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SHORT COMMUNICATION

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Universality and individuality as complementary factors to optimize and reproduce cell populations

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Abstract Irreversible thermodynamics allows us to formulate the law of mass action for a large number of reactions running off in open systems. By applying it to cell populations, these ensembles are supposed to be installed by defined increments. Stationary ensembles are then predicted to be optimized in the sense of thermodynamics of irreversible processes. General relations for describing the cell size distribution or the intracellular length distribution of proteins are deduced. During cell growth and multiplication, the cell size distributions change systematically in the course of time; yet, they are all reproduced by only adjusting the standard energy. This phenomenon is considered to originate with process-dependent constraints according to irreversible thermodynamics characterized by hidden variables. In agreement with the theoretical demands, all the different realizations belong to the same universal cell size distribution. Moreover, the universal stationary cell size distribution is classified by a single parameter, p. It is then of great importance that the stationary size distributions of cells of bacteria, yeast and human melanocytes all belong to the $p = 3$ type, irrespective of the external conditions and of the individual chemical structure of the constituents. Universality also classifies the intracellular length distribution of proteins. The results discussed are enough to uncover as to how the genetically perfect production of the individual constituents and the thermodynamic optimization of the whole cell population are logistically coordinated.

Keywords Bacteria · Yeast · Melanocytes · Cell size distribution · Length distribution of proteins · Universality

Introduction

The genetic code guarantees that enzyme-activated reactions produce with outstanding accuracy the constituents of living systems. In this way, topological factors, like the shape and the internal properties of cells, are exactly predestined. Yet, growing cell populations as open systems have continuously to adapt themselves to the external conditions. Here, nature has developed ingenious logistics: depending on the external conditions many different metastable stationary states can be installed, de facto built up with the same individual units. The law of mass action formulated within the framework of irreversible thermodynamics allows us to identify these stationary states as optimized patterns with universal properties. The ensemble structures of stationary states adjusted under different external conditions belong to the same type. This is also true for different systems, like bacteria, yeast and human melanocytes. This universality traces back to general principles of thermodynamics. In this respect individual properties of chemical units are of secondary importance. With different genetic codes there

are thus boundless possibilities of generating perfectly organized, completely different cell ensembles that belong to the same class.

Essentials

Cell populations are open systems [1, 2, 3]. Under fixed external conditions cell ensembles approach rapidly stationary states via many enzyme-activated chemical reactions [4]. These states can be characterized by applying thermodynamics of irreversible processes [5, 6]. The course of each reaction is described by an extra variable, $\xi_k(t)$, often called a "hidden variable".

If biomass is relatively slowly exchanged the differential of the Gibbs energy at constant temperature T and pressure p may approximately be set equal to

$$(\mathrm{d}G)_{T,P} = -\sum_k A_r(T,p,\xi_r)\,\mathrm{d}\xi_r, \tag{1}$$

where

$$A_r = -\sum_r \nu_{kr}\mu_r. \tag{2}$$

The term on the right-hand side of Eq. (1) gives the differential contribution of the "chemical energy" of the many reactions that are running off. ν_{kr} is the stoichiometric coefficient of the particles k in the reaction r. μ_r is the chemical potential. Under stationary conditions the affinities $A_r[T,P,\xi_r(t)]$ are positive and constant. The affinity measures the "distance" from an adequate state of reference.

Under the given circumstances, stationary states are bound to the conditions

$$\begin{aligned} &A_k(T,p,\xi_k) = \text{constant} > 0\\ &\omega_k = \text{constant}\\ &\qquad\Downarrow\\ &\Psi = \sum_k \omega_k A_k = \text{constant} > 0. \end{aligned} \tag{3}$$

The dissipation function, Ψ, is invariant owing to the constant entropy production. $G(T,p,\xi_k)_{T,p},\xi_k$ characterizes a "metastable constrained stationary state" with optimum properties [1].

Let us introduce the set of elementary reactions

$$\sum_i \nu_i[m_i] \underset{\kappa'}{\overset{\kappa}{\rightleftarrows}} \sum_j \nu_j[m_j]. \tag{4}$$

where ν_i and ν_j are the stoichiometric coefficients and $[m_i]$ or $[m_j]$ indicates different reacting "molecules". κ and κ' are the rate constants of the reactions as indicated. The total reaction rate, w, related to the production rate of the newly formed entities ($\omega > 0$) has been shown to be given by [6]

$$w = \omega\{1 - \exp[-\beta A(\xi)]\}\ . \tag{5}$$

Hence, under stationary conditions [w = constant, ω = const, $A(\xi)$ = const], the mass of the constituents like the many intracellular proteins or the number of cells of the ensemble increases steadily.

