other processes possibly related to MTS/PS0/PS:

Etiolation. Submitting growing chloroplasts to intermittent or flashing light stops their development in the *etiolated phase*, in which oxygen evolution is absent, the antenna is smaller and the RC density in the membrane is higher.

Stacking and swelling. In the stacked state many thylakoid membranes are flattened and resemble a pile of empty balloons. The stacks store light harvesting complexes. This storage must result in a high RC density, as required by PS0, in the remaining unstacked part of the membrane. Destacking then

³⁴ R. Carpentier, R.M. Leblanc, M. Mineault, On the nature of the photosynthetic energy storage monitored by photoacoustic spectroscopy, "Photosynthesis Research" 1990, v. 23, p. 313-318.

³⁵ J. Barber, Membrane surface charges and potentials in relation to photosynthesis, "Biochimica et Biophysica Acta" 1980, v. 594, p. 253-304.

³⁶ A.W.J. Muller, A mechanism for thermosynthesis..., op. cit.

³⁷ J.M.G. Torres-Pereira, H.W. Wong Fong Sang, A.P.R. Theuvenet, R. Kraayenhof, Electric surface charge dynamics of chloroplast thylakoid membranes. Temperature dependence of electrokinetic potential and aminoacridine interaction, "Biochimica et Biophysica Acta" 1984, v. 767, p. 295.

³⁸ S.S. Brody, G.S. Singhal, Spectral properties of chloroplast membranes as a function of physiological temperatures, "Biochemical and Biophysical Research Communications" 1979, v. 89, p. 542-546.

would be equivalent to an increase of capacitor area, and a lesser RC density, suggesting a switch from PS0 to PS.

Induction. Upon a dark-light switch chloroplasts show transients and oscillations, a phenomenon called induction. Both in contemporary organisms during a dark-light switch and in evolving organisms in the past the PDP may have been generated first, and quinones may have diffused across the membrane later.

Oscillations. Chloroplasts can oscillate, with a t_{cyc} range ~4-60 s. The oscillations comprise many phenomena: changes in fluorescence, ATP synthesis and hydrolysis, ΔpH , stacking and swelling, oxygen evolution and CO_2 assimilation³⁹. These oscillations may be related to PS0, which is also a cyclic process.

State 1 – State 2 transitions. Chloroplasts contain two different RCs or photosystems, PSI and PSII, with different light-absorption properties. When irradiated with light preferably absorbed by resp. PSI and PSII, different states are assumed, named State 1 and State 2. The temperature⁴⁰ and the light intensity have been implicated in transitions between these states. The cyanobacterium *Synechococcus* assumes State 2 in the dark, State 1 in the shade, and State 2 again at high light intensity⁴¹; these dark resp. shade states may be relics from the dark-PS0 resp. light-PS0 states.

Clearly, many dynamic phenomena in chloroplasts can be related to MTS/PS0.

6.2. MIMICKING THERMAL CYCLING DURING ISOTHERMY

In the TS/PS0 model the metabolism of the first organisms depends on cyclic conditions. TS and PS0 depend on a critical temperature resp. a critical light-induced polarization. Active variation of this parameter permits mimicking cyclic conditions, and metabolism can have freed itself from this dependency by such variation.

PTS. Phosphorylation-dephosphorylation of proteins is achieved by appropriately activating kinases / phosphatases. It is extremely widespread. It can temporarily change a folding temperature of a protein⁴², forcing at constant

³⁹ D.A. Walker, Concerning oscillations, "Photosynthesis Research" 1992, v. 34, p. 387-395.

⁴⁰ E. Weis, Light and temperature induced changes in the distribution of excitation energy between photosystem I and photosystem II in spinach leaves, "Biochimica et Biophysica Acta" 1985, v. 807, p. 118-133.

⁴¹ D. Rouag, P. Dominy, State adaptations in the cyanobacterium *Synechococcus* 6301 (PCC). Dependence on light intensity or spectral composition?, "Photosynthesis Research" 1994, v. 40, p. 107-117.

