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Anomalous diffusion and non-Gaussian velocity distribution of *Hydra* cells in cellular aggregates

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Abstract

We study the center of mass motion of single endodermal *Hydra* cells in two kinds of cellular aggregates: endodermal and ectodermal. The mean square displacement displays anomalous super-diffusion with $\langle x^2\rangle \sim t^{\alpha}$ where $\alpha > 1$. The velocity distribution function is non-Gaussian and fits well the q-distribution function of velocities within the framework of the non-extensive thermostatistics proposed by Tsallis. Our results indicate that cell motion in two-dimensional cellular aggregates can be described by a "correlated-type" anomalous diffusion. © 2001 Published by Elsevier Science B.V.

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1. Introduction

Morphogenesis and wound healing in multicellular organisms require movement of individual cells or tissue masses from one part of the body to another. Cell migration also plays a significant role in many pattern formation phenomena like cell-sorting, tissue engulfment, aggregation of amoeba and collective motion of bacteria.

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Previous studies of cell locomotion of eukaryotic cells have focused on the dynamics of single cells or groups of cells on adhesive substrates. Typically, these studies employ non-interacting cells in well-defined surroundings. Most earlier work on cell motion has observed normal diffusive motion [1,2] and Gaussian velocity distributions. Recently, Czirok et al. [3] found exponential velocity distributions for non-interacting cells on substrates.

Cell motion is driven by protrusions that probe the local environment. These are due to the polymerization–deploymerization of cytoskeletal actin filaments near the leading edge of the cell membrane. How a cell moves may depend strongly on what kind of environment it is in. During *in vitro* cell-sorting or *in vivo* cellular reorganization during development [4], cells directly contact other cells of the same or different types. Cells moving in a compact aggregate of other cells interact strongly with one another and each cell's movement depends on the local environment within the tissue. Many factors, including adhesion (due to adhesion molecules on the surface of cells), internal cytoskeletal dynamics, elasticity of the cell membrane and collective motion due to the close packed nature of the aggregates, all affect cell motion. A detailed study of the dynamics of cell motion within a surrounding tissue is lacking and several questions are unanswered. Little is known about the statistical mechanics of single cell motion in cellular aggregates of different types. Does the type of surroundings qualitatively affect the nature of the observed statistics or does it only quantitatively alter specific parameter values? In this paper, we have attempted to obtain a complete physical description (dynamics and thermodynamics) of cell motion within aggregates.

We study the statistical properties of the two-dimensional motion of single endodermal *Hydra* cells in two different aggregate types—endodermal and ectodermal. As our experimental system, we use the freshwater polyp *Hydra viridissima*—a simple organism consisting of only two main cell types. *Hydra* is also a good model for development in general, as a dissociated and reconstituted mixture of *Hydra* tissues eventually regenerates to form a functional animal. A *Hydra* consists of a cylindrical body column with two layers of cells (inner—endoderm and outer—ectoderm) separated by extracellular matrix—the mesoglea which holds the two layers together. Previous experiments with *Hydra* tissues have shown that, from initial random mixtures of the two tissues, endodermal cells sort to the center of the aggregates and ectodermal cells form a surrounding layer [5]. Further, if an endodermal aggregate is placed in contact with an ectodermal aggregate, the ectoderm will engulf the endoderm. Indirect measurement of relative surface adhesivity show that the adhesion energies are in the order $E(\text{endo–endo}) > E(\text{endo–ecto}) > E(\text{ecto–ecto})$ [5]. Here, the endo–endo contacts are the most cohesive. Rieu et al. tracked cell motion in *Hydra* aggregates during sorting of the two cell types in randomly mixed aggregates [6]. Our simpler experiment analyzes the motion of single cells in non-sorting environments to elucidate the mechanism of cell motion independent of sorting.

2. Experimental methods

We cultured the *Hydra* at 18°C in *Hydra* medium [7], fed them five times a week with *Artemia naupli* shrimp and starved them for 24–36 h before experiments. We dissociated the *Hydra* into single cells using a standard protocol and formed aggregates by pelleting [6]. After removing the heads and feet from a group of 8–10 animals, we separated the inner and outer layers of the body column using Procaine-HCl [8]. We dissociated the endoderm and ectoderm separately, then filtered the cell suspension using a 53 μm nylon mesh and centrifuged it at 250 g for 5 min to sediment and collect the epithelial cell pellets.