Universality

The crucial point is now that the concentration of the constituents of stationary ensembles should be invariant, showing a unique and invariant cell size distribution while the mass increases. The generalized formulation of the law of mass action furnishes evidence of this claim [6, 7]. All that happens is that the reaction constant, K, is modified according to

$$K\exp[-\beta A(\xi)] = \prod_k x_k^{\nu_k}. \tag{6}$$

At any defined distance from the stationary state of reference [$\xi = \text{constant} > \xi_\infty$, $A(\xi) = \text{constant} > 0$] the concentrations x_k are fixed. Moreover, the type of the distribution is the same since the coefficients ν_k are not affected. According to Montroll et al. [8], these stationary distributions should be "maximum entropy patterns".

On the basis of the previous conception, the frequency of cells with y contacts (composed of $y_a = y + 1$ units), n_y/n_0, is deduced to be given by the Γ-distribution [7, 9]:

$$\begin{aligned} &\frac{n_y}{n_0} = y^p\exp(-y\Delta u_0) = \exp[-(y\Delta u_0 - k_B T p\ln y)],\\ &\frac{n_y}{n_0} = \exp\left(-\Delta f_{0y}\right) = \exp\left[-(y\Delta u_0 - T\Delta s_{0y})\right],\\ &\Delta s_{0y} = k_B p\ln y, \end{aligned} \tag{7}$$

with

$$\begin{aligned} &\Delta u_0 = \Delta u_0^*(\xi_\infty) + A(\xi),\\ &n_0 = \sum_y n_y\ ;\ \xi = \text{const}\ :\Delta u_0 = \text{const},\\ &y = y_{\text{tot}} - y_b\ ;\ \beta = \frac{1}{k_B T}. \end{aligned} \tag{8}$$

By calling the "cell birth-volume" y_b, the effective number of contacts between the units of a cell is written as $y = y_{\text{tot}} - y_b$. Δu_0 is the standard energy of these contacts that seems to correspond to an amino acid plus a "decent local environment". $\Delta u_0^*(\xi_\infty)$ is the contact energy in the stationary state of reference, $A(\xi_\infty) = 0$. It is a

crucial point that the standard energy of units Δu_0 (formed by different amino acids) seems to show the same value. Δu_y of a cell with y contacts can thus be cast into the "increment version" $\Delta u_y = y\Delta u_0$.

By rewriting Eq. (7) in terms of the variable η_y the universal distribution of the cell sizes of stationary populations comes out:

$$\frac{h(\eta_y)}{h_0} = \eta_y^p \exp(-\eta_y), \tag{9}$$

with

$$\eta_y = y\beta\Delta u_0 = y\beta\left[\Delta u_0^*(\xi_\infty) + A(\xi)\right]; \quad \xi = \text{const.} \tag{10}$$

h_0 is a normalization constant. Each value of p defines a "universal class" of stationary ensembles. $p > 0$ accounts for additional contributions to the ensemble entropy (see Eq. 7), in that way characterizing the entropy-relevant topology of the ensemble structure (isotropic ensemble composed of anisotropic cells, their anisotropy growing with the size: $p = 2$; constrained intracellular metastable configurations: $p = 1$).

All the different stationary states (different $\xi > \xi_\infty$) with the same p are equivalent, including different states of reference (ξ_∞). Cell ensembles have thus de facto unbounded possibilities to adjust to external conditions and to optimize themselves without losing their topological identity.

Results

Representative experiments are now described deliberately emphasizing individual and universal factors. The transmission electron microscopy (TEM) image of a population of *Escherichia coli* (prokaryote) [4] with rod-shaped cells is shown in Fig. 1a, while a TEM image of yeast (eukaryote) [4] cells with an ellipsoidal shape is shown in Fig. 1b. The enormous variance of the shape of the cells as a hallmark of individuality is demonstrated by the microscopic images of cultivated populations of human melanocytes [10] in Fig. 2 showing differently branched dendrites. The individual properties, very different intracellular structures included (e.g., bacteria are cells without a nucleus!), are genetically predestined; yet, these very different cell ensembles have common features. All of them exhibit, for example, a cell size distribution. This is qualitatively evidenced by the

