Biophysical growth and reproduction mechanisms of cells and first principles of life origin and development

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Abstract

There are evidences that organic life cannot be reduced to a single biomolecular realm, but, similar to other natural phenomena, rather involves different mechanisms acting at different scale levels, which together define evolvement of the living world. Here we show that a certain biophysical mechanism of general nature, acting at a cellular scale level and above, is also an influential factor in life phenomena. In inherent unity with biomolecular mechanisms, it governs growth and reproduction of cells, organs and whole organisms, and secures irreversibility of the life cycle progression. For validation of proposed concepts, we study the growth and division of B. subtilis, Staphylococcus, E. coli, S. pombe and Amoeba using extensive experimental observations. A well founded hypothesis is presented why and how cells grow and divide. The study also sheds light on other pertinent problems of biology, biophysics, and first principles of life origin and development.
**Introduction**

Understanding the first principles of *why* and *how* cells grow and reproduce, what kind of *fundamental* mechanisms so universally and persistently govern cellular growth and reproduction, is of great importance for both the practical needs (medical, crop production, growth and productivity of domesticated animals, biotechnological, etc.) and scientific studies.

Most studies on the subject of cell growth and division explore biochemical mechanisms, representing chains of biochemical reactions implementing transitions through successive growth and division phases. Examples can be works (Marshall et al., 2012; Moseley et al., 2009; Moseley and Nurse, 2010; Conlon and Raff, 2003; Tyson and Csikasz-Nagy, 2002; Jorgensen and Tyers, 2004).

Another direction of research was inspired by ideas to find *systemic* level mechanisms responsible for the cell growth and division. In the review (Mitchison, 2003) the author says with regret: "It would be satisfying if the main parameters of cell cycle growth had been established in the earlier work. Not surprisingly, however, there were still major uncertainties left when people moved from this field to the reductionist approaches of molecular biology." Review (Jorgensen and Tyers, 2004) accentuates specific properties of cellular growth and reproduction, which are unlikely to be resolved exclusively at a biomolecular level.

Many concepts with regard to general growth and division mechanisms were proposed. Such are the "sizing" and "timing" hypotheses, claiming accordingly the priority of a cell size and of a certain time period as primary factors defining the cell cycle progression. P. Fantes (1977) found experimentally that actually both "sizing" and "timing" homeostasis takes place. Note, in all instances, it is implicitly assumed that some *biochemical mechanisms* are at the core of such hypothetical systemic mechanisms, sensing the cell size, or time, or other cellular *macro*-characteristics.

Another group of studies suggests that there are fundamental growth and reproduction mechanisms at *higher than molecular level* (Thompson, 1992; Stewart, 1998).

Here, we consider a certain biophysical mechanism (called also as a "general growth law") that works at higher than molecular levels, and, based on supporting experimental
data, appears to be an influential player in the cell growth and reproduction. This mechanism (a) reconciles known facts about cellular growth and division; (b) predicted certain growth and reproduction effects, some of which already found experimental confirmations; (c) it is seamlessly integrated with the cellular biochemical machinery; in fact, both work in tight cooperation.

Methods

**Forces shaping biological phenomena**

Although the main paradigm of modern biology is a biochemical one, life as such does not exclude mechanisms acting at other scale levels. Such an arrangement can be observed in a physical world, when a multitude of different mechanisms shapes the same phenomenon, each mechanism acting at a different scale level.

Living organisms represent an uninterrupted continuation of an inorganic world, and so there are no fundamental reasons that the life cycle and evolution of living species should be defined entirely by biomolecular mechanisms. It is intuitively clear that the objective causes which led to appearance of living organisms existed before the biochemical mechanisms, and DNA in particular, were created. These "founding" (often called *first principles*) mechanisms belonged to an inorganic world. It is much due to their action that living organisms originated and progressed through their evolutionary paths. DNA of an evolutionarily developed single cell could contain nothing related to multicellular structure, like a balanced growth of organs and systems in multicellular organisms. There should be other forces of nature which took care of such tasks at appropriate scale levels, besides the workings of biomolecular machinery.

The well defined set of cell shapes, how did it happen? Was it only a random play of chemical reactions? Very unlikely, given the optimal functionality of microbes and other microorganisms, supported, besides the other macro-characteristics, also by certain geometrical shapes, like rods, spheres. What about the level of tissues, organs, systems, whole multicellular organisms? Should we still assume that these multi-scale constructions are managed from a molecular level? Maybe the shape of living organisms is also defined by some unknown mechanisms at higher than molecular level? Why not?
This is how the physical world we know is arranged, and the living creatures present an inherent part of it.

**Biophysical growth mechanism**

The growth and reproduction mechanism which we consider in this paper was first introduced in (Shestopaloff, 2008, 2009), with the following advancements and applications in (Shestopaloff, 2010a, 2010b, 2011a, 2012a, 2012b, 2013, 2014a, 2014b, 2015, 2016; Shestopaloff and Sbalzarini, 2014) and other publications. The principal foundation of this mechanism is that it distributes nutrients, acquired by an organism, between the biomass synthesis and maintenance needs in a certain well defined way. In other words, using the mathematical representation of this general growth mechanism, the growth equation, we can find how much nutrients are used for biomass synthesis, and how much for the organism's maintenance needs, *at each moment of organism's life cycle*. The implications such knowledge provides are of fundamental value for biology.

Growth equation for a simple growth scenario, when nutrients are acquired through the cell surface, is as follows.

\[
p_c(X) dV(X,t) = k(t) \times S \times \left( \frac{R_s}{R_g} - 1 \right) dt
\]

where \( X \) represents a spatial coordinate, \( p_c \) is the density of the cell (units of measure \( \text{kg} \cdot \text{m}^{-3} \)), \( t \) is time in \( \text{sec} \), \( k \) is a specific nutrient influx (per unit surface per unit time) measured in \( \text{kg} \cdot \text{m}^{-2} \cdot \text{sec}^{-1} \), \( S \) is the total surface (in \( m^2 \)) nutrients are acquired through.

