

Exponential Distribution of Locomotion Activity in Cell Cultures

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In vitro velocities of several cell types have been measured using computer controlled video microscopy, which allowed the recording of the cells' trajectories over several days. On the basis of our large data sets we show that the locomotion activity displays a *universal* exponential distribution. Thus, motion resulting from complex cellular processes can be well described by an unexpected, but very simple, distribution function. A simple phenomenological model based on the interaction of various cellular processes and finite ATP production rate is proposed to explain these experimental results. [S0031-9007(98)07256-1]

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Tissue cell migration [1] has been studied in a number of biological processes, including the development of the nervous system [2], immune reactions [3], and tumor spreading [4]. In addition to the inherent biological importance, cell locomotion plays a key role in a number of interesting large scale pattern formation phenomena such as cell sorting [5–7] or aggregation of amoebae [8]. Although the behavior of swimming bacteria has been well studied [9] and served as a basis to understand collective microbiological phenomena [10], rather little is known about the basic statistical features of eukaryotic cell migration on time scales comparable to the time of the pattern formation processes.

The locomotion of cells emerges from parallel cyclic subprocesses including cell attachment to and detachment off the substrate, growth and collapse of filopodia/lamellopodia, the dynamic maintenance of appropriate cell surface composition, and displacement of the cell mass. This complexity suggests two consequences. On one hand, strong correlation cannot be expected between the actual cell velocity and the concentration of a single (or a few) cell biological regulatory factor(s) [11]. On the other hand, interacting complex units often results in a characteristic macroscopic behavior which can be understood by mathematical models [5–8,10], thus some sort of statistical regularities might be expected in the cellular behavior as well.

In order to obtain data providing a basis for an extensive statistical analysis, we have developed a computer controlled system which automatically collects data and maintains the cell culture conditions in a chamber attached to the microscope for several days. We found that the locomotion activity displays an *exponential distribution*; i.e., in spite of the complex cellular processes, the motion of a cell can be well described by a simple universal distribution function. This observation can be explained in terms of a phenomenological model based on the competition of various cellular processes for the finite free energy [adenosine 5'-triphosphate (ATP)] resources.

With the aim of applying statistical approaches in the study of cellular locomotory activity, long-term cell mi-

gration patterns were recorded in low-density monolayer cultures (see Table I). The cellular trajectories (Fig. 1) were tracked using a computer controlled phase contrast videomicroscope system. Because of a high degree of automatization, we could collect and analyze a large set of data (about 100 000 cell positions), significantly exceeding the amount of information evaluated in previous works on cell motility [2,6,7,15–19].

Average velocities were calculated over a time period of one hour (t_0) as $v_i(t) = |\vec{x}_i(t + t_0/2) - \vec{x}_i(t - t_0/2)|/t_0$, where the location of the i th cell at time t is denoted by $\vec{x}_i(t)$. Remarkable fluctuations were found in the locomotion activity of individual cells, a phenomenon which has also been reported in living slices of developing cortex [2], *in vitro* migration of neurons [20], and fibroblasts [15]. The characteristic time scale of the fluctuations was estimated to be a few hours, a value similar to that reported for fibroblasts [15].

Because of the low cell density, most cells migrated freely, but the effects of various cell interactions could be also investigated. Since it is known that high cell density can significantly decrease the cell locomotion activity [21], we investigated whether the observed velocity fluctuations were caused by changes in local intercellular connections. Cell to cell contacts were identified on the images and the locomotion velocities $v_i(t)$ were sorted into two sets (“solitary”/“interacting”) depending on whether the cell in question had or had not visually observable contacts with other cells. Cumulative velocity distribution functions $F(v)$ were calculated for time periods of 20 hours giving the probability of the relation $v_i(t) < v$ to hold for a randomly selected cell i and a random time t . Comparing $F(v)$ of solitary cells to that of interacting cells (Fig. 2a), two almost overlapping ranges of velocities (0–80 and 0–100 $\mu\text{m/h}$, respectively) were found. It is also known [1] that during migration cells leave various extracellular molecules and other polypeptides attached to the substrates. To elucidate the effect of these cell trails on the velocity fluctuations, we filtered our database in such a way that we kept only those cell positions separated by

TABLE I. The most important features of the cultures investigated.

