

An Early and Anaerobic Scenario for the Transition to Undifferentiated Multicellularity

Juan Carlos Aledo

Received: 20 December 2007 / Accepted: 15 May 2008 / Published online: 15 July 2008
© Springer Science+Business Media, LLC 2008

Abstract Glycolysis, an ancient energy-processing pathway, can operate either under an efficient but slow regime or, alternatively, under a dissipative but fast-working regime. Trading an increase in efficiency for a decrease in rate represents a cooperative behavior, while a dissipative metabolism can be regarded as a cheating strategy. Herein, using irreversible thermodynamic principles and methods derived from game theory, we investigate whether, and under what conditions, the interplay between these two metabolic strategies may have promoted the clustering of undifferentiated cells. In the current model, multicellularity implies the loss of motility, which represents a hindrance rather than an improvement when competing with mobile single-celled organisms. Despite that, when considering glycolysis as the only energy-processing pathway, we conclude that cells endowed with a low basal anabolic metabolism may have benefited from clustering when faced to compete with cells exhibiting a high anabolic activity. The current results suggest that the transition to multicellularity may have taken place much earlier than hitherto thought, providing support for an extended period of Precambrian metazoan diversification.

Keywords Cambrian explosion · Cooperation · Energetic metabolism · Glycolysis · Metazoan evolution

Introduction

The key problem for the evolution of cellular cooperation is the fitness cost to the cooperating individual. Cooperation within a group of cells produces a public good that benefits all group members, regardless whether or not they cooperate, but only the cooperators pay the cost of producing the public good (Pepper and Smuts 2001). Those cells that do not cooperate but gain advantage from the cooperative behavior are called cheaters. Multicellular life may have evolved partly to exclude cheaters from clusters of cooperative cells. In other words, forcing cells to collaborate may have been the initial advantage of multicellularity (Pfeiffer and Bonhoeffer 2003). In this way, cooperation among cells provides one mechanism to drive the evolution of simple undifferentiated cell clusters. These clusters of undifferentiated cells may have been the raw material for the later evolution of increasingly more sophisticated division of labor.

Although it is easy to imagine a mutation, or series of mutations, leading to the formation of undifferentiated cell clusters, the precise nature of the cooperative behavior driving the transition is unclear. Furthermore, it should be emphasized that whatever the selective advantages of clustering undifferentiated cells might have been, they had to outweigh disadvantages such as hampered mobility and high local cell densities. Nevertheless, and despite these obstacles, multicellularity has evolved independently on several occasions and became abundant after the accumulation of oxygen in the primitive atmosphere (Lalucque and Silar 2003; Pfeiffer and Bonhoeffer 2003). This enrichment in oxygen opened the way for the apparition of oxidation reactions that were used as energy sources for the synthesis of ATP. Aerobic respiration represents an efficient, although somewhat slow, way to obtain ATP. In contrast,

J. C. Aledo (✉)
Departamento de Biología Molecular y Bioquímica, Facultad de Ciencias, Universidad de Málaga, 29071 Málaga, Spain
e-mail: caledo@uma.es

the yield of anaerobic fermentation is much lower than that of respiration (2 mol versus 32 mol of ATP per mole of glucose, approximately), while the rate of ATP formation through fermentation can be up to 100 times faster than that of oxidative phosphorylation. Although the trade-off existing between yield (mole of ATP per mole of substrate) and rate (mole of ATP per unit of time) in energy metabolism has long been recognized (Westerhoff et al. 1982; Aledo and Esteban del Valle 2002), Pfeiffer and coworkers were the first to realize that trading an increase in yield for a decrease in rate represents a cooperative behavior (Pfeiffer et al. 2001) that may have helped in promoting the transition from single-cell to multicellular beings (Pfeiffer and Bonhoeffer 2003). The kind of advantage they propose for multicellularity means that it should have occurred after the Earth's atmosphere was invaded by oxygen and aerobic respiration had evolved.

Anaerobic glycolysis is an ancient energy-processing pathway, which probably appeared when the planetary environment was strictly anaerobic. Thus, glycolysis had been performed for hundreds of millions of years before aerobic respiration evolved. Recently, we have shown that anaerobic glycolysis can operate either under an efficient but slow (ES) regime or, alternatively, under a dissipative but fast (DF)-working regime (Aledo and Esteban del Valle 2004). Briefly, cells endowed with a genotype allowing a high basal anabolic metabolism will lead to a relatively low stationary ATP concentration. This phenotype is optimal for rapid but inefficient use of glucose (DF regime). In contrast, those cells exhibiting low ratios between anabolic and catabolic activities will maintain relatively high ATP concentrations that, in turn, will allow glycolysis to perform under an efficient although slower-working regime (ES regime).

In the current study, these working regimes are considered two different strategies in the struggle for life: a competitive (DF) and a cooperative (ES) usage of glucose. Using irreversible thermodynamic principles and methods derived from game theory, we investigate whether, and under what conditions, the interplay between these two metabolic strategies might have promoted the clustering of undifferentiated cells earlier than oxygen accumulated in the atmosphere. Without appealing to aerobic metabolism, we conclude that under conditions of resource scarcity, cells endowed with a low basal anabolic metabolism may have benefited from clustering when forced to compete with cells exhibiting a high anabolic activity.