⁴² D.W. Urry, Free energy transduction in polypeptides and proteins based on inverse

temperature unfolding followed by folding, and therefore mimicking a thermal PTS cycle.

MTS. The thermotropic phase transitions and membrane potential changes during MTS may be mimicked by variation of the internal Ca^{2+} concentration, $[\text{Ca}^{2+}]_i$.

PS0. The stability of light-induced dipoles depends on many factors, and may very well be actively regulated.

Some enzymes may remain sensitive to accidental temperature changes. The heat shock response may have evolved to protect against such disruption: heat shock proteins can, in addition to preventing unfolding, renature proteins that have become unfolded. Proteins might be forced to fold and to unfold by variation of the activity of heat shock proteins, another method for isothermally mimicking thermal cycling.

6.3. THERMAL CYCLING DURING CELL DIVISION AND DEVELOPMENT

At present the just mentioned processes of phosphorylation-dephosphorylation, regulation by calcium, and heat shock proteins regulate and control metabolism and development. However thermal cycling still plays a role in induction of germination of microorganisms and plants, induction of flowering, synchronized cell division and diapause breaking. Many organisms in natural waters that contain a temperature gradient perform diurnal vertical migrations; other organisms – including man – vary their body temperature daily. Daily temperature cycling enhances the growth rate of plants and arthropods. The mechanisms behind these phenomena may involve processes that are relics from TS.

7. DISCUSSION

Thermosynthesis greatly simplifies the problem of the origin of life. Experimentally, one only has to demonstrate the emergence of a pF_1 during thermal cycling of a solution of heated amino acids. The theoretical problem is solved in terms of concepts from many branches spanning physics, chemistry and biology: thermodynamics, enzymology, bioenergetics, physical chemistry, surface science, origin of life research, photosynthesis research, molecular biology, ecology and even solid state physics and electronics. Thermosynthesis touches numerous biological phenomena: the origin of life, the genetic code, the root of the phylogenetic rRNA tree lying near those organisms that nowadays live in boiling, i.e. plausibly convecting, water, the chemiosmotic

temperature transitions, "Progress in Biophysics and Molecular Biology" 1992, v. 57, p. 23-57.

mechanism, the binding change mechanism, variable stoichiometries of ATPases/synthases, the split operon for the F_0 and F_1 ATPsynthase genes, catalytic and non-catalytic nucleotide binding sites in F_1 , synchronized cell division, germination, flowering, budding, vertical migrations in natural waters, high productivity in thermoclines, the protoplasm stream, interface niches, biofouling on heat transfer surfaces, protein phosphorylation, effects of Ca^{2+} , heat shock, hysteresis in membrane potential during thermal cycling, effects of fluctuating light, the stalk in bacterial photosynthetic reaction centers, entropy changes in these reaction centers, inactive reaction centers, chloroplast oscillations, etiolation, photosynthesis induction, State 1 – 2 transitions, possible life beneath ice on the Moon, Mercury, Mars, Europa and Titan. Moreover, the thermosynthesis theory identifies the first enzyme, and the dissipative structure in which it operated. The components of the chloroplast are acquired in the order: F_1 moiety of ATPsynthase (PTS stage); asymmetric biomembrane, F_0 moiety of ATPsynthase, surface protein RC0 (MTS stage); addition to RC0: Chl_2 dimer, phaeophytin, temporary charge carriers, cyt *c*-containing stalk, tightly RC-bound quinones (PS0 stage); loosely bound quinones, *bc_1* complex; RC duplication and diversification yields two RC types (PS stage). Further research in molecular biology, e.g., sequence data, may confirm the proposed stepwise acquirement of the components of ATPsynthase and the photosynthetic system. Further research in enzymology may confirm the proposed ancestry of phosphorylation-dephosphorylation processes and the heat shock response from the switch to isothermy.

Architects and engineers commonly combine concepts from different disciplines in the construction of buildings, planes, rockets and computers. They draw blueprints. A similar blueprint for the origin of life – based on well established experimental data from many scientific disciplines – is drawn here, yielding a model that has a large explanatory power. "In a theory that has given results like these, there must certainly be a great deal of truth".

FIGURE CREDITS

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