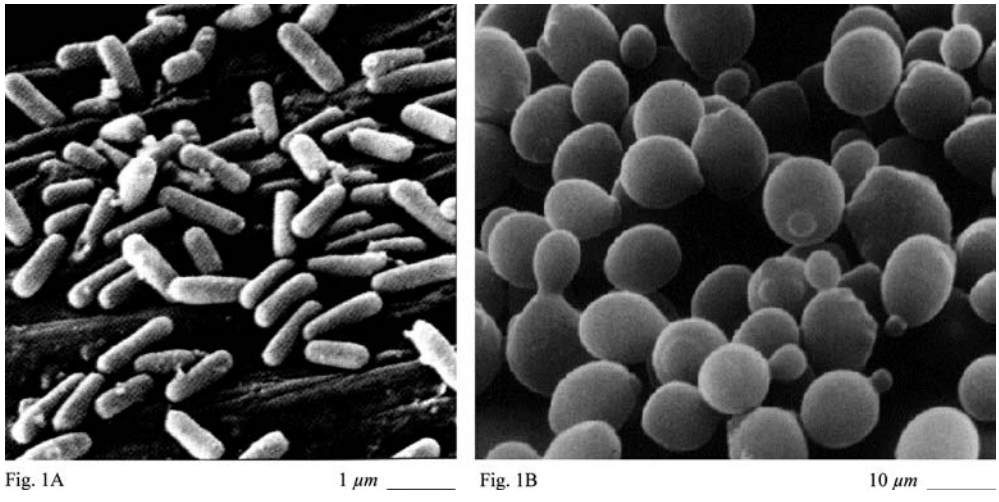

Fig. 1 **a** Transmission electron microscope (*TEM*) image of *Escherichia coli* [4]. **b** TEM image of yeast [4]

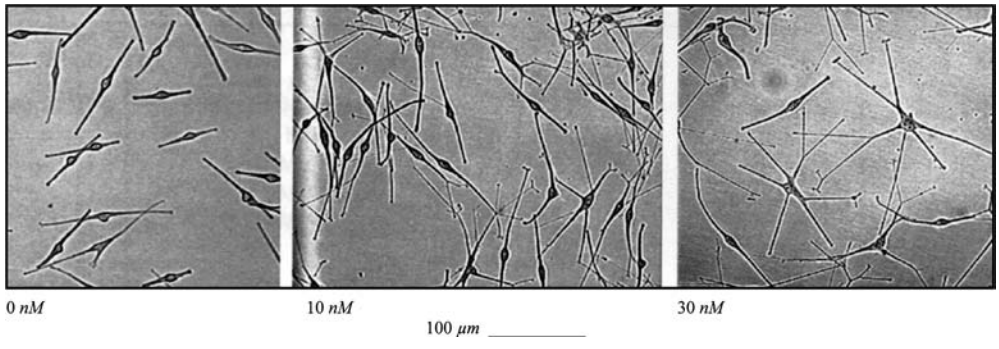

Fig. 2 Microscopic images of cultivated melanocyte populations with increasing concentrations of staurosporin (inhibitor protein kinase) [10]

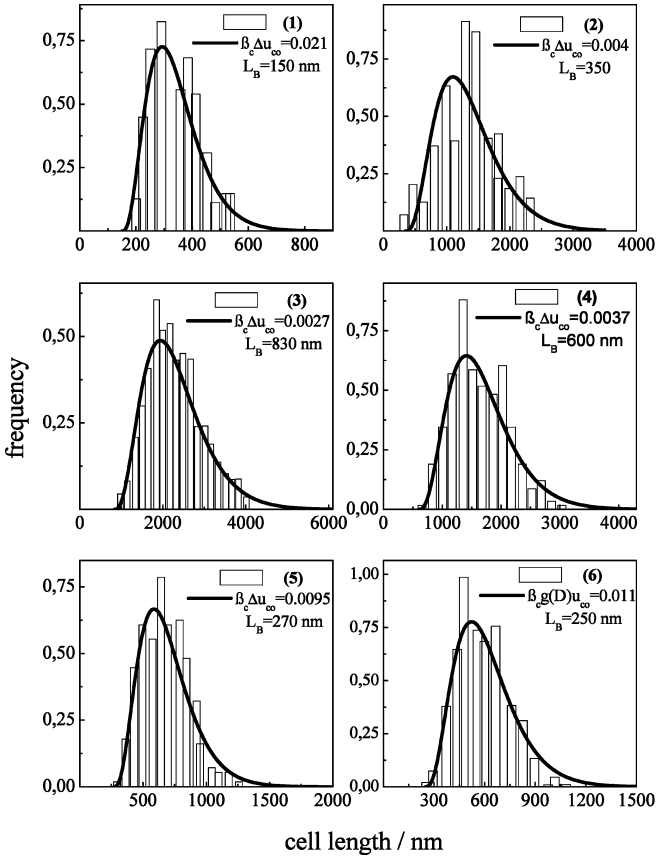

Fig. 3 Histograms of cell lengths of *E. coli* in different stages of a growing cell ensemble according to data from Refs. [7, 11]. The *solid lines* are computed with Eq. (7), $p = 3$ and the parameters indicated