Thermodynamic Aspects of the Model

In the glycolytic pathway, the splitting of glucose to pyruvate is coupled with the formation of ATP. However,

only part of the energy released from glucose is captured in ATP bonds, while the remainder is dissipated as heat. The rate at which energy is extracted from glucose is referred to as input power (W_i). On the other hand, the rate at which energy is conserved in the form of ATP is designated output power (W_o). The efficiency (η) of the conversion process is given by the ratio between the output power and the input power. In addition to the output power and efficiency, another relevant factor is the dissipation function or entropy production (Φ), which can be interpreted as the rate at which the energy available for biological processes is reduced due to metabolic activities.

In this context, glycolysis can be envisaged as an energy converter susceptible to formal analyses (Aledo and Esteban del Valle 2004). Using such an approach, we have shown that glycolysis can perform either under a DF regime or under an ES regime, depending on the stationary ATP concentration (Esteban del Valle and Aledo 2006). Table 1 summarizes in biophysical terms the main characteristics of both working regimes. By comparing the two states, it can be noted that there is a trade-off between output power and efficiency (low dissipation). The Appendix gives a brief account of the theory behind these working regimes. In the current study, these regimes are considered two different strategies: competitive (DF) and cooperative (ES) usage of glucose.

Game Theoretical Aspects of the Model

We consider a model world that has 100×100 available sites. Every lattice patch contains maximally one cell that can exhibit either an ES or a DF phenotype. At each time step, the performance of each cell was scored as the sum of the payoffs obtained in the encounters with the four orthogonal neighbors, according to a payoffs matrix (Table 2). In order to assess the payoffs, we consider the contribution of intrinsic and environmental energetic factors as described somewhere else (Aledo et al. 2007). Briefly, on one side, the performance of a cell is positively related to its own ability to withdraw energy from its

Table 1 Glycolytic strategies

Working regime	Output power	Efficiency	Dissipation function
DF	1/4	1/2	1/2
ES	2/9	2/3	1/3

Note: DF, dissipative but fast; ES, efficient but slow. The description of glycolysis as a free-energy converter allows the identification and analysis of two different stationary working regimes that have opposing properties in terms of rate (power) and efficiency (for further details, see the Appendix). The output power and the dissipation function (Φ) are expressed as a fraction of the maximum attainable input power

Table 2 Payoffs matrix

Site owner	Neighbor site		
	Empty	ES	DF
ES	$2/9 - 1/(3\varepsilon)$	$2/9 - 2/(3\varepsilon)$	$2/9 - 5/(6\varepsilon)$
DF	$1/4 - 1/(2\varepsilon)$	$1/4 - 5/(6\varepsilon)$	$1/4 - 1/\varepsilon$

Note: ES, efficient but slow; DF, dissipative but fast. Six different types of encounters are possible. The corresponding payoffs obtained by the site owners are indicated as a function of ε . These payoff functions can be calculated after substituting the proper output power and dissipation function values (given in Table 1) into Eq. 1 as explained in the text. Depending on the values given to ε , the two metabolic strategies will be playing different games (Aledo et al. 2007). It can be proved that when $6 < \varepsilon < 12$, the cells are engaged in a Prisoner’s Dilemma game

surroundings (output power). On the other hand, it is reasonable to think that a cell surrounded by dissipative organisms will perform worse than a cell surrounded by efficient neighbors. Among other reasons, the energetic resource will be exhausted much earlier in the first case. In this sense, calorimetric studies have shown that heat dissipation can be considered an index of stress (Barros et al. 2004). Thus, the local entropy production, given by the dissipation function, is considered an indicator of the competitive pressure of the environment and, therefore, is acting negatively. Because the influence of the local entropy production will be less when the resources are high, this second term is normalized by the energy content of the environment (ε). This can be formalized as follows:

$$\text{Payoff} = W_o - \Sigma\Phi/\varepsilon \tag{1}$$

Here, W_o and Φ stand for the output power and the entropy production, respectively. ε is given in arbitrary units, where the amount of energy required to duplicate a cell is taken as 1 unit. Now, using Eq. 1 and the data given in Table 1, the payoff matrix can be calculated. For instance, when an efficient cell interacts with a dissipative cell, the payoff obtained by the former is

$$\text{Payoff} = 2/9 - 5/(6\varepsilon) \tag{2}$$

where $2/9$ being the output power corresponding to the ES phenotype, and $5/6$ the summation of the entropy production of both cells: the site owner, being a ES cell, contributes one-third, and its neighbor, exhibiting a DF phenotype, accounts for one-half. Similar reasonings were used to complete Table 2.

It should be noted that when the energetic resource is abundant (high values of ε), the competition for extracellular energy becomes less important. In other words, the consumption of free energy due to biological activities (entropy production) has a minor role in determining the cellular performance. In such circumstances the performance will be mainly determined by the output power.

At the start of the next time step, each lattice site will be occupied by a cell with a phenotype equal to that of the cell with the highest score among the previous owner and the immediate neighbors. If the evaluated site was empty, it will remain empty only in the case that the immediate-neighbor sites were also empty. Otherwise, it will be filled with a new cell exhibiting the phenotype corresponding to the neighbor cell with the highest score.

In our model, the cellularity and metabolic strategy are independent traits determined by the genotype. Therefore, any given cell, besides being either ES or DF, can be a single-celled organism itself or belong to a cluster of cells. In the latter case, the score of the cells forming the cluster are individually computed and used to calculate an average value that is then assigned as the score of each cell belonging to the cluster. One cluster occupies as many lattice sites as cells form the cluster. These clusters can grow or shrink depending on how the averaged score compares to the score of those cells found in the neighborhood, as described above. In all cases, the cells forming part of a cluster, when surviving, remain glued together and are not allowed to move freely. By contrast, before starting a new iteration, single-celled organism can either move randomly to empty sites or interchange positions with other single-celled organism within the lattice. A flowchart of the algorithm used to carry out the simulations is given as Supplementary Information.