The left part of Eqn 1 is the mass increment. The right part is the product of the total influx through the surface (the term \( k(t) \times S \)), by a parameter \( \left( \frac{R_s}{R_g} - 1 \right) \), called the *growth ratio* \( (G) \); it defines which fraction of the total nutrient influx is used for biomass production. Thus, the right part represents the *amount* of nutrients used for biomass synthesis.

The most important parameter in Eqn 1 is the growth ratio. Why the nutrient distribution between the biomass synthesis and maintenance in nature has to be so definitive? The answer is this. The primary evolutionary goal of any living organism is its successful reproduction. No reproduction - no organism. For that purpose, the organism must use acquired resources *optimally*. If the biomass synthesis is non-optimal
- for instance, too slow, then the reproduction process is in jeopardy. If non-optimal insufficient amount of nutrients is directed to maintenance, then the organism won't be able to produce biomass efficiently, and then the reproduction will be delayed too. And so nature, as is the case with other laws of nature, goes on an optimal path, thus securing the fastest reproduction time for the given conditions. What is also extremely important, this optimal path provides the greatest stability possible for a given phenomenon (Shestopaloff, 2010c). (The fundamental stability of the world we know by and large is the consequence of such optimality of laws of nature.)

This optimality is tied to a certain geometrical form. All organisms do have some geometrical form, which is the base of why such a nutrient distribution could be universal for all species and their constituents, from the cellular level to organs to whole multicellular organisms. The growth ratio is a mathematical representation of this optimal distribution of nutrients, implemented in Nature.

In physics, the same principle of maximum stability due to optimality is behind the facts that acceleration of a body in mechanics is directly proportional to applied force and inversely proportional to mass, or electric current in a circuit is directly proportional to applied voltage and inversely proportional to resistance (Shestopaloff, 2010c). The growth ratio and the general growth mechanism are from the same category of fundamental parameters and relationships between them, although unknown so far to a wide audience. It was shown in (Shestopaloff, 2010c) that when relationships between fundamental parameters deviate even a little from such an optimum, the world as we know would be unlikely to exist, because of the inherent instability brought by these deviations.

Note that the growth ratio was discovered heuristically, which is the only way for discovering fundamental parameters and relationships between them - recall famous "Eureka!" by Archimedes (Shestopaloff, 2014b). It is defined as follows. Suppose that a cell can grow to a maximum volume $V_{\text{max}}$, which has a maximum surface $S_{\text{max}} = S(V_{\text{max}})$. The dimensionless parameters, which we call a relative surface $R_s$ and a relative volume $R_v$, are as follows:

$$R_s = \frac{S(V)}{S(V_{\text{max}})} \quad (2)$$

$$R_v = \frac{V}{V_{\text{max}}} \quad (3)$$
Then, the dimensionless growth ratio $G$ is as follows:

$$G = \frac{R_x}{R_y} - 1$$  \hspace{1cm} (4)

As a function of volume, the growth ratio monotonically decreases when the organism's volume increases. According to Eqn 1, it means that the more the organism grows, the less nutrients are available for biomass synthesis, and more nutrients are used for maintenance. This is understandable, since the growing biomass requires more nutrients for maintenance. Eventually, this conflict stops the growth.

The maximum size can change during growth depending on nutrients availability and other parameters. Such, a cell that begins to grow in an environment with low nutrient content, is destined to have a smaller final size. However, if, at some phase of growth, the environment is enriched in nutrients, then the cell grows bigger, which experimental observations confirm (Maaloe and Kjeldgaard, 1966). In many instances, the maximum size can be known upfront, if all growth conditions are defined at the beginning and do not change unpredictably later. Otherwise, the maximum size can change. This mathematical specific of the growth equation does not mean that it has some defects or it is of approximate nature. This is just an adequate mathematical reflection of the growth phenomena, when the change of parameters during the growth alters the final size of a grown organism. In the same way, we can compute the trajectory of a thrown stone. However, if the stone accidentally hits the tree branch, then its trajectory will change.

The possible variability of the maximum possible volume can be addressed by adding the dependence of its value from other parameters; for instance, from nutrient influx, temperature. Below we will discover that in certain types of growth scenarios knowing the maximum volume is not required.

**Finding nutrient influx**

The next important parameter in Eqn 1 is nutrient influx $k$. Note that the product $k \times S$ represents the total nutrient influx $K$. This fact reflects the property of the growth equation that it does not matter which way the nutrient influx was acquired. Such, in a
growing budding yeast part of nutrients comes from the mother cell; in an apple nutrients come through the fruit's stem.

In (Shestopaloff, 2016) amount of nutrients required for cellular transportation depending on the shape of cells was found, while in (Shestopaloff, 2015) the overall amount of nutrients required for the growth of *S. pombe* and *amoeba* was obtained. It was discovered in (Elliot and McLaughlin, 1978; Gourse, 1996) that in some elongated cells, like *E. coli, S. cerevisiae*, the rate of RNA synthesis is twice the rate of protein synthesis. Taking into account the double rate of nutrient consumption for RNA synthesis, we can write for the nutrient influx $K_{\text{min}}$ required for the biomass synthesis and maintenance (without transportation costs) the following.

$$K_{\text{min}}(v) = N\left(C_{p}^{s}v + C_{r}^{s}v^{3}\right) \quad (5)$$

Here, $C_{r}^{s}$ and $C_{p}^{s}$ are fractions of nutrient influx required for RNA and protein synthesis; $v$ is the relative increase of organism's volume (the ratio of the current volume to the volume at the beginning of growth, so that $v \geq 1$).

For an elongating cylinder-like cell, whose diameter remains constant, volume is proportional to the relative increase of length $L$. Using the same consideration as in (Shestopaloff, 2015, 2016) about proportionality of transportation costs to the traveled distance, and substituting $K_{\text{min}}$ from Eqn 5, we obtain an equation for the total nutrient influx.

$$dK(L) = C_{i}K_{\text{min}}(L)dL \quad (6)$$

where $C_{i}$ is a constant.

