Physical Nature of Bacterial Cytoplasm

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We track the motion of individual fluorescently labeled mRNA molecules inside live *E. coli* cells. We find that the motion is subdiffusive, with an exponent that is robust to physiological changes, including the disruption of cytoskeletal elements. By modifying the parameters of the RNA molecule and the bacterial cell, we are able to examine the possible mechanisms that can lead to this unique type of motion, especially the effect of macromolecular crowding. We also examine the implications of anomalous diffusion on the kinetics of bacterial gene regulation, in particular, how transcription factors find their DNA targets.

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The bacterial cell has traditionally been viewed as a ''concentrated soup'' where intermolecular interactions are governed by diffusion [1]. In other words, the conditions inside a prokaryotic (non-nucleated) cell are often assumed to be very close to those in a test tube. This picture is changing with the recent findings of a dynamic cytoskeleton in prokaryotic cells [2]. In addition, theoretical arguments have long been raised pointing to the discrepancies between presumed diffusion-dominated processes and the observed kinetics of protein/DNA interactions [1,3,4].

Little is actually known about the movement of macromolecules in the bacterial cell. In pioneering experiments, Elowitz and co-workers quantified the mobility of several proteins in the *E. coli* cytoplasm [5], finding that their motion was consistent with simple diffusion on time scales of $< \sim 1$ sec and length scales of the bacterium, ~ 1 μm. However, when examining the value of the diffusion coefficient D for different proteins, they found that a single cytoplasmic viscosity could not explain all the experimental values of D. Recently, Deich *et al.* [6] tracked individual fluorescently labeled proteins in the membrane of *C. crescentus* and characterized their two-dimensional motion as diffusive.

Here we examine the random motion of individual molecules inside the cell by tracking fluorescently labeled mRNA molecules in *E. coli*. We find that cytoplasmic motion is subdiffusive on the time scale of seconds to minutes. That is, the mean squared distance $\langle \delta^2 \rangle$ traveled by the molecule in a time interval τ can be written as $\langle \delta^2 \rangle = \Gamma \tau^\alpha$, where $\alpha < 1$ is the subdiffusion exponent and Γ is the generalized diffusion coefficient [7,8]. This behavior implies that on these time scales the motion of the RNA molecule is dominated by interactions with obstacles in the medium surrounding it. While Γ varies considerably between cells in the population and between different experimental conditions, the exponent α, a physically more important characteristic of the motion, is very robust, with little or no dependence on particle size, growth conditions, and genetic background, including cytoskeletal mutants. These findings suggest that the cytoplasmic medium is ''superdense'': the density of obstacles hindering molecular motion is probably much higher that the threshold density leading to the onset of subdiffusion [9].

The mRNA detection system [10–12] consists of the bacteriophage MS2 coat protein (henceforth referred to as MS2 protein) fused to green fluorescent protein (GFP), and a reporter RNA containing 96 tandemly repeated MS2-binding sites. When the MS2-GFP fusion is coexpressed with the target RNA, a large number of the tagging proteins bind to each RNA molecule [$\sim$3 kb long, with an estimated physical size of the order $\sim$100 nm [12]], forming bright fluorescent particles which can be followed under the microscope. The labeled RNA messages are much longer lived than endogenous mRNA [11], allowing us to follow them for many minutes.

Here we focus on the motion of mRNA molecules released from their template DNA and free to move in the cytoplasm. In a typical experiment, a sample was taken from a growing culture of cells producing a low level of target mRNA, as well as saturating numbers of MS2-GFP tagging proteins. Cells were tracked for up to 30 minutes at 1 frame/sec [Fig. 1(a), see also movie in [13]]. The tagged RNA moves randomly in the cell, spanning the complete cell length multiple times within the 30 min period. A first hint of the unique nature of the motion is found in the observation that it is discontinuous, made up of periods of almost localized motion, separated by fast jumps to a new position [Figs. 1(b) and 1(c)].

We characterize the motion by measuring the mean squared displacement $\langle \delta^2(\tau) \rangle$, where $\delta = |\mathbf{r}(t+\tau) - \mathbf{r}(t)|$ is the particle displacement between two time points, and δ^2 is averaged over all pairs of time points with difference τ between them. For Brownian motion one expects the Einstein-Smoluchowski relation $\langle \delta^2(\tau) \rangle = 2dD\tau$, where d is the spatial dimension and D is the diffusion coefficient of the moving particle [14]. Measuring $\langle \delta^2(\tau) \rangle$ for multiple RNA molecules, however, reveals very different behavior [Fig. 2(a)]: $\langle \delta^2(\tau) \rangle \sim \tau^\alpha$, with $\alpha = 0.70 \pm 0.07$ (21 trajectories). This subdiffusive behavior is well known in the physics of random systems. It arises when a particle inter-