Pairwise Competition Simulations

Since in the current model the cellularity and metabolic strategy are independent traits, four types of organisms can be distinguished: dissipative unicellular and multicellular organisms (U_{DF} and M_{DF} , respectively) and their efficient counterparts (U_{ES} and M_{ES}). Therefore, up to six different types of pairwise competition experiments were carried out (U_{DF} vs. M_{DF} , U_{DF} vs. U_{ES} , U_{DF} vs. M_{ES} , M_{DF} vs. U_{ES} , M_{DF} vs. M_{ES} , U_{ES} vs. M_{ES}). A typical experiment was carried out as follows. After setting the energetic conditions of the environment (value of ε), equal numbers of each type of the competing organisms were randomly distributed through the grid and allowed to proliferate according to their ability to withdraw and use the energy, as described above. Once a stationary state was reached, the number of each type of cell was recorded.

Results

In the current model, multicellularity implies loss of motility. Congruently, when agents exhibiting the same metabolic phenotype but different cellularity had to compete for limited space, mobile unicellular organisms always

prevailed over clustering cells, regardless the energetic content of the environment and the metabolic strategy being considered (Fig. 1). These results stress the importance of cell motility in determining the output of competition and highlight the fact that whatever the

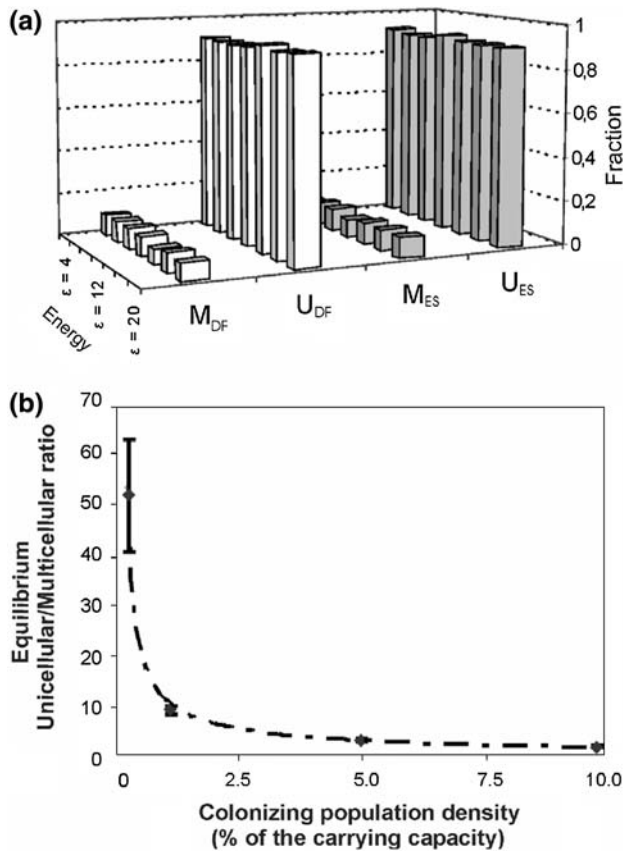


Fig. 1 Undifferentiated multicellularity per se represents a hindrance rather than an improvement. In experiments where organisms with the same metabolic phenotype but different cellularity had to compete for limited space, the output was always consistently the same: single-celled organisms performed better than their multicellular counterparts under all energetic conditions tested, ranging from situations of abundance ($\epsilon = 20$) to those of energetic limitation ($\epsilon = 4$). **(a)** A typical pairwise competition experiment was carried out as follows. Equal numbers of either dissipative unicellular (U_{DF}) and dissipative multicellular (M_{DF}) or efficient unicellular (U_{ES}) and efficient multicellular (M_{ES}) organisms were randomly distributed through the grid and allowed to proliferate, as described in the text. Once a stationary state has been reached, the number of each type of cell was recorded and used to plot the fraction of the total biomass that it represents (ordinate). **(b)** A multifactorial analysis of variance ruled out any main effect of extracellular energy or the metabolic strategy on the equilibrium ratio between unicellular and multicellular biomasses. However, as illustrated here, the starting population density showed a pronounced effect. Each point represents the mean \pm SE of 18 simulations carried out under six different conditions (environmental energy and metabolic strategy) in triplicate. It can be noted that the advantage of single-celled organisms is remarkable when colonizing (at low densities) a new ecosystem. Nevertheless, even when the colonizing population represented 10% of the carrying capacity, the unicellular organisms yielded 2.5 ± 0.3 times more biomass than their multicellular counterparts

selective advantages of clustering cells might have been, they had to outweigh disadvantages such as hampered mobility and high local cell densities. According to this line of thinking, one should expect that these disadvantages become less pronounced if the competing unicellular population is also present at high cell densities. This prediction was indeed confirmed by the results presented in Fig. 1b.

Although it is easy to imagine a mutation, or series of mutations, leading to the formation of undifferentiated cell clusters, it is unclear what the benefits of clustering cells over their unicellular ancestor could have been. In order to search for such advantages, we started analyzing the ecological niches of the single-celled ancestors. Thus, we first assessed the effect of cell motility and external energy on the competition between efficient and dissipative single-celled organisms. As can be deduced from Fig. 2, the key variable in determining the output of the competition was the energy content of the environment. In this way, DF always dominated when the resource was abundant. In contrast, limited fuel led to the dominance of ES. On the other hand, cell motility exhibited a modulating effect on the energy threshold beyond which DF outnumbers ES. Such a critical value of energy defines the parameter referred to as $\epsilon_{0.5}$. When unicellular organisms employing different metabolic strategies face competition for a limited space and a shared resource, the lack of mobility favors the efficient use of the resource, as indicated by a higher $\epsilon_{0.5}$ than that observed when the cells are fully mobile (Fig. 2).