Solving Eqn 6, we find

$$K(L) = N_{1}C_{i}\left(\frac{1}{2}C_{p}^{s}L^{2} + \frac{1}{3}C_{r}^{s}L^{3}\right) = A\left(C_{p}^{s}L^{2} + C_{r}^{s}L^{3}\right) \quad (7)$$

where $N_{1}$ is a constant; $A = N_{1}C_{i}/2$; $C_{p} = C_{p}^{s}$; $C_{r} = (2/3)C_{r}^{s}$.

Similarly, we can find the total required nutrient influx for a disk and a sphere. We assume that a disk grows in two dimensions (height remains constant); a sphere increases proportionally in three dimensions.

$$K(v)_{\text{disk}} = \left(C_{p}^{s}v^{3/2} + C_{r}^{s}v^{5/2}\right) \quad (8)$$

$$K(v)_{\text{sph}} = \left(C_{p}^{s}v^{4/3} + C_{r}^{s}v^{7/3}\right) \quad (9)$$
Obtaining analytical solutions as Eqns 7, 8 and 9, is not always possible. Such, there is no analytical solution for an elongating ellipsoid. In this case, the following growth equation should be solved numerically (Shestopaloff, 2012b, 2014b).

\[
p_c dV(r) = k_{\text{min}}(V(r)) \times (r/r_0) \times S(r) \times \left( \frac{R_0}{R_r} - 1 \right) \frac{dV}{dr} dt
\]  

(10)

Here, \( r_0 \) is the beginning radius in the same direction of growth, which is defined by a radius-vector \( r \).

Results

Growth and division model of amoeba

Growth

We will start from a simpler and, apparently, evolutionarily the earliest growth scenario, which is implemented in amoeba. Let us define parameters of the growth equation.

Density and mass calculation. For all considered microorganisms, we assume the density to be constant during the growth and equal to \( 1 \text{ g} \cdot \text{cm}^{-3} \). This assumption is a reasonable approximation (Prescott, 1955). The mass of a grown amoeba used in calculations corresponds to the last experimental measurement.

Maximum possible volume. A parameter "spare growth capacity" (SGC) was introduced for the characterization of the maximum possible volume in (Shestopaloff, 2014b). It is defined as \( SGC = 1 - V_d/V_f \). Here, \( V_d \) is the volume when cell divides; \( V_f \) is the maximum possible volume, which the growth curve asymptotically approaches. For available experimental data, SGC value was in the range 1.0 - 2.8%. Two amoebas did not divide and, indeed, increased their mass by about 2% after missing division, so that SGC is a real value. In calculations, we used the maximum possible volume which exceeded the last measurement by 2%.

Geometrical form. Amoeba is modeled by a disk whose height \( H \) is equal to the initial disk radius \( R_b \). The maximum possible disk radius is \( R_0 \). Such a model was chosen based on analysis of amoeba’s images from different sources, which indicate rather two-dimensional increase of this species. Substituting the above parameters into Eqns 2 - 4, we obtain:
\[ R_{sd} = \frac{R(R+H)}{R_0(R_0+H)}; \quad R_{vd} = \frac{R^2}{R_0^2}; \quad G_d = \frac{R_{sd}}{R_{vd}} - 1 = \frac{R_0(R+H)}{R(R_0+H)} - 1 \] (11)

where index \( d \) denotes 'disk'.

**Nutrient influx.** The rate of nutrient consumption for RNA and protein synthesis are assumed to be the same for amoeba (Shestopaloff, 2014b, 2015), which transforms Eqn 8 into

\[ K(v)_{disk} = v^{3/2}(C_p + C_r) \] (12)

**Model verification.** Using the above parameters, we first computed the growth curves, and only then compared them with experimental data. So, this comparison is not a data fitting procedure in the usual sense, but actually a much more rigorous verification of the model's adequacy. The same verification was used for all other models, for which experimental data were available.

**Solution of the growth equation.** Substituting the above parameters into Eqn 1, we obtain the following differential equation.

\[
4p\pi dR = \frac{k(R_0-R)R}{(R_0+H)R_b^3} dt
\] (13)

Solution of this equation is as follows.

\[
t = \frac{2p\pi(R_0+H)R_b^3}{kR_0} \ln \left( \frac{R(R_0-R_b)}{R_b(R_0-R)} \right)
\] (14)

Certainly, we can use Eqn 14 to draw the growth curve. However, it would be better to find a direct analytical solution for the radius \( R \) as a function of time \( t \). The solution, indeed, is a remarkable one.

\[
R = \frac{R_0 \exp(t / c_0)}{(R_0 / R_b - 1) + \exp(t / c_0)}
\] (15)

where \( c_0 = \frac{2p\pi(R_0+H)R_b^3}{kR_0} \).

The remarkable thing about Eqn 15 is that this is a **generalized** solution of the famous logistic equation. Unlike the classic solution, when the equation's constants are chosen using ad hoc considerations, Eqn 15 produces these values **naturally**, as functions of the model's input parameters, which is a significant advancement. The fact that the heuristically introduced growth equation produced a generalized solution of the logistic equation (which is also used for modeling growth phenomena), should be considered as a
remarkable result, which works in favor of validity of the growth equation. (Note that the growth equation Eqn 1 represents a *new type of mathematical equation*, so that obtaining such an interesting and significant result is a good start for its mathematical explorations too.)

Figure 1 shows the computed growth curve for *amoeba* versus experimental data from (Prescott, 1955), and the corresponding growth ratio. We can see that the computed growth curve corresponds to experimental measurements very well. Comparison with other experiments shows slightly more dispersion of experimental points relative to the computed growth curves. However, the experiment in Figure 1 was chosen not for the least deviation from the computed growth curve, but for the *stability* of growth conditions compared to other experiments, in which nutrient influx was not so stable. If we could know the actual nutrient influx, we would compute the growth curves for other experiments more accurately too.