We observed two situations: (A) endodermal cells in an endodermal aggregate and (B) endodermal cells in an ectodermal aggregate. For case (A), we used pure endodermal aggregates and for case (B), we mixed a small percentage of endodermal cells into ectodermal aggregates. We cut the pellets into fragments about 1 mm in diameter and clamped them between cover-glass pairs with 25 μm width spacers to form essentially two-dimensional aggregates. We placed the cover-glass assembly in a petri dish containing culture medium and observed it with a confocal microscope (Olympus 1×70(KrAr-SP1), Japan). *Hydra viridissima* shows good contrast between unstained endodermal and ectodermal cells due to the auto-fluorescence of endodermal cells. We imaged the endodermal cells in the aggregate interior to track the cell center-of-mass and membrane fluctuations. As only about 50% of the endodermal cells fluoresce, we could also study pure endodermal aggregates.

We recorded the two-dimensional motion of several sets of cells for both the cases (A) and (B). The magnification was adjusted to obtain a set of 15–30 cells in one field of view. A confocal microscope took digitized images directly at intervals of 30 s. There is almost no auto-fluorescence in the ectodermal cells or the non-fluorescent population of endodermal cells, thereby making the background quite dark and producing very good contrast. We also took transmitted light images where all the cells are visible in order to visualize the general movement patterns in a particular part of the aggregate. We analyzed the images using NIH-Image, FORTRAN and MATLAB software to obtain the time series of the center of mass of each fluorescently labeled cell and used these coordinates to calculate several relevant statistics.

3. Results

From the time-lapse transmitted light images of all cells in a particular field of view, it is evident that several neighboring cells in parts of the aggregate display highly correlated motion. Qualitatively, we can see this correlation as regions of the aggregate moving together collectively. Cell motion within different types of aggregates, in the presence or absence of collective modes, has never been quantified before. In the following, we present our detailed analysis of the statistical properties of the observed motion.

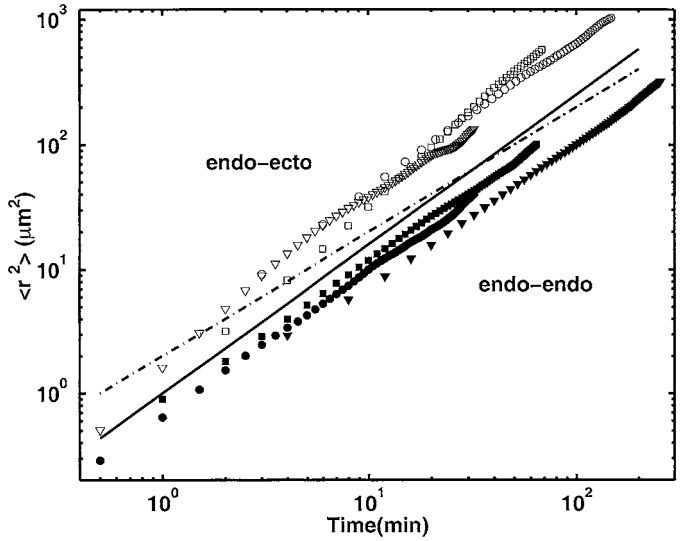

Fig. 1. $\langle r^2 \rangle$ vs. t plot for endodermal cells in an endodermal aggregate (filled symbols) and endodermal cells in an ectodermal aggregates (open symbols). The solid line has a slope of 1.2 while the dashed line has a slope of 1.0 (which would represent normal diffusion).