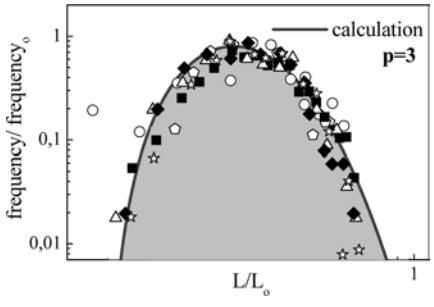

Fig. 4 Master curve of cell size distributions of the ensembles depicted in Fig. 3

image in Fig. 1a. In the following, the universal aspects of such common features are deliberately underlined as the unique global properties of stationary cell ensembles. The size distributions deduced in different stages of a growing cell population [11] are depicted in Fig. 3. They can be fitted with Eq. (7) (parameters are indicated). The different values of $\beta\Delta u_0(\xi_\infty)$ should indicate according to Eqs. (8) and (10) different distances from the stationary

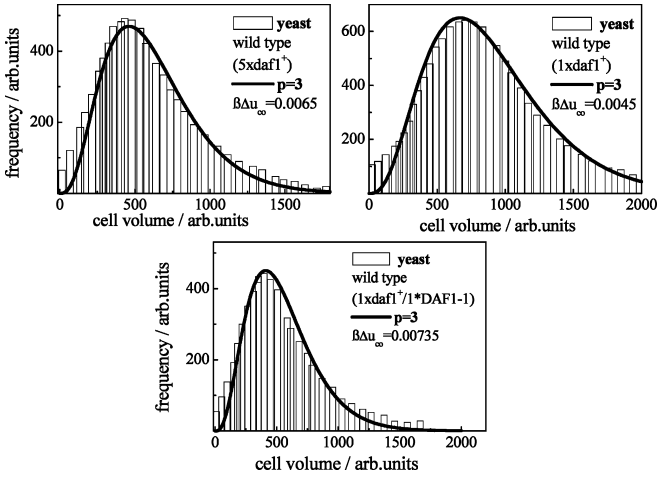

Fig. 5 Mutants of yeast according to Ref. [12]. The *solid lines* were computed with Eq. (7) and the parameters indicated [7]

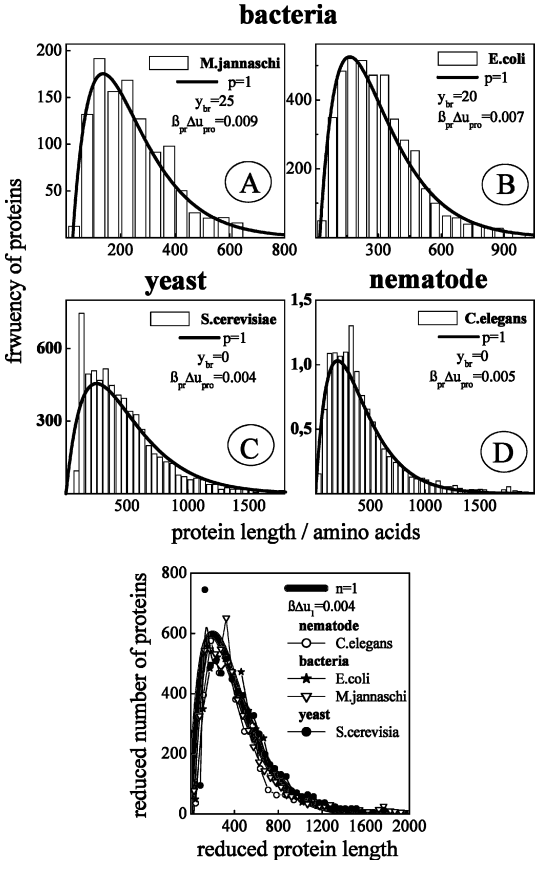

Fig. 6 Protein length distributions of living systems [13]. The *solid lines* were computed with Eq (7) and the parameters indicated [7]

state of reference. All these distributions belong, nevertheless, to the universal $p = 3$ class falling according to Eq. (9) on the master curve in Fig. 4.