![Figure 1. Computed amoeba's growth curve versus experiment, and the growth ratio depending on time. Data are from (Prescott, 1955).](image)

*Division*

A continuous redistribution of nutrient influx between maintenance needs and biomass production, enforced by the general growth law, explains deceleration of the growth rate and subsequent stopping of growth. Indeed, growing biomass requires more nutrients for maintenance to support it, and so fewer nutrients are available for biomass synthesis. The
decrease of the growth ratio during growth is just a reflection of this fact in a mathematical form. This arrangement of the growth phenomena, however, has far reaching important implications. Here is why. Organismal biochemical machinery represents a single unity. There are no separate biochemical machineries for maintenance and for biomass production, but all biochemical reactions interrelate; they are arranged in such a way that output substances of previous reactions become inputs for the next. Success of methods of metabolic flux analysis is based entirely on this arrangement, when through such interdependencies, described by a system of stoichiometric equations, it is possible to unambiguously find how much of each substance participates in the biochemical interchange (Stephanopoulos et al., 1998; Shestopaloff, 2012b).

According to works on metabolic flux analysis, the solution of a system of stoichiometric equations produces the most adequate results when this solution is optimized for a maximum amount of produced biomass. Indeed, evolutionary development shaped the composition of biochemical reactions in the direction prioritizing fast reproduction; in other words, making the amount of produced biomass a leading parameter, which the composition of biochemical reactions is tied to. So, if the amount of synthesized biomass changes, the composition of biochemical reactions changes too. In which direction though? The answer is: in the direction that secures successive transitions through the entire growth period, and the fastest reproduction.

This is how the growth and reproduction is regulated from the standpoint of the general growth mechanism. Let us reiterate this. The general growth law, through the growth ratio, imposes constraints on the amount of produced biomass. The increasing biomass requires more nutrients for maintenance, and so inevitably less nutrients is available for biomass synthesis. Quantitatively, this continuous nutrient redistribution is defined by the growth ratio. Composition of biochemical reactions is evolutionarily tied to the amount of produced biomass, so that such forced changes in the amount of produced biomass are accompanied by changes in the composition of biochemical reactions, so that the newer composition corresponds to the new amount of produced biomass. (In particular, such composition's change is very likely one of the main factors triggering cell specialization.)

We know that growth processes and organisms' life cycles are generally irreversible, including such phenomenon as aging of multicellular organisms. The described
arrangement explains irreversibility during growth. The decreasing amount of nutrients diverted to biomass production acts as a ratchet, preventing the current composition of biochemical reactions to revert to the previous state, when more biomass was produced. For such a reversion to happen, the fraction of nutrients used for biomass production has to increase. However, the grown biomass already took for maintenance the part of nutrient influx, which was earlier used for biomass synthesis; and the entire biochemical machinery was adjusted accordingly. In order to increase the fraction of nutrients for the biomass production, the growth ratio has to be increased. It can happen through the size reduction or by substantially changing the geometrical form, which is not impossible, but would cause certain energetic, functional and developmental complications. If the said is true, then at least some simpler organisms might be able to "rejuvenate" through the decrease of biomass. Indeed, in (Prescott, 1956; Hartmann, 1928) the authors acknowledged that by periodically resecting part of amoeba's cytoplasm it is possible to indefinitely prevent it from entering division. However, in general, the growth cycle is difficult to reverse for the reason explained above, and this is the price for the smooth and persistent proceeding through the entire growth and reproduction cycle. (The objection to the said above can be that there are cells that divide without growth. However, these cells reside within multicellular organisms, whose other parts increase their biomass and can send appropriate signals to other cells.)

Quantitatively, the leading role of biomass production can be confirmed as follows. Let us rewrite Eqn 1 to explicitly show the amount of produced biomass $m_b$.

$$dm_b = k(X,t) \times S(X) \times \left( \frac{R_s}{R_v} - 1 \right) dt$$

(16)

As we will see later, the specific nutrient influx $k$ for amoeba at the end of growth changes little, as well as its volume and consequently the surface area. From three terms in Eqn 16, the growth ratio $G = \left( \frac{R_s}{R_v} - 1 \right)$ changes by far the quickest, so that the changes in the amount of produced biomass are defined mostly by changes of the growth ratio. It is important to understand that it is not the absolute, but relative changes which alter the composition of biochemical reactions the most. 50% decrease of small amount of produced biomass affects composition of biochemical reactions more than 25% decrease of a bigger amount of synthesized biomass. If our assertion is correct, then the...
most drastic changes in composition of biochemical reactions should be supported by significant relative changes of produced biomass, like the division phase.

The other candidate for the role of such a division trigger is often assumed to be the size of an organism. Figure 2 presents graphs of relative change of growth ratio and volume during the whole growth period for equal time intervals $\tau$, that is the values $(V(t + \tau) - V(t))/V(t)$ and $(G(t + \tau) - G(t))/G(t)$. We can see that relative changes of volume before the division are substantially smaller, about sixty times, than the relative changes of the growth ratio. Moreover, volume's relative change decreases, while in case of the growth ratio the relative change remains constant. Apparently, a triggering mechanism, which reacts to a greater and increasing parameter, will work better than a trigger reacting on a small and decreasing value.

![Figure 2. Relative change of volume and growth ratio for amoeba during the growth.](image)

So, the growth ratio in this competition for the cellular cycle control by far supersedes the organism's volume (which is equivalent to mass in our assumptions). Thus, this is not the size of an organism, which eventually triggers the division and enforces ordered changes of compositions of biochemical reactions through the growth cycle, but rather the change in the amount of produced biomass relative to the total nutrient influx. This arrangement is a little bit off the mainstream biological paradigm, but unlike other hypotheses, it explains all known facts about growth and reproduction phenomenon, without a single exception, and already allowed predicting new effects which later found experimental confirmations. Unlike the other hypotheses, it is supported by a mathematical apparatus,
which produces results very accurately corresponding to experiments. None of the other hypotheses about fundamental growth and reproduction mechanisms has this. So, despite the fact that the solution of this important problem resides rather in the area nobody was expecting it to be at, it should not be discarded on that ground. Important scientific breakthroughs, like the military ones, usually originate in unexpected directions.