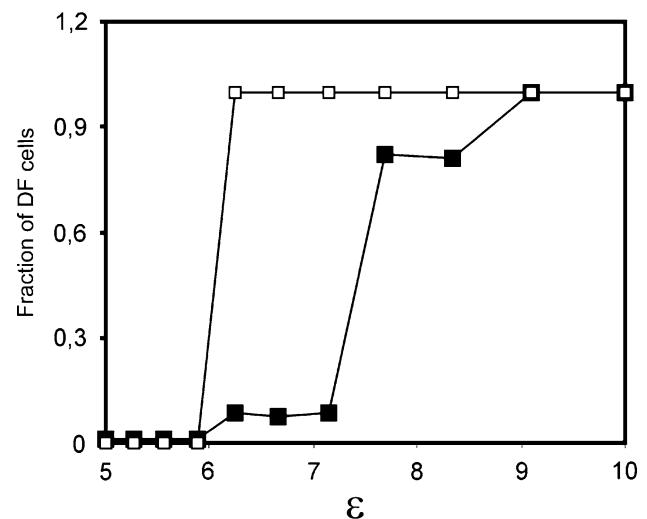


Fig. 2 Fuel abundance and motility favor a dissipative strategy. Competition experiments between single-celled organisms with different metabolic strategies were carried out under different conditions. In one set of experiments the competing cells were considered to be mobile (open squares). In the other set, the cells were not allowed to move (filled squares). At the end of each experiment the fraction of cells exhibiting a dissipative metabolism was calculated and plotted as a function of the extracellular energy (ϵ)

Table 3 Multicellularity favors the efficient strategy when competing with dissipative organisms

	U_{ES}	M_{ES}
U_{DF}	6.0	7.4
M_{DF}	11.4	7.4

Note: Competition between the two metabolic strategies was comprehensively studied. U_{ES} and U_{DF} : efficient and dissipative unicellular organisms, respectively. M_{ES} and M_{DF} : their multicellular counterparts. All the possible pairwise competition experiments were run as described under Materials and Methods. Data were plotted as in Fig. 2, and for each curve the abscissa of the point with an ordinate of 0.5 yielded the parameter defined as $\varepsilon_{0.5}$. This parameter can be interpreted as the extracellular energy that represents a threshold beyond which DF outcompetes ES. The table shows the $\varepsilon_{0.5}$ values corresponding to the indicated situations. It can be noted that multicellularity increases the energy threshold, in this way favoring the efficient strategy

Next, competition between both strategies was comprehensively studied. All the possible pairwise competition experiments were carried out, but now single-celled organisms were always mobile. Table 3 shows that clustering efficient cells led to an enhanced $\varepsilon_{0.5}$, even when these clusters competed with highly motile single-celled dissipative organisms.

According to the results obtained from pairwise competition experiments, when extracellular free energy is high enough to yield a value of $\varepsilon > 6$, then dissipative unicellular organisms (U_{DF}) thrive at the expense of their efficient counterpart (U_{ES}) (see Table 3). Regardless of the energy levels, the latter are favored when competing for space against efficient multicellular forms (Fig. 1b), which, in turn, are better endowed to face competition against U_{DF} if $\varepsilon < 7.4$ (Table 3). As illustrated in Fig. 3a, these three agents (U_{DF} , U_{ES} , and M_{ES}) seem to have relationships analogous to the game of rock-scissors-paper, with the first outcompeting the second, the second outcompeting the third, and the third outcompeting the first. Therefore, we next investigated the outcome of the competition when all three types of organisms are simultaneously present and $6.0 < \varepsilon < 7.4$. To this end, we performed cellular automata simulations on a two-dimensional lattice, where the three agents were initially randomly distributed in space and their population densities were followed over time. We carried out two different set of experiments. In one set, all the sites of the model world were always occupied from the beginning, but the starting proportions of each agent were variable from one experiment to another. In the other set of simulations, the starting proportions were also variable, but only 10% of the available sites were occupied at the beginning of each experiment. The dynamics of the proportions among the three agents is shown in Fig. 3b. As can be observed, the trajectory of the proportions is influenced by both the initial proportions and the starting total density. However, eventually the output was the