For each experiment, we averaged the cell displacements, $\langle r^2(t) \rangle = \langle (x(t_0 + t) - x(t_0))^2 + (y(t_0 + t) - y(t_0))^2 \rangle$, over all cells in the set (typically 15–30). The mean squared displacement (or variance) is given by

$$\langle r^2(t) \rangle = Dt^{\alpha} , \tag{1}$$

where D is an effective diffusivity; and α is an exponent which can be greater than, less than or equal to one. Previous studies of cell motion on substrates [1] or cell motion in three-dimensional aggregates [2] found normal diffusive behavior ($\alpha = 1$). In our experiments, we obtain $\alpha = 1.23 \pm 0.1$. α is always greater than or close to 1, indicating that these cells undergo normal to super-diffusive motion. In experiments with α close to 1, cells execute a biased random walk because the drift velocity $V = \langle r \rangle / t$ is non-zero. In fact, the drift is very small compared to $\sqrt{\langle r \rangle^2}$ (less than 10%). Fig. 1 shows the variance of displacement as a function of time, $\langle r^2 \rangle$ vs. t, for sample experiments. These plots show that the effective diffusion constant (y-axis intercept) of the endo–endo (ed) case is smaller than the endo–ecto (ec) case. The endodermal cells have higher root mean squared speeds in an ectodermal aggregate than in an endodermal aggregate, $V_{ec} \sim 100\,\mu\text{m/h}$, $V_{ed} \sim 60\,\mu\text{m/h}$, consistent with our intuition that cells will move faster in a less cohesive environment. Endo–endo cell contacts are more adhesive than endo–ecto contacts, increasing the effective viscosity of the cellular medium traversed by the endodermal cell. Recent experiments by Forgacs et al. [9] have shown that an aggregate of cells behaves like a viscoelastic medium. More adhesive cells show a higher effective viscosity, consistent with our observations.

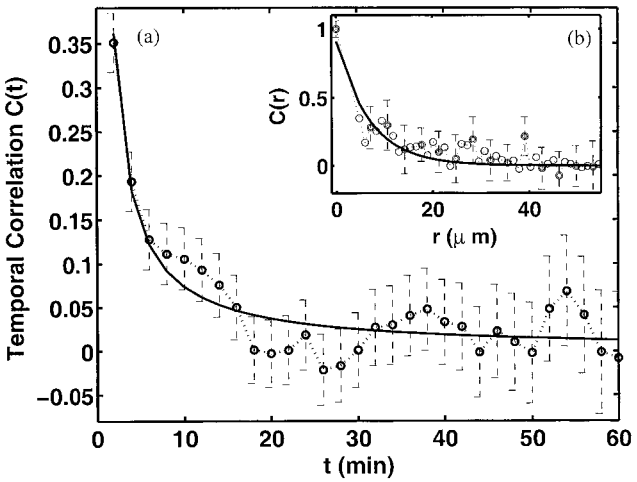

Fig. 2. (a) Temporal auto-correlation function, $C(t)$, of the cell velocities, as a function of time interval (t). The solid line is a fit to a power law. (b) Average spatial correlation function, $C(r)$, of the cell velocities as a function of the distance (r) between cell pairs. The solid line is a fit to an exponential function.

Examples of anomalous diffusion can be found in diffusion of tracers in turbulent fluids [10], diffusion of micelles [11], surface growth and diffusion in porous media [12]. The anomalous exponent can either be $\alpha<1$, corresponding to *sub-diffusion*, or $\alpha>1$, which corresponds to *super-diffusion*. Super-diffusion (if $\alpha>1$, as in our experiments) can be of two kinds: Levy-type (broad distributions with diverging first or second moment) and correlated type (due to long-range correlations). Anomalous diffusion can be induced by temporal or spatial correlations [13]. If the correlations decay more rapidly than t^{-1}, they do not affect the dynamics, diffusion is normal. On the other hand, if the correlations scale as t^{-k} with $k<1$, they modify the typical behavior of the variance, enhancing diffusion. In our experiments, we calculated the temporal auto-correlation functions of the velocities, $C(t)$, and fit it to a power law. For large t, we observe $C(t)\sim t^{-k}$ with $k\sim 0.9$, suggesting that temporal correlations could be the cause of anomalous diffusion. Fig. 2(a) shows the data for temporal correlations with a power-law fit. Spatial correlations can also change the dynamics. For space dimensions, d, larger than 2, only long range correlations give rise to anomalous diffusion. But if $d\leqslant 2$, even weak correlations can induce anomalous diffusion [13]. Fig. 2(b) shows short range spatial correlations of the cell velocities. The correlation length is on the order of 10–15 μm (one to two cell diameters).