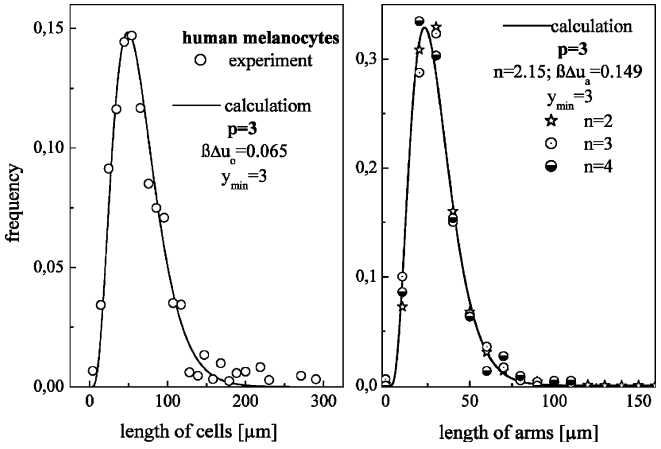

Fig. 7 Experimental data of a cultivated dispersion of human melanocytes [10]. The *solid lines* were computed with Eq. (7) and the parameters indicated

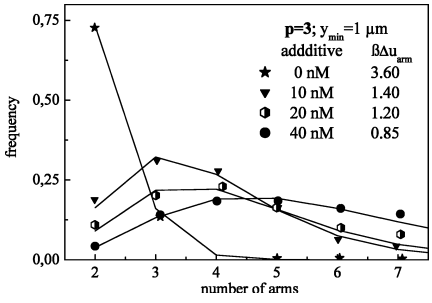

Fig. 8 The number of arms per cell in melanocyte dispersions at different concentrations of staurosporin [10]. The *solid lines* were computed with Eq. (7), $p = 3$ and the standard energies indicated

Our conception is also supported by reproducing the cell-length distributions of mutants of yeast [12] (eukaryotes). Compared with bacteria the shape of these cells (Fig. 1b) or the intracellular structure is completely different. Nevertheless, the topology of the ensemble structure is the same since the best fits to the data shown in Fig. 5 are arrived at with $p = 3$.

Keeping to the same principles, the length distribution of the intracellular proteins of bacteria, yeast and a nematode are all found to be an optimized pattern belonging to the $p = 1$ class [7, 13]. This is demonstrated by the plots in Fig. 6. The large number of intracellular proteins seem, in any case, to be bound to or to built into an intracellular scaffold [4] so as to confine the conformational entropy analogously. The presence of a nucleus and the endo-membrane system do not seem not to reduce the possibilities of optimizing the collective intracellular properties of eucaryote cells [7]. Moreover, the analogous intracellular organization might also contribute to securing defined functions of each cell.

We were astonished to observe that cell size distribution of layers of melanocytes [10, 14] belong to the $p = 3$ class (Fig. 7). For two-dimensional cell populations with an invariant shape of the anisotropic cells (their anisotropy growing with the size) p should have a value of 2. $p = 3$ must be related to the extra ensemble entropy originating from the fluctuations of the shape of the cells. The length as well as the length distributions of the arms are observed to be the same for all the differently branched dendrites as can be seen by the plot on the right-hand side of Fig. 7.

Moreover, the nearly perfect fits to the discrete frequency distribution of differently branched dendrites shown in Fig. 8 are arrived at by using Eq. (7), really proving that the frequency of cells with different numbers of arms fits to an entropy-maximized stationary pattern. Defined variations are enforced with different concentrations of the staurosporin, demonstrating in this way that the optimized stationary ensemble structure can be "triggered from outside". This elucidates one of the many possibilities cell ensembles have to install stationary states under varying external conditions. The topological identity of cell ensembles abstractly defined on a "global level" is the same for the differently branched melanocyte populations. They occupy optimized patterns of the same class ($p = 3$). It is not too surprising that the degree of branching decreases when the concentration of the staurosporin, for example, is reduced [10].

Final comments

These results illustrate the very different features cell ensembles may have. The genetic code guarantees the individual profile of each one and controls this during every cell cycle (not considering mutations). From the plots depicted in Fig. 6, the stationary distributions of the lengths of the proteins are identified via the law of mass action and the resulting relations (Eq. 7 or Eq. 9) to be universal $p = 1$ patterns irrespective of "individual factors". This is analogously true for the cell size distribution of the cell ensembles ($p = 3$) (Figs. 4, 5, 7). The power of irreversible thermodynamics is evidenced.

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