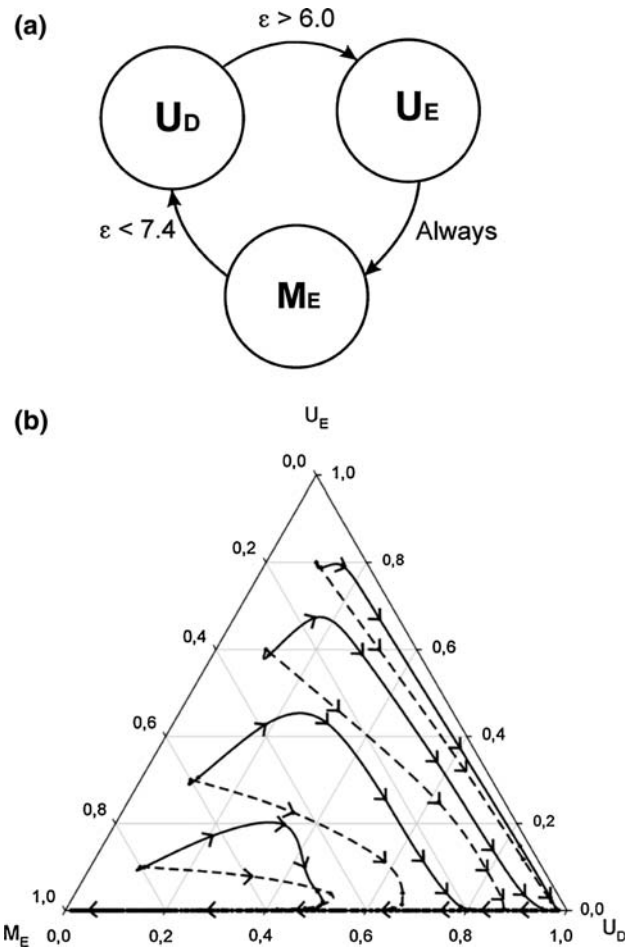


Fig. 3 The high degree of aggressiveness exhibited by dissipative single-celled organisms drives the dynamics of a rock-scissors-paper game that leads to the flourishing of undifferentiated clusters of efficient cells. (a) Under certain conditions ($6.0 < \varepsilon < 7.4$) U_E , U_D , and M_E may be engaged in a cyclic competition hierarchy of the rock-scissors-paper type. In an infinite population, this competitive loop should lead to a cyclic dynamic of the population densities of each agent. However, in finite populations the situation can be different since a particular population can go extinct. (b) Simulations where the three types of organisms were initially present were carried out in a model world with $\varepsilon = 6.3$. The dynamics of the proportion of each agent is plotted in the ternary phase diagram. Here U_E stands for the proportion of efficient single-celled organisms and ranges linearly from zero along the base of the triangle to unity at its peak. Similarly, U_D and M_E (the proportions of dissipative unicellular and efficient multicellular organisms, respectively) increase to unity at their respective corners. Two set of experiments were conducted. In one set only 10% of the available sites were occupied at the beginning of each experiment (continuous line). In the other set of simulations, all the sites were occupied from the beginning (dashed line)

same in all the cases. In words, U_{DF} rapidly drove U_{ES} extinct, and then M_{ES} thrived slowly, while U_{DF} shrank in parallel. Experiments where all compete against all were also carried out. The results obtained were qualitatively the same as those described above. At intermediate energetic levels the system tends to a homogeneous state where efficient multicellular forms are the winner.

Finally, in order to gain a better understanding of the forces imposing this dynamics, we focused our attention on those game theoretical aspects that might be relevant. In this sense, Frean and Abraham have shown that ecosystems formed by three species without strict competitive hierarchies can exhibit a counterintuitive phenomenon: the species that is the least competitive will eventually have the largest population and will be the least likely to die out. In contrast, those species exhibiting higher aggressiveness are more likely to become extinct (Frean and Abraham 2001). To test whether that was the case in our model, we assessed, in pairwise competition experiments, the time required by the winner to decrease the proportion of its competitor from 50% to less than 10%. In this way, the time required by U_{DF} to reduce the U_{ES} population was one order of magnitude smaller than that of M_{ES} against U_{DF} , suggesting that the predominance of undifferentiated aggregates of efficient cells is, paradoxically, an indirect consequence of the high competitiveness of the opposing dissipative strategy.

Discussion

In extant organisms, the most obvious benefits of multicellularity arise from the division of labor between differentiated cells. However, since these organisms are believed to have evolved from undifferentiated pluricellular ancestors, the division of labor cannot be invoked as a force driving the transition from single cells to multicellular beings. Furthermore, these primitive clusters of undifferentiated cells most probably had to face disadvantages such as locally increased cell densities and hampered mobility. This is indeed reflected in our model by the predominance, regardless of the energetic content of the environment, of mobile single-celled organisms when competing against their corresponding multicellular counterparts. The fact that, in the absence of labor division, the hindrances of multicellularity are well reflected in our model suggests its validity to search for the selective advantages that could have outweighed such hurdles, driving the transition to undifferentiated multicellularity. In that way, we conclude that the interplay between two glycolytic working regimes might have promoted the clustering of undifferentiated cells in an early period of life, as supported by the observation that clustering efficient cells led to an increase in the $\varepsilon_{0.5}$ parameter (Table 3). In other words, aggregation of undifferentiated cells represents a selective advantage for the nondissipative strategy when competing against the dissipative one. Nevertheless, under extreme conditions, that is, when the energy levels of the environment are high or low, single-celled organisms are better endowed to compete in such circumstances.

However, as deduced from Fig. 3b, at intermediate energetic levels the system tends to a homogeneous state where unicellular forms of life are competitively excluded.

When two beneficial traits are linked by a trade-off relationship, natural selection cannot improve one without impairing the other. Under certain conditions, grouping cells differing in these traits may benefit from the so-called covariance effect (Michod 2006). In other words, the group fitness is augmented beyond the average fitness of component cells. In the current model, all the cells forming an organism exhibit the same metabolic phenotype, precluding the covariance effect. However, since velocity and efficiency are two useful attributes, we could wonder whether multicellular organisms encompassing DF and ES cells may lead to a favorable covariance effect. In extant metazoans, most of their tissues are engaged in viability functions and rely on an efficient metabolism. In contrast, those tissues requiring a rapid proliferative capacity, such as challenged lymphocytes, behave as dissipative devices (Aledo 2004). Therefore, maintaining both metabolic strategies within an individual seems to be advantageous. However, it should be emphasized that this selective advantage is only possible once differentiation has arisen, allowing the coordination of the proliferative activity of each cell lineage (Soto and Sonnenschein 2004). In our model, organisms multiplied without other restrictions than those imposed by their own ability to deal with energetic resources. Under this condition, when a few DF cells were harbored in an ES multicellular organism, the efficient cells were rapidly outnumbered by DF cells (results not shown), which eventually drove the individual to extinction. It is remarkable that this situation is reminiscent of that observed during cancer development, including the fact that tumor cells exhibit a more dissipative metabolism than their healthy counterparts (Warburg 1956; Aledo 2004).