Culture	Ref.	Cell density ^a [cells/mm ²]	Duration [h]	Positions	$\langle v_i(t) \rangle$ [$\mu\text{m/h}$]
Mouse neuroepithelial progenitor (NE-4C)	[12]	20, 40, 200	24, 24, 20	2000, 4000, 12 500	6.7, 8.7, 17
Mouse fibroblast (NIH-3T3)	ATCC ^b	170	20	5000	12.1
Human glioblastoma (HA)	OITI ^c	48, 65	50, 50	13 500, 19 500	11.1, 11.6
Human glioblastoma (HC)	OITI	126, 80	50, 50	37 800, 24 000	6.5, 7.0
Human breast adenocarcinoma (MDA MB231)	ATCC, [13]	40	24	2500	5
Rat primary astroglia	[14]	200 ^d	50, 50, 36, 30	3500, 3500, 3000, 2500	8

^aAt seeding; ^bAmerican Type Culture Collection; ^cNational Institute of Neurosurgery, Hungary; ^dMigration from cell aggregates.

more than 20 μm (i.e., approximately twice the typical cell size) of the trajectory of other cells. Again, no qualitative difference was found in the velocity fluctuations suggesting that they are an *inherent property of the cells*.

One of our main findings is that $F(v)$ of all cultures investigated could be very well fitted by an exponential distribution

$$F(v) = 1 - e^{-\alpha v} \quad (1)$$

within measurement errors in the *entire* velocity range studied (Fig. 2b). This result indicates that the exponential velocity distribution is likely to be a general characteristic feature of *in vitro* cell motility for a broad class of surface attached cells.

The above velocity distributions were found for relatively large (≈ 100 cells) populations. On the level of individual cells, the exponential behavior (1) can be interpreted in two ways: (i) The culture is inhomogeneous; i.e., slower and faster cells can be distinguished on the bases of well preserved phenotypic properties. In this case the exponential $F(v)$ distribution can reflect the ra-

tio of the slow and fast cells in the culture, while the velocity fluctuations of the individual cells can show an arbitrary distribution. (ii) If the culture is homogeneous, then almost all cells exhibit the same distribution of velocity fluctuations; i.e., $F_i(v) \approx F(v)$ holds for each cell i , where $F_i(v)$ denotes the distribution function of the cell i . In this scenario the average velocity $\langle v_i(t) \rangle$ of each cell would be the same if we could calculate the time averages over an infinitely long time. Since the time averages are calculated over a finite time τ only, for the distribution of the average velocities we can expect a gamma distribution with a parameter $s = \tau/t_0$, where the correlation time of the process is denoted by t_0 [22].

To decide between the above alternatives, $F_i(v)$ and the average velocity $\langle v_i(t) \rangle$ over a period of $\tau = 16$ h were calculated for each cell i . Figures 3a and 3b show that the experimental data clearly support the homogeneous alternative. As an example, the data shown in Fig. 3b can be well fitted by a normalized gamma distribution $F_\Gamma(u, s) = \int_0^u dz s^s z^{s-1} e^{-sz} / (s-1)!$ with a parameter value of $s = 9$. The fitted value of s is consistent with the finding that the correlation time scale of the velocity fluctuations is in the order of hours.

The fact that the distribution of cellular velocities follows a *simple* exponential function is an unexpected finding. Several subprocesses of cell locomotion (receptor binding, membrane exocytosis, lamellopodium/filopodium formation, etc.) display either Poisson distribution [18] or periodic behavior [23] if studied on time scales short enough to exclude nonstationarities of the cell state. However, the fluctuations of cell velocities recorded in our experiments were observed on significantly longer time scales than those listed above. Since in a few hours many lamellopodia are formed and many vesicles are fused to the membrane, etc., the relative frequency fluctuation of such stationary processes must be small and close to Gaussian. In agreement with this expectation, previous works on cell locomotion—although not calculating $F(v)$ explicitly—have set up phenomenological models predicting Gaussian velocity distribution [15,16,18]. Also, a close to Gaussian distribution was found in [6], where motion within a cell aggregate was studied. In that case,

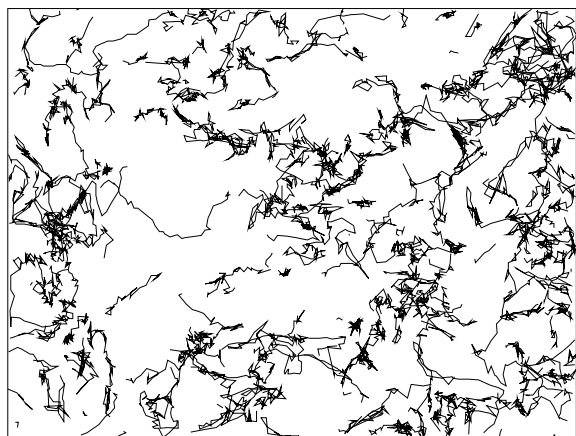


FIG. 1. Cellular trajectories of NE-4C cells in a field of $1110 \mu\text{m} \times 840 \mu\text{m}$, during the first 20 hours in culture. Microscopic images were taken with $10\times$ objective magnification every 3–4 min. The cells in each third image were tracked manually with a precision of about $5 \mu\text{m}$, which is comparable with the size of the cells ($10 \mu\text{m}$).