A note about the constant value of the relative change of the growth ratio in Figure 2. This is a surprising result. Its mathematical proof is as follows. Let us substitute the value of $R$ as a function of $t$ from Eqn 15 into the expression for the growth ratio $G$ in Eqn 11.

$$G(t) = \frac{(R_0 / R_b - 1)H}{(R_0 + H) \exp(t / c_0)} = \frac{(R_0 / R_b - 1)H}{(R_0 + H)} \exp(-t / c_0)$$

(17)

So, the growth ratio as a function of growth time is an exponential function. Recall that the growth ratio is the ratio of the relative surface to the relative volume (Eqns 2 and 3) (minus one), and none of them, of course, contains exponents. Obtaining such an entirely unexpected result in the given circumstances rather means that we found some important new geometrical property of the real world (which is, in the first place, a geometrical one).

The first derivative of an exponential function is also an exponential function, so that the relative change of the growth ratio (its first derivative) is an exponent. However, for illustrative purposes, let us consider equal discrete time intervals $\tau$. Then, we can find the relative change of the growth ratio as follows.

$$\frac{G(t + \tau) - G(t)}{G(t)} = \exp(-\tau / c_0) - 1$$

(18)

So, for the equal time intervals the relative change of the growth ratio, indeed, remains constant. What is the meaning of this relationship in the real world? It is an interesting and a very natural one. It means that at equal time intervals the amount of nutrients that is diverted to biomass production is reduced by the same fraction from the ending amount of the previous time interval. There are many such natural processes defined by fundamental laws of Nature, like decaying electric current in certain electrical circuits (Shestopaloff, 2010c, 2011b). So, now we can say with certainty that the heuristic growth equation Eqn 1 and its main "wonder" the growth ratio are definitely associated with the realm of natural processes.
Amoeba’s metabolic properties

Once we know the total nutrient influx \( K(t) \) and the growth ratio \( G(t) \), we can find separately nutrient influxes for growth - \( K_g(t) \), and maintenance - \( K_m(t) \).

\[
K_g(t) = K(t)G(t) \quad \text{(19)}
\]
\[
K_m(t) = K(t)(1 - G(t)) \quad \text{(20)}
\]

Also, we can find nutrient influxes per unit of surface \( k_s(t) = K(t)/S(t) \) and per unit of volume \( k_v(t) = K(t)/V(t) \); accumulated amount of nutrients used for biomass synthesis \( M_g \), maintenance \( M_m \), and the total amount of consumed nutrients \( M_{tot} \) during the time period \((t_1, t)\). We will use units of measure for the influx \( pg \cdot min^{-1} \) (\( 1 pg = 10^{-12} g \)), except for amoeba, for which the unit of measure is \( \mu g \cdot min^{-1} \). Influx \( k_s(t) \) is measured in \( pg \cdot min^{-1} \cdot \mu m^{-2} \); \( k_v(t) \) in \( pg \cdot min^{-1} \cdot \mu m^{-3} \) (for amoeba accordingly \( \mu g \cdot min^{-1} \cdot \mu m^{-2} \) and \( \mu g \cdot min^{-1} \cdot \mu m^{-3} \)).

\[
M_g(t) = \int_{t_1}^{t} K_g(\tau)G(\tau)d\tau \quad \text{(21)}
\]
\[
M_m(t) = \int_{t_1}^{t} K_m(\tau)G(\tau)d\tau \quad \text{(22)}
\]
\[
M_{tot}(t) = \int_{t_1}^{t} K(\tau)G(\tau)d\tau \quad \text{(23)}
\]

Application of Eqns 19 - 23 to the growth curve in Figure 1 produces metabolic characteristics presented in Figure 3.
Figure 3. Nutrient influx and accumulated amount of nutrients for amoeba, depending on time. A - Specific nutrient influx $k_s(t)$ per unit of surface ($\mu g \cdot \text{min}^{-1} \cdot \mu m^{-2}$) and per unit of volume $k_v(t)$ ($\mu g \cdot \text{min}^{-1} \cdot \mu m^{-3}$). B - Accumulated amount of nutrients used for growth and maintenance, and the total amount, in $\mu g$.

Metabolic properties of studied organisms will be compared in Table 1. For now, note that (a) amoeba consumes about 28 times more nutrients for maintenance than for growth; (b) we can find amount of synthesized biomass directly, while finding this critical for biotechnological applications parameter, in particular by methods of metabolic flux analysis, is a big problem today.

**Amoeba's growth and division mechanism from the evolutionary perspective**

Geometrical form is an inherent property of any living organisms. Since the growth ratio is inherently tied to the geometrical form, the growth and division mechanism based on direct changes of growth ratio is probably the most ancient one. (We call it as the growth and division mechanism of the first type.) Its characteristic features are: (a) the growth proceeds almost through the entire possible growth period (corresponding to the growth curve described by the growth equation); (b) the rates of protein and RNAs synthesis are the same; (c) the value of the spare growth capacity is small, about 2%.

**Fission yeast S. pombe. Growth and division**

This model organism represents the second type of the growth and division scenario. An organism does not go through the whole possible growth cycle, but uses only the fastest part of the whole growth curve, switching to division much earlier. This enhancement substantively reduced reproduction period. Evolutionarily, such mechanism very likely was developed on top of more primitive ones, like the one studied in amoeba, since it requires a set of advanced features, which unlikely appeared simultaneously.

For illustration, we used experiment from (Baumgartner and Tolic-Norrelykke, 2009). Earlier, in (Shestopaloff, 2015), similar results were obtained for 85 experiments from the same work, for the temperatures of $32 \, ^\circ\text{C}$, $28 \, ^\circ\text{C}$ and $25 \, ^\circ\text{C}$, and also for
experimental graphs from (Sveiczer et al., 1996). So, the presented results can be considered as statistically meaningful.