Life can be traced unambiguously to approximately 2.7 Ga ago. Beyond this point it is difficult to declare with certainty what the oldest traces of life are. Putative biosignatures found in rocks dated between 3.0 and 3.8 Ga are the subject of ongoing discussions (López-García et al. 2006). In any event, these early manifestations of life contrast with the late apparition of most animal phyla in the fossil record about 550 million years ago (Morris 2000). It has long been recognized that atmospheric oxygen levels may have played a key role in the evolution of metazoans (Falkowski et al. 2005). In this sense, it is believed that in order to set the stage for the emergence of animals, the continuous toil of nearly 3 billion years by photosynthesizing organisms was required (Dismukes et al. 2001). Then, nearly every extant phylum of the kingdom Animalia emerged within the very short time span of approximately 10 million years (Ohno 1996). In other words, metazoans, highly complex multicellular creatures with specialized

organs, abruptly appear fully formed in the fossil record, without any available intermediates to link them to their unicellular ancestors. This leaves the Cambrian explosion as one of the great, as yet unsolved mysteries of evolution. Our results allow us to devise a hypothetical scenario for the emergence of the earliest metazoan. The sequence of events we hypothesize is as follows. Before oxygen built up in the Earth’s atmosphere, the competition among different anaerobic strategies in the use of fuel could have favored the apparition of small clusters of undifferentiated cells. These clusters of undifferentiated cells had billions of years to evolve increasingly more sophisticated division of labor. Although the low energy yield characteristic of anaerobic metabolism may have precluded these forms of life to grow large and complex (Catling et al. 2005), the wealth of genes and functions that these soft-bodied and microscopic multicellular organisms may have reached at the beginning of the Cambrian, could have been enough to enable a fast development of diversity once oxygen levels triggered a growth in both body size and metabolic complexity (Raymond and Segrè 2006). Furthermore, our hypothesis is in line with a new and growing body of evidence, derived from molecular analyses, that suggests that animal phyla diverged much earlier than when they appear in the fossil record (Bromham et al. 1998; Wang et al. 1998). Therefore, our results suggesting that the transition to multicellularity may have taken place earlier than hitherto thought provide additional support for an extended period of Precambrian metazoan diversification.

Acknowledgments The author thanks Alicia Esteban del Valle, Juan Antonio Pérez-Claros, and Miguel Angel Medina for thoughtful discussions. The author is also grateful to three anonymous referees who have helped to improve the original manuscript. This work was supported by the Universidad de Málaga and Grant CGL2007-65010 from the Ministerio de Educación y Ciencia de España.

Appendix: Insight into the Thermodynamic Working Regimes

In the framework of nonequilibrium thermodynamics, the glycolytic fluxes of ATP formation (J_1) and glucose consumption (J_2) are proportional to the chemical affinities for ATP synthesis (X_1) and glucose breakdown (X_2):

$$J_1 = L_{11}X_1 + L_{12}X_2 \tag{A1}$$

$$J_2 = L_{12}X_1 + L_{22}X_2 \tag{A2}$$

where the proportionality coefficients ($L_{ij} \geq 0$) incorporate kinetic (enzymatic) attributes. The rate at which energy is extracted from glucose is referred to as input power (W_i), which is computed by multiplying the energy released per mole of glucose breakdown (X_2) by the flux of glucose consumption (J_2): $W_i = J_2X_2$. Under physiological

conditions, the chemical affinity for ATP synthesis takes negative values, slowing down glucose consumption. Therefore, the maximum attainable input power is $L_{22}X_2^2$, achieved under the hypothetical condition that ATP synthesis and glucose breakdown are uncoupled, $L_{12} = 0$ (Aledo 2007). On the other hand, the rate at which energy is conserved in the form of ATP is referred to as output power, W_o , which can be calculated as the rate of ATP formation times the energy needed to produce 1 mol of ATP: $W_o = -J_1X_1$. Since X_1 is negative (energy that has to be provided), the negative sign in the former equation has been arbitrarily introduced to give a positive output power.

Although far from equilibrium we lose the mathematical guarantee of linearity, this does not mean that linear flow-force relations cannot be established. In fact, empirical and theoretical analyses suggest that linear relations between fluxes and forces are much more common than perhaps expected (Westerhoff and van Dam 1987).