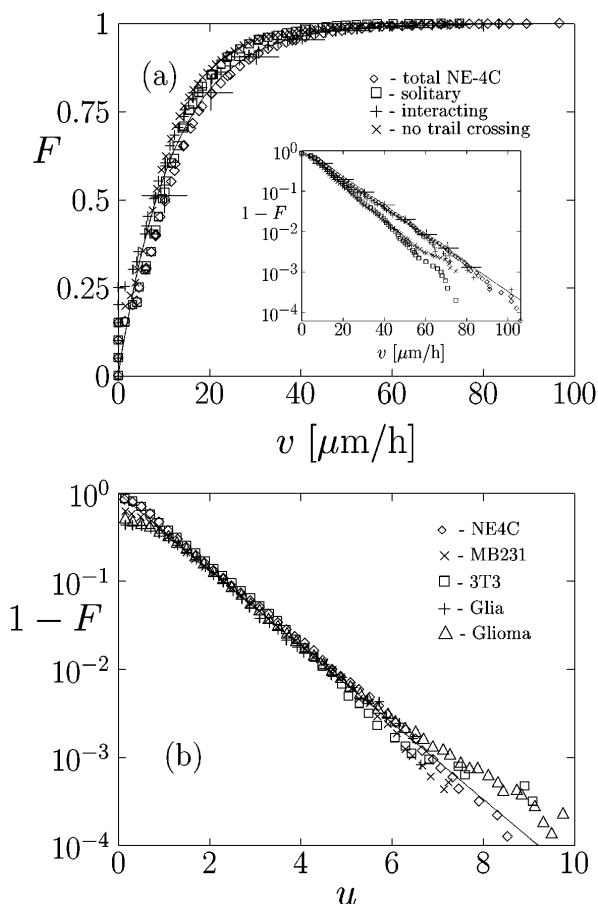


FIG. 2. (a) Cumulative velocity distribution functions $F(v)$ calculated from the trajectories of Fig. 1 for three subsets of cells: interacting, solitary, and cells which do not cross the trails left on the substrate (see text for details). The similarity of the curves indicates that the fluctuations are an inherent property of the cells. The horizontal bars correspond to the error of velocity determination due mainly to the method of cell positioning. The vertical bars mark the systematic error ($\approx 5\%$) of $F(v)$, which estimation was based on the difference between the distribution functions calculated for the first and the second 10 hours of the record. The solid line is a fitted exponential distribution function. The inset shows the same distribution functions on a linear-logarithmic plot. (b) The velocities $u = v/\langle v_i(t) \rangle$ normalized by the average velocity of the population show an exponential distribution in all of the 13 cultures investigated, demonstrated by the linear region covering 4 decades in the linear-logarithmic plot. The figure shows a typical result for each cell line.

however, there was a strong interaction between the cells and the displacement of a single cell was also a result of the activity of the neighboring cells.

Within the time scale of the velocity fluctuations both the cellular environment and the pattern of gene expression in the cells can be considered as stationary. This time, on the other hand, is long enough to permit changes in cell motility as a consequence of changes in the concentration of several intracellular regulatory factors. In the following, we demonstrate with a very simple cell model that the