We studied the underlying thermodynamics of the motion, by calculating the probability distribution function of the velocities. Over small time scales ($\Delta t=$ 30 s), the velocity distributions are non-Gaussian (unlike Boltzmann thermodynamics). Fig. 3(a) shows a histogram of the speeds with a fit to a Maxwell distribution. The experimental distribution has a heavier tail, with a significantly greater number of high velocity events than predicted by Maxwell–Boltzmann thermodynamics. We also

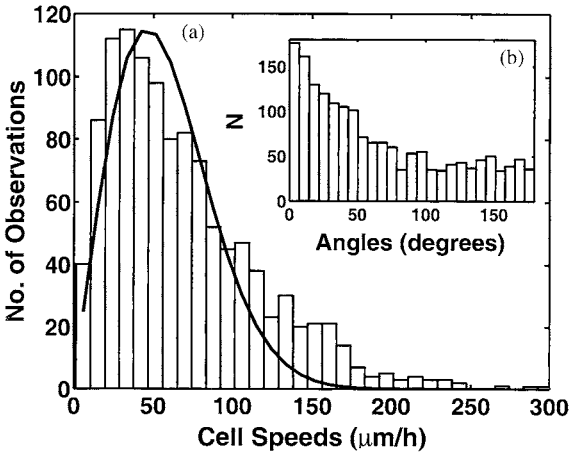

Fig. 3. (a) Probability distribution function (PDF) of cell speeds. The solid line is a fit to the Maxwell distribution of speeds for a Brownian particle: $F(V) = aV\exp(-bV^2)$. (b) PDF of the angles between successive orientations of the cell displacements.

calculated the histogram of angles between orientations of the velocity vector at successive observation times, as shown in Fig. 3(b). A large percentage of events have angles less than 30°, rather than a flat distribution which would result from randomly distributed successive orientations. Thus the cell velocities have non-random direction and speed components. Clearly, Boltzmann thermodynamics are not sufficient to explain our observed results.

Generalization of Maxwell–Boltzmann thermodynamics provides a framework for understanding anomalous diffusion of Levy-type distributions [14] and correlated-type diffusion [15,16]. This generalized thermodynamics derives from the non-extensive entropy introduced by Tsallis [17]:

$$S_q = \frac{1 - \int \mathrm{d}x[f(x)]^q}{q-1}\,, \tag{2}$$

where q is a parameter quantifying the degree of non-extensivity and $f(x)$ is the probability distribution function. The limit of $q \to 1$ recovers the regular Gibbs entropy $S = -\int f\ln(f)\,\mathrm{d}x$. Optimizing S_q under normalization and mean energy constraints yields a generalized probability density function as well as the q-distribution of velocities [18]:

$$F(\mathbf{v}) = A_q\left[1 - (1-q)\frac{\beta m\mathbf{v}^2}{2}\right]^{1/1-q}\,. \tag{3}$$

where $\mathbf{v}$ is the velocity of the diffusing species. Here β and m do not represent the actual temperature or mass, but rather denote an effective mobility. The temperature is not that of the surroundings, as the motion is not thermally driven—it is an effective temperature representing the cytoskeletal activity driven by nucleotide hydrolysis. Again, the case of $q \to 1$ recovers regular Maxwell–Boltzmann thermodynamics with Gaussian velocity distributions.

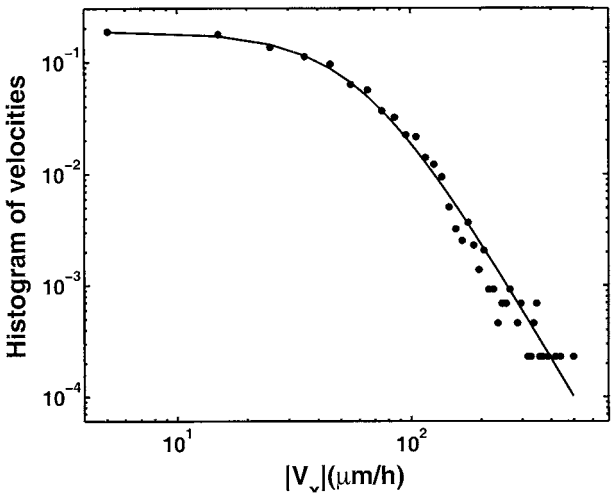

Fig. 4. PDF for the horizontal component of velocity for endodermal cells in an ectodermal aggregate. The solid curve is a fit to Eq. (4).