For our purposes, it will be convenient to express the output power as a function of the efficiency. To this end, and considering that the efficiency is defined as minus the ratio between input and output power, we can start by writing $W_o = \eta J_2X_2$. Now, substituting J_2 by (A2), we obtain

$$W_o = \eta J_2X_2 = \eta X_2^2(L_{12}(X_1/X_2) + L_{22}) \tag{A3}$$

This equation can be simplified taking into account some constraints that enforce certain relationships between their variables. In this respect, two interesting concepts that assist in the study of linear energy converters are the degree of coupling, $q = L_{12}/(L_{11}L_{22})^{1/2}$, and the phenomenological stoichiometry, $Z = (L_{11}/L_{22})^{1/2}$. For glycolysis, where ATP formation and glucose breakdown are tightly coupled processes ($q = 1$), with a fixed stoichiometry of two molecules of ATP formed per molecule of glucose split ($Z = J_1/J_2 = 2$), the following equalities hold: $-\eta/2 = X_1/X_2$ and $L_{12} = (L_{11}L_{22})^{1/2}$. When translating these substitutions into Eq. (A3), we obtain $W_o = \eta X_2^2 ((L_{11}L_{22})^{1/2} (-\eta/2) + L_{22})$, an expression that can be reorganized to yield $W_o = \eta X_2^2 L_{22} ((L_{11}/L_{22})^{1/2} (-\eta/2) + 1)$, which can be further simplified when considering the constraint $Z = (L_{11}/L_{22})^{1/2} = 2$:

$$W_o = L_{22}X_2^2(-\eta^2 + \eta) \tag{A4}$$

Thus, the output power is a negative quadratic function of the efficiency. The value of η for which a maximum output power is achieved can now be calculated:

$$\frac{dW_o}{d\eta} = -2\eta + 1 = 0 \Leftrightarrow \eta = 1/2 \tag{A5}$$

In the current paper, cells performing under conditions optimizing the output power are said to exhibit a DF phenotype. Although the ability to maximize the output

power is an important trait, for biological systems a high degree of fitness may imply not only high output powers but also high efficiencies (Aledo et al. 2007). Consequently, we also considered a function representing a compromise between high output power and high efficiency. Such a function can be obtained, as proposed by Stucki (1980), multiplying the efficiency by the output power. The resulting new function is referred to as efficient output power, EW_o (Stucki 1980; Aledo et al. 2007):

$$EW_o = L_{22}X_2^2(-\eta^3 + \eta^2) \quad (A6)$$

It is easy to prove that $\eta = 2/3$ represents an optimum for this function. Organisms that operate under conditions optimizing the efficient output power are said to exhibit an ES regime.

In order to calculate the output power obtained by DF and EF organisms, we only have to evaluate the function (A4) at the points $\eta = 1/2$ and $\eta = 2/3$. In this way, it can be concluded that the output power of DF and EF cells are 1/4 and 2/9 of the maximum attainable input power, respectively.

In addition to the output power and efficiency, another relevant factor is the dissipation function, Φ . This thermodynamic function can be formulated as the sum of the products of all the fluxes and their corresponding driving forces. In our model:

$$\Phi = J_1X_1 + J_2X_2 + J_3X_3 \quad (A7)$$

where J_3 is the flux of all the ATP-consuming processes lumped together. Since these processes are driven by the hydrolysis of ATP, $X_3 = -X_1$. Thus, $J_3 = -L_{33}X_1$ and (A7) can be expressed as

$$\Phi = J_1X_1 + J_2X_2 + L_{33}X_1^2 \quad (A8)$$

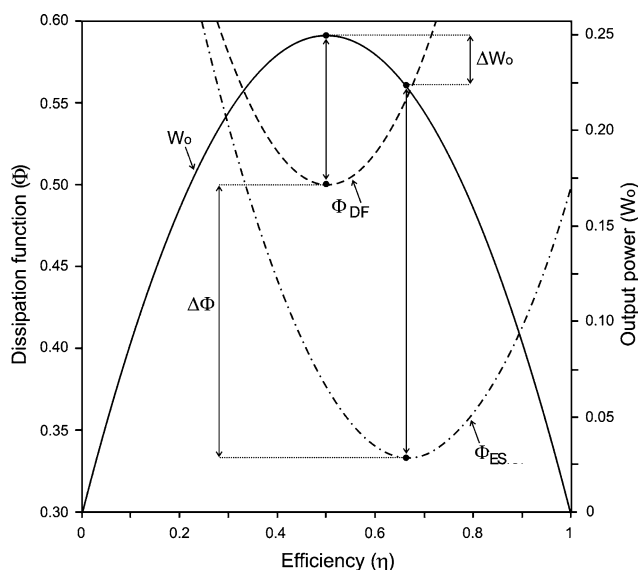
According to the definitions given previously, $J_1X_1 = -W_o$, $J_2X_2 = W_o/\eta$, and $X_1 = -\eta X_2/2$. Thus, (A8) can be rewritten $\Phi = -W_o + W_o/\eta + L_{33} \eta^2 X_2^2/4$. Finally, when we substitute the output power by its corresponding function of the efficiency, the result is

$$\Phi = L_{22}X_2^2 [(1 + L_{33}/4L_{22})\eta^2 - 2\eta + 1] \quad (A9)$$

In order to evaluate Φ in the cases of DF cells ($\eta = 1/2$) and ES cells ($\eta = 2/3$), we need to know the value of L_{33}/L_{22} . To this aim we will take advantage of the minimum entropy production theorem, which claims that in the steady state the entropy production must be minimal. That is, $d\Phi/d\eta = 0 = 2(1 + L_{33}/4L_{22})\eta - 2$, from which we obtain the following constraint: $\eta = 4 L_{22} / (4 L_{22} + L_{33})$. Herein, it may be convenient to remember that DF and ES organisms operate at efficiencies of 1/2 and 2/3, respectively. This means that $L_{33} = 4 L_{22}$ for dissipative cells, whereas in the case of efficient organisms the relation is $L_{33} = 2 L_{22}$ (Aledo and Esteban del Valle 2005).

Bearing these considerations in mind, Eq. A9 leads to a straightforward conclusion: The dissipation functions of DF and EF cells are one-half and one-third of the maximum attainable input power, respectively.