We use the same geometrical model of \textit{S. pombe} as in (Shestopaloff, 2014b, 2015). This organism is modeled by a cylinder with a length $l$, radius $r$, with hemispheres at the ends; beginning length is $l_b$, ending length is $l_e$. In these notations, using Eqns 2 - 4, the relative surface, relative volume and the growth ratio can be found as follows.

$$R_{Sc} = \frac{(2r + l)}{(2r + l_e)}; \quad R_{yc} = \frac{((4/3)r + l)}{((4/3)r + l_e)}; \quad (24)$$

$$G_c = \frac{R_{Sc}}{R_{yc}} - 1 = \frac{((4/3)r + l_e)(2r + l)}{((4/3)r + l)(2r + l_e)} - 1 \quad (25)$$

where index 'c' denotes 'cylinder'.

We will also need the relative lengths' increases $L = l/l_b$ and $E = l_e/l_b$, and a relative radius's increase $R = r/l_b$. Then, the growth ratio from Eqn 25 can be written as follows.

$$G_c = \frac{(2/3)R(E - L)}{((4/3)R + L)(2R + E)} \quad (26)$$

The volume $V$ of a cylinder with hemispheres is $V = (4/3)\pi r^3 + \pi r^2 l$. The differential is $dV = \pi r^2 dl$. The nutrient influx is defined by Eqn 7.

Note that Eqn 26 uses the \textit{relative} length's increase for the cylindrical part of the organism, not for the \textit{whole} length. The rationale is that the cell's volume increases through the elongation of the \textit{cylindrical} part.

Substituting these parameters into Eqn 1, we obtain the following differential growth equation.

$$p \pi r^2 l_b dL = A(C_p L^2 + C_v L^3) \frac{(2/3)R(E - L)}{((4/3)R + L)(2R + E)} dt \quad (27)$$

The analytical solution of Eqn 27 was considered in (Shestopaloff, 2014b, 2015). It is as follows.

$$t = \frac{B}{A} \left( f \left( \frac{1}{L_b} - \frac{1}{L} \right) + d \ln \left( \frac{L}{L_b} \right) + g \ln \left( \frac{1 + CL}{1 + CL_b} \right) + h \ln \left( \frac{E - L_b}{E - L} \right) \right) \quad (28)$$

where

$$B = 3 p \pi R l_b^3 (2R + E) / (2C_p); \quad f = \frac{4R}{3E}; \quad d = \frac{1 + f - fCE}{E}; \quad h = \frac{d + fCE}{CE + 1}; \quad g = C(h - d) \quad (29)$$
Unlike in *amoeba*, the rate of RNA synthesis in *S. pombe* is about double of the rate of protein production (Shestopaloff, 2014c, 2015). This is why we obtained the cube of length in Eqn 26. It is often assumed (Baumgartner and Tolic-Norrelykke, 2009) that the double rate of RNA synthesis triggers after completing S phase, while before that the rates of protein and RNA synthesis are the same. In this case, \( K(L) = AL^2(C_p + C_r) \), and the solution of the growth equation is as follows (Shestopaloff, 2015).

\[
 t = \frac{B_S}{A_S} \left[ f_s \left( \frac{1}{L_0} - \frac{1}{L} \right) + d_s \ln \left( \frac{L}{L_0} \right) + g_s \ln \left( \frac{E-L_0}{E-L} \right) \right] 
\]  

(30)

where \( B_s = 3\pi \pi R^3_s (2R + E) / (2(C_p + C_r)) \); \( f_s = 4R/(3E) \); \( g_s = d_s = (1 + f)/E \).

Model's input parameters are listed in Table 1. A diameter and a fraction of nutrients used for RNA synthesis were estimated based on the fact of fast growth and large initial size of the considered species. Unfortunately, these parameters were not measured. As we can see from the Figure 4A, *S. pombe*, unlike *amoeba*, does not proceed through the whole possible growth cycle, defined by the full growth curve, but switches to the division phase at a maximum rate of biomass production, at inflection point. This significant evolutionary enhancement secures much faster growth.

The value of the spare growth capacity (SGC) for *S. pombe* is much greater than *amoeba*'s 2%, and resides in the range of 30-40%. However, knowledge of SGC for computing growth curves in case of *S. pombe* and similarly growing organisms (including *B. subtilis*, *E. coli*) is not required, since comparison with experimental data is based on the beginning of division, which coincides with the inflection point.

The amount of produced biomass remains the leading parameter which defines composition of biochemical reactions through the growth cycle. However, in *S. pombe*, it triggers the beginning of division phase at the inflection point of the growth curve.
Figure 4. *S. pombe*‘s growth’ and metabolic characteristics. A - Full growth curve for *S. pombe* versus experiment 1 from 32 °C dataset from (Baumgartner and Tolic-Norrelykke, 2009). Maximum of the first derivative of the growth curve corresponds to the beginning of division phase and inflection point of the growth curve. B - Change of the growth ratio, and the relative changes of the growth ratio versus the relative change of volume, for equal time intervals. C - Nutrient influx per unit of surface $k_s$ (measured in $\text{pg} \cdot \mu\text{m}^2 \cdot \text{min}^{-1}$) and per unit of volume $k_v$ (measured in $\text{pg} \cdot \mu\text{m}^3 \cdot \text{min}^{-1}$). D - Accumulated nutrients for maintenance, growth and the total amount, in $\text{pg}$. High value of SGC (and accordingly the possibility of continuing to grow beyond the inflection point) for *S. pombe* is not a mathematical ad hoc. Many cells can grow substantially bigger than their normal size, when the division is suppressed (Jorgensen and Tyers, 2004). For *S. pombe*, it was confirmed experimentally in (Baumgartner and Tolic-Norrelykke, 2009). Computations in (Shestopaloff, 2015) on the basis of Eqn 30 confirmed this too, and produced a growth curve similar to the experiments.
Metabolic properties of *S. pombe* were studied using Eqns 19-23. Figure 4B is presenting further evidence that the amount of produced biomass is that leading indicator which drives growth and division process of *S. pombe*. Indeed, we can see that the relative change of the growth ratio computed at equal time intervals is *substantially greater* than the relative change of volume (by 3.8 times at the division point). Also, the rate of change of the growth ratio quickly *increases* at the beginning of the division phase, while the relative change of volume *decreases*. So, it is very unlikely that changes in volume (or mass) could be the factor triggering the division.