We find that, the experimental velocity distributions for both the endo–endo and endo–ecto cases have the functional form of the q-distribution of velocities:

$$F(V_x) = \frac{a}{(1 + bV_x^2)^c} \,. \tag{4}$$

We fit the x and y components of the velocity vectors (V_x and V_y) with the above form (Fig. 4). From the fits, we obtain q using $q = c + 1/c$. Averaging the results of several experiments, we get $q \sim 1.5 \pm 0.05$.

How can we distinguish whether the observed non-Gaussian statistics arise due to correlated or Levy-type anomalous diffusion? Pure Levy flight dynamics have a broad distribution of the elementary displacements X during time t without any correlated jumps: $P(X,t) = 1/X^a$ with $a < 3$. In correlated-type anomalous diffusion, the distribution $P(X,t)$ can be narrow ($a > 3$) or Gaussian, with a non-linear growth of the variance. We measured the exponent a for the probability distribution of cell displacements and obtained $a \sim 4$. This value of a is consistent with $q \sim 1.5$, if we approximate the Tsallis form of $P(X,t)$ by a power law for large X. The narrow distribution of elementary displacements and the observed temporal and spatial correlations of the velocities suggests that the dynamics may be described by a correlated type anomalous diffusion.

To verify what dynamics gives rise to the observed statistics, we need to find a connection between q and the exponent α of the mean squared displacement. To this end, we consider an alternate way of obtaining probability distribution functions for anomalous diffusion—by solving the Fokker–Planck equations. A diffusion equation with fractional derivatives may be used to describe Levy-type diffusion [19,20]. On the other hand, a non-linear Fokker–Planck equation [15,16,21] has been proposed for those systems with correlated anomalous diffusion. The explicit form of the non-linear

Fokker–Planck equation is given by

$$\frac{\mathrm{d}f^{\mu}}{\mathrm{d}t} = -\frac{\mathrm{d}}{\mathrm{d}x}(Kf^{\mu}) + Q\frac{\mathrm{d}^2}{\mathrm{d}x^2}(f^{v})\,. \tag{5}$$

Interestingly, the solutions of the nonlinear Fokker–Planck equation are same as the distributions that optimize the generalized entropy [15,16].

In our case, we are interested in the time evolution of the probability distribution function, which implies $\mu = 1$. As has been shown before [15], for correlated anomalous diffusion, $q = 1 + \mu - v$ and $\alpha = 2\mu/(\mu + v)$, this gives us $\alpha = 2\mu/(2\mu - q + 1)$. The experimentally obtained values are $\alpha = 1.24 \pm 0.1$ and $q = 1.5$. For $\mu = 1$ and $q = 1.5$ we obtain $\alpha = 1.3$ which is close enough to our observed α within experimental error. On the other hand, as shown by Zanette and Alemany [14], for two-dimensional Lévy flights, for $q = 1.5$, $\alpha = q - 1 = 0.5$ which is not consistent with our observations. The above arguments indicate that our experimental system displays correlated type of anomalous diffusion as described by a non-linear Fokker–Planck equation.

4. Discussion

We have shown that in addition to a regular biased random walk, cell motion in aggregates shows a novel type of statistics. Cell velocities have non-Gaussian probability distributions, analogous to physical examples with spatial or temporal correlations. Cells move faster in a less adhesive cellular environment, although the type of observed dynamics is the same in both kinds of surroundings. The motion of each cell is governed by its intrinsic machinery and also depends on the motion of neighbors. The physics possibly resembles a granular material with very high packing density. Experiments and simulations of two-dimensional vibrated beds of granular media showed anomalous diffusion with exponent ~ 1.2 and non-Gaussian velocity distributions [22]. Since cells are in such close contact with each other, they cannot move independently. The coupling of the probability distribution of packing densities with that of cell velocities can give rise to non-Gaussian statistics [23]. The natural inhomogeneity of the cell population leads to fluctuations in the packing density and the strength of interactions, possibly giving rise to the different kinds of behavior—random and correlated. In the present experiments, the strong interactions between cells induce correlations which modify the dynamics. In fact, using renormalization group arguments, Mendes and Tsallis have shown that in systems with long range interactions, the $q > 1$ case appears as one fixed point of the renormalization group in the (q, temperature)-space, the other fixed point being $q = 1$ [24]. In our case, the interactions between cells may lead to long range correlations which could explain our experimentally obtained value of $q > 1$.