An intuitive (graphical) insight into the thermodynamics underlying these working regimes (DF vs. ES) can be provided by plotting the output power and dissipative functions against the efficiency [$W_o = L_{22}X_2^2 (-\eta^2 + \eta)$, solid line; $\Phi_{DF} = L_{22}X_2^2 (2\eta^2 - 2\eta + 1)$, dashed line; $\Phi_{ES} = L_{22}X_2^2 (1.5\eta^2 - 2\eta + 1)$, dotted-dashed line; the y axis is in units of $L_{22}X_2^2$]; it can be graphically deduced that an ES regime represents an excellent compromise between high output power and low entropy production. In other words, an ES strategy trades a low decrease in output power for a high decrease in entropy production.



References

- Aledo JC (2004) Glutamine breakdown in rapidly dividing cells: waste or investment? *BioEssays* 26:778–785
- Aledo JC (2007) Coupled reactions versus connected reactions. Coupling concepts with terms. *Biochem Mol Biol Educ* 35: 85–88
- Aledo JC, Esteban del Valle A (2002) Glycolysis in Wonderland: the importance of energy dissipation in metabolic pathways. *J Chem Educ* 79:1336–1339
- Aledo JC, Esteban del Valle A (2004) The ATP paradox is the expression of an economizing fuel mechanism. *J Biol Chem* 279:55372–55375
- Aledo JC, Pérez-Claros JA, Esteban del Valle A (2007) Switching between cooperation and competition in the use of extracellular glucose. *J Mol Evol* 65:328–339
- Barros N, Gallego M, Feijóo S (2004) Calculation of the specific rate of catabolic activity (Ac) from the heat flow rate of soil microbial reactions measured by calorimetry: significance and applications. *Chem Biodivers* 1:1560–1568

- Bromham L, Rambaut A, Fortey R, Cooper A, Penny D (1998) Testing the Cambrian explosion by using a molecular dating technique. *Proc Natl Acad Sci USA* 95:12386–12389
- Catling DC, Glein CR, Zahnle KJ, McKay CP (2005) Why O₂ is required by complex life on habitable planets and the concept of planetary “oxygenation time”. *Astrobiology* 5:415–438
- Dismukes GC, Klimov VV, Baranov SV, Kozlov Yu N, DasGupta J, Tyryshkin A (2001) The origin of atmospheric oxygen on Earth: the innovation of oxygenic photosynthesis. *Proc Natl Acad Sci USA* 98:2170–2175
- Esteban del Valle A, Aledo JC (2006) What process is glycolytic stoichiometry optimal for? *J Mol Evol* 62:488–495
- Falkowski PG, Katz ME, Milligan AJ, Fennel K, Cramer BS, Aubry MP, Berner RA, Novacek MJ, Zapol WM (2005) The rise of oxygen over the past 205 million years and the evolution of large placental mammals. *Science* 309:2202–2204
- Frean M, Abraham ER (2001) Rock-scissors-paper and the survival of the weakest. *Proc R Soc Lond B* 268:1323–1327
- Lalucque H, Silar P (2003) NADPH oxidase: an enzyme for multicellularity? *Trends Microbiol* 11:9–12
- López-García P, Moreira D, Douzery E, Forterre P, Van Zuilen M, Claeys P, Prieur D (2006) Ancient fossil record and early evolution (ca. 38 to 05 Ga). *Earth Moon Planets* 98:247–290
- Michod RE (2006) The group covariance effect and fitness trade-offs during evolutionary transitions in individuality. *Proc Natl Acad Sci USA* 103:9113–9117
- Morris SC (2000) The Cambrian “explosion”: slow-fuse or megatonnage? *Proc Natl Acad Sci USA* 97:4426–4429
- Ohno S (1996) The notion of the Cambrian pananimalia genome. *Proc Natl Acad Sci USA* 93:8475–8478
- Pepper JW, Smuts BB (2001) Agent-based modeling of multilevel selection: the evolution of feeding restraint as a case study. In: Pitt WC (ed) *Swarmfest (2000), Proceedings of the 4th Annual Swarm User Group Conference Natural Resources and Environmental Issues*, vol XIII. S. J. and Jessie E. Quinney Natural Resources Research Library, Logan, UT, pp 57–68
- Pfeiffer T, Bonhoeffer S (2003) An evolutionary scenario for the transition to undifferentiated multicellularity. *Proc Natl Acad Sci USA* 100:1095–1098
- Pfeiffer T, Schuster S, Bonhoeffer S (2001) Cooperation and competition in the evolution of ATP-producing pathways. *Science* 292:504–507
- Raymond J, Segrè D (2006) The effect of oxygen on biochemical networks and the evolution of complex life. *Science* 311:1764–1767
- Stucki JW (1980) The optimal efficiency and the economic degrees of coupling of oxidative phosphorylation. *Eur J Biochem* 109:269–283
- Wang DY-C, Kumar S, Hedges SB (1998) Divergence time estimates for the early history of animal phyla and the origin of plants, animals and fungi. *Proc R Soc Lond B* 266:163–171
- Warburg O (1956) On the origin of cancer cells. *Science* 123:309–314
- Westerhoff HV, van Dam K (1987) *Thermodynamics and control of biological free-energy transduction*. Elsevier, Amsterdam, pp 269–283
- Westerhoff HV, Hellingwer KJ, van Dam K (1982) Thermodynamic efficiency of microbial growth is low but optimal for maximal growth rate. *Proc Natl Acad Sci USA* 80:305–309