Figure 4C shows change of nutrient influxes $k_s(t)$ and $k_v(t)$. Unlike in *amoeba* (see Figure 3A), the increase of these influxes accelerates all the time. This is also a factor contributing to fast reproduction. Figure 4D shows amount of accumulated nutrients for growth and maintenance, and the total amount of consumed nutrients. Note that maintenance requires about 18.2 times more nutrients than biomass production.

**Growth and division mechanism of the second type**

The considered second type of growth and division very much differs from *amoeba's* one. Both types of growth also have very different characteristics of population growth (Shestopaloff, 2013).

The following features are characteristic for the second type of growth: (a) species do not go through the entire possible growth cycle, but switch to division much earlier, at the inflection point of the growth curve; (b) the growth curve has a well expressed inflection point; (c) such species are elongated (the inflection point is better expressed for the elongated forms); (d) the rate of RNA synthesis is double the rate of protein synthesis (which is also a factor contributing to a better expression of an inflection point and faster growth).

Note that among all elongated forms a cylinder has the fastest growth time due to a higher value of the growth ratio (Shestopaloff, 2014b, 2015). This explains why so many elongated microorganisms are cylinder-like. The second fastest is a cone (recall a carrot.) Also, the cylinder form is less restrictive with regard to the maximum length, since the value of the growth ratio changes slowly towards the end of growth. Overall, all known
facts about growth of *S. pombe* and its cylindrical form are well explained by the general growth mechanism.

**Growth and division of *B. subtilis* and *E. coli***

*S. pombe, B. subtilis* and *E. coli* exercise the growth and division mechanisms of the second type. Nutrient influx is defined by Eqn 7, since, as it was previously discussed, *E. coli* has a double rate of RNA synthesis compared to protein synthesis. There are no such data for *B. subtilis*, but it can be assumed to be the same with very high probability, given the similarity of geometrical forms of *E. coli* and *B. subtilis* and their fast growth. Both factors, according to the results for *S. pombe*, strongly correlate with a double rate of RNA synthesis.

Figure 5A,B shows computed growth curves for *E. coli* (by Eqns 28 and 30) versus the two experimental data sets from (Reshes et al., 2008). Figure 5C shows a similar growth curve for *B. subtilis* versus the exponential data fit from (Godin et al., 2010). In all instances, we see a very good correspondence between the computed growth curves and experiments. Model's input parameters are listed in Table 1. The fraction of nutrients for RNA synthesis was estimated based on the rate of growth (the higher the rate of growth is, the greater this fraction) and the possible range of this value (0.035 to 0.246 for *E. coli*, according to (Maaloe and Kjeldgaard, 1966)). The diameter was estimated based on geometrical proportions of organisms.

It was suggested in (Reshes et al., 2008) that *E. coli*’s growth curve is approximated as a bilinear or tri-linear one. If we take into account rounding of the tip of a divided microbe in the first minutes of growth, which is the cause of faster length's increase at the very beginning, then, the computed growth curves actually correspond to experiments better than the authors' bi- and tri-linear approximations. The length's increase due to the tip rounding at the beginning of growth was proved in (Baumgartner and Tolic-Norrelykke, 2009), and later was confirmed in (Shestopaloff, 2015).

For *E. coli* and *B. subtilis*, metabolic properties (the graphs for nutrient influx and accumulated nutrients for growth and maintenance) are similar to ones for *S. pombe*, that is they are quickly increasing convex curves. For *Staphylococcus*, the appropriate metabolic curves resemble the concave curves for *amoeba*.
Figure 5. Computed growth curves for *E. coli* and *B. subtilis* versus experimental data, and the computed growth curve for *Staphylococcus*. Experimental data for *E. coli* are from (Reshes et al., 2008). For *B. subtilis* - from (Godin et al., 2010). A - The growth curve for *E. coli* vs. the data the authors suggested to model by a bilinear curve. B - The same for the data the authors suggested to model by a tri-linear curve. C - A computed growth curve for *B. subtilis* versus the experimental data. D - A computed growth curve and the growth ratio for *Staphylococcus*.

**Growth and division model of Staphylococcus**

*Staphylococcus's* growth was modeled by a growing sphere. The rate of RNA and protein synthesis was assumed to be the same. (Although, there is a possibility that the rate of RNA synthesis can be greater, similar, for instance, to *S. cerevisiae* (Elliot and McLaughlin, 1978)). Then, Eqn 9 transforms into

\[
K(v) = (C_p + C_r)v^{4/3}
\]

(index 's' denotes 'sphere').
The growth ratio can be found as follows.

\[
R_{ys} = \frac{R^2}{R_0^2}; \quad R_{ys} = \frac{R^3}{R_0^3}; \quad G_s = \frac{R_s}{R_0} - 1 = \frac{R_0 - R}{R}
\]  

(32)

where \( R_0 \) is the maximum possible (asymptotic) radius; \( R \) is the current radius.

Substituting \( G_s \) from Eqn 32 and nutrient influx from Eqn 31 into Eqn 1, we obtain the following equation.

\[
4p\pi dR = \frac{kR(R_0 - R)}{R_b^4} \frac{dt}{dR}
\]

(33)

where \( R_b \) is the beginning radius of the sphere.

Solution of Eqn 33 is as follows.

\[
t = \frac{4p\pi R_0^4}{kR_0^2} \ln \left( \frac{R(R_0 - R)}{R_b(R_0 - R)} \right)
\]

(34)

It is very similar to Eqn 14 for amoeba. Denoting \( c_{0s} = 4p\pi R_0^4/(kR_0) \), and solving Eqn 34 for radius \( R \), we obtain a similar generalized form of logistic equation as Eqn 15 for amoeba, with the same interesting properties.