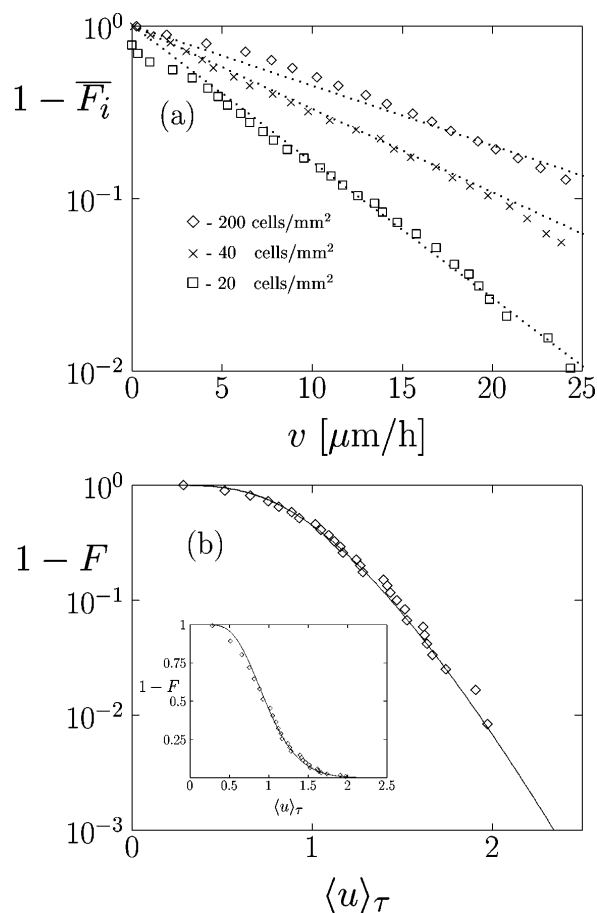


FIG. 3. (a) The average function of the single-cell velocity distributions $\overline{F}_i(v) = \sum_{i=1}^n F_i(v)/n$ shows the same exponential behavior as the cumulative distribution function $F(v)$ of the whole cell population of NE-4C cells. The dotted lines are an exponential fit of $F(v)$ in the corresponding velocity regime. (b) The distribution function of the normalized average cellular velocities $\langle u_i \rangle_\tau = \int_0^\tau v_i(t) dt / (\tau \langle v_i(t) \rangle)$ is presented, both as linear and linear-log plots. The solid line is a fitted gamma distribution.

exponential distribution of cell velocity fluctuations can indeed reflect such stochastic intracellular changes.

Since the cell locomotion processes are cyclic and dissipative, the observed velocity is proportional to the amount J_0 of free energy dissipated (number of ATP molecules hydrolyzed) in the appropriate chemical reactions contributing to cell motility:

$$v(t) = \mu J_0(t), \quad (2)$$

where μ denotes the conversion factor between velocity and energy dissipation. It is natural to assume that μ is constant; i.e., the subprocesses are synchronized in such a manner that a close to optimal distribution of the energy inflow is maintained.

The individual reaction rates are determined on one hand by the ATP concentration c and on the other hand by the concentrations of various regulatory factors. Some

of these factors maintain the motility efficiency μ , while other factors are products of different cellular processes, with the potential of altering the cell locomotion activity. These latter factors can change *independently* from each other: while some of the changes increase, some others decrease the cell motility. Thus, we assume that the temporal changes of J_0 can be represented as

$$\frac{dJ_0}{dt} = g_0 \frac{dc}{dt} + \xi_0, \quad (3)$$

where $g_0(t) > 0$ and $\xi_0(t)$ is a stochastic variable with zero mean and Gaussian distribution.

Finally, we assume that the cellular ATP production rate P is limited by the ATP production capacity of the cell, so the various cellular processes (among them cell locomotion, protein synthesis, DNA duplication, etc.) compete for the ATP available. Since the changes of c can be calculated as a difference in the production and the consumption rates, the equations

$$\frac{dJ_\ell}{dt} = g_\ell \left(P - \sum_{k=0}^N J_k \right) + \xi_\ell \quad \ell = 0, 1, \dots, N \quad (4)$$

are obtained, where J_ℓ denotes the rate of free energy dissipation in a given cellular process ℓ , while g_ℓ and ξ_ℓ are analog quantities to g_0 and ξ_0 , respectively.

It can be seen that Eqs. (4) describe a diffusion process in the vicinity of the $\sum_{k=0}^N J_k = P$ hyperplane. Accordingly, the probability distribution of the "cell state" $\vec{J} = (J_0, J_1, \dots, J_N)$ on this hyperplane is uniform. Integrating the distribution of \vec{J} and taking into account Eq. (2), we obtain the experimentally observed exponential behavior

$$F(v) = 1 - \left(1 - \frac{v}{\mu P} \right)^N \approx 1 - e^{-v/\mu p}, \quad (5)$$

where $p = P/(N + 1)$ is the average free energy consumption rate of a given cellular process.

There is a formal similarity between the distribution given by Eq. (5) and the Boltzmann energy distribution within systems in thermal equilibrium. In the latter case, the uniform microcanonical distribution can be derived using either quantum mechanics or Liouville's theorem for Hamiltonian systems while here it is an interesting feature of the interacting regulatory processes.

Although Eq. (4) is certainly oversimplified and the free energy dissipation rates J_ℓ are not identified with actual biochemical reactions, the exponential behavior of Eq. (5) is expected to hold for a wide class of models (e.g., nonconstant P , considering explicitly the interactions of regulatory networks, etc.). Our simple calculation also predicts that exponentially distributed fluctuations can be expected in numerous other cellular activities as well.

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