We can hypothesize the following scenario for cell motion in aggregates. The motion of each cell is characterized by (1) regimes where it is trapped in a cage of its nearest neighbors, all of which are fluctuating, (2) the cell has a finite probability of a sudden escape from the cage. This corresponds to the almost linear parts of the observed

trajectories. The above process could also occur at different length scales e.g. trapping and escape of a cluster from surrounding clusters. The escape probability would depend on the adhesion and the membrane fluctuation amplitude; the size of typical clusters formed may also depend on the cell–cell adhesivities. Spatial correlations between cells due to such mechanisms may lead to temporal correlations.

More careful work is needed to characterize the difference in spatial correlation functions for the two cell types considered. The role of cell membrane protrusions driven by internal actin dynamics is a key factor in understanding the various motile processes. We are currently investigating the role of membrane fluctuations as an internal temperature, and its effect on diffusion and correlations. Future work will involve analysis of data at different time scales, experiments with different cell type combinations (ecto in ecto, ecto in endo) and development of a model to explain the observed phenomena and understand the appearance of correlations.

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References

[1] G.A. Dunn, A.F. Brown, J. Cell. Sci. Suppl. 8 (1987) 81.
[2] J.C.M. Mombach, J.A. Glazier, Phys. Rev. Lett. 76 (1996) 3032.
[3] A. Czirok et al., Phys. Rev. Lett. 81 (1998) 3038.
[4] D. Godt, U. Tepass, Nature 395 (1998) 387.
[5] U. Technau, T. Holstein, Dev. Biol. 151 (1992) 117.
[6] J.P. Rieu, N. Kataoka, Y. Sawada, Phys. Rev. E 57 (1998) 924.
[7] A. Gierer et al., Nature (London), New Biol. 239 (1972) 98.
[8] Y. Kishimoto, M. Murate, T. Sugiyama, J. Cell Sci. 109 (1996) 762.
[9] G. Forgacs et al., Biophys. J. 74 (1998) 2227.
[10] T.H. Solomon, E.R. Weeks, H.L. Swinney, Phys. Rev. Lett. 71 (1993) 3975.
[11] A. Ott et al., Phys. Rev. Lett. 65 (1990) 2201.
[12] H. Spohn, J. Phys. I 3 (1993) 69.
[13] J.P. Bouchad, A. Georges, Phys. Rep. 195 (1990) 127.
[14] D.H. Zanette, P.A. Alemany, Phys. Rev. Lett. 75 (1995) 366.
[15] C. Tsallis D.J. Bukman, Phys. Rev. E 54 (1996) R2197.
[16] L. Borland, Phys. Rev. E 57 (1998) 6634.
[17] C. Tsallis, J. Stat. Phys. 52 (1988) 479.
[18] R. Silva, A.R. Plastino, J.A.S. Lima, Phys. Lett. A 249 (1998) 401.
[19] C. Tsallis, S.V.F. Levy, A.M.C. Souza, R. Maynard, Phys. Rev. Lett. 75 (1995) 3589.
[20] A. Compte, Phys. Rev. E 53 (1996) 4191.

[21] A. Compte, D. Jou, J. Phys. A 29 (1996) 4321.
[22] Y. Murayama, M. Sano, J. Phys. Soc. Japan 67 (1998) 1826.
[23] Y.H. Taguchi, H. Takayasu, Europhys. Lett. 30 (1995) 499.
[24] R.S. Mendes C. Tsallis, Renormalization Group Approach to Nonextensive Statistical Mechanics, preprint, 2000 [cond-mat/0003365].