Division mechanism of Staphylococcus is very likely of the first type, as in amoeba. The reasons for such a suggestion is that the growth curve of a sphere does not have a well expressed inflection point, or other specific features, which could serve as the checkmarks for starting an earlier division without going through the whole possible growth curve. We can see from Figure 4D that the growth ratio is much higher in Staphylococcus at the beginning of growth (a value of 0.27 versus 0.117 for S. pombe). This accordingly means a 2.3 times higher rate of biomass production at the beginning, which compensates for the slower growth at the end.

Discussion

Metabolic properties of cells. Allometric scaling

Table 1 presents the summary of metabolic properties of considered organisms and input parameters of their models.

In addition to ones earlier introduced, the following metabolic parameters were calculated: average and maximal metabolic rates per unit surface \( k_{Suv} \) and \( k_{Smax} \); average
and maximal metabolic rates per unit volume $k_{\text{rav}}$ and $k_{\text{rmax}}$; the total maximal, average and minimal nutrient influxes, accordingly $K_{\text{max}}$, $K_{\text{av}}$, $K_{\text{min}}$.

Table 1 considers nutrient influxes, but not the actual metabolic rates. On one hand, using nutrients has an advantage over the conventional methods, which may not account for all metabolic mechanisms. In particular: (a) it becomes possible to compare the consumed amount of food with the measured metabolic output; (b) knowing metabolic mechanisms of particular organisms, it is possible to transform the amount of consumed nutrients into the metabolic output. On the other hand, metabolic output for the same amount of nutrients can differ in different organisms. Besides, different types of nutrients could provide different metabolic outputs.

Table 1. Summary of metabolic properties of considered organisms and input parameters for their growth models.

|                   | B. subtilis | Staphylococcus | E. coli, 3-Linear | E. coli, 2-Linear | S. pombe | Amoeba |
|-------------------|-------------|----------------|------------------|------------------|---------|--------|
| $k_{\text{rav}}$, pg $\cdot \mu m^{-2} \cdot \min^{-1}$ | 0.083        | 0.135          | 0.1053           | 0.2194          | 0.0957  | 0.493  |
| $k_{\text{rmax}}$, pg $\cdot \mu m^{-2} \cdot \min^{-1}$ | 0.134        | 0.147          | 0.284            | 0.433            | 0.222   | 0.545  |
| $V_{\text{av}}$, pg $\cdot \mu m^{-3} \cdot \min^{-1}$ | 0.672        | 0.5336         | 1.0073           | 0.8933           | 0.0866  | 0.01215|
| $V_{\text{rmax}}$, pg $\cdot \mu m^{-3} \cdot \min^{-1}$ | 1.066        | 0.552          | 2.65             | 1.71             | 0.195   | 0.013  |
| $V_{\text{max}}$, $\mu m^{-3}$                | 0.617        | 2.145          | 3.999            | 16.975           | 325.4   | 1.88E+7|
| Diameter, $\mu m$                           | 0.536        | 0.446          | 1.09             | 5                |         |        |
| Diameter beg. $\mu m$                        | 1.27         |                |                  |                  | 285.7   |        |
| Diameter end, $\mu m$                        | 1.6          |                |                  |                  | 409.5   |        |
| Beginning length, $\mu m$                    | 1.608        | 1.003          | 2.39             | 10.1             |         |        |
| Ending length, $\mu m$                       | 2.915        | 3.999          | 4.957            | 18.24            |         |        |
| Asymptotic length, $\mu m$                   | 4.7          | 6.563          | 7.641            | 24.9             |         |        |
Note the large variations of nutrient influxes per unit of surface and per unit of volume between different organisms in Table 1.

Figure 6. Dependence of metabolic rates for the studied organisms on volume (mass) on a logarithmic scale. A - maximal metabolic rates for the entire cells; B - metabolic rates per unit of volume.

Figure 6A shows dependence of maximal metabolic rates for the studied organisms on volume (equivalent to mass in our case). On the graphs, the order of organisms is the
same as in Table 1. A line fits data for the maximal total nutrient influx reasonably well. A corresponding allometric exponent is 0.757. The obtained value of allometric exponent complies with available experimental data on allometric exponent for unicellular organisms, whose value can be in the range from 2/3 to more than one (Glazier, 2014, on p. 473). Work (Tang and Peters, 1995) confirms this range too.

Figure 6B shows dependence for the maximal metabolic rate per unit volume. It decreases when organisms' mass grows, but we can find no definitive relationships. Comparison of Figure 6A and Figure 6B strongly supports the idea that the allometric scaling is rather the property of the entire organism than its constituents.

**Conclusion**

We introduced a general growth mechanism, called the general growth law; developed growth models for unicellular organisms on its basis, and validated the models by available experimental data. Comparison of computed growth curves with experiments showed high models' adequacy. We also studied metabolic characteristics of considered organisms and discovered that the corresponding allometric exponent complies with experimental observations. These results confirm the validity of the general growth law.

How much can one trust the obtained results? The answer is, to the same extent as the general growth mechanism and its mathematical representation the growth equation. The general growth law, given the presented proofs, can be considered as a very likely valid one. Besides, there are many other proofs of its validity in earlier works.

It might seem embarrassing and elusive finding so many important characteristics from a single equation. However, this is how fundamental laws always work. What is needed to compute the trajectory of a thrown stone? Only the Newton's Second law.

There are many biological phenomena, which originate at a higher than biomolecular level. The work (Shestopaloff, 2016) gives an example of how the cell size matters for the metabolic properties of multicellular organisms; the result which cannot be derived from biomolecular mechanisms. In (Shestopaloff, 2014a; Shestopaloff and Sbalzarini, 2014) the adequate models of liver transplants' growth in dogs and humans, developed on the basis of the general growth law, allowed obtaining results, which *principally* cannot be derived on a biomolecular basis.
It takes time and efforts to understand the value of this really existing mechanism, which much defines the evolvement of living organisms and their constituents, proving that biological phenomena are driven not only by biomolecular machinery.

At some point, presently much fragmented biochemical mechanisms have to be at a systemic level. The general growth law is a good candidate for the role of such a unifying mechanism at all scale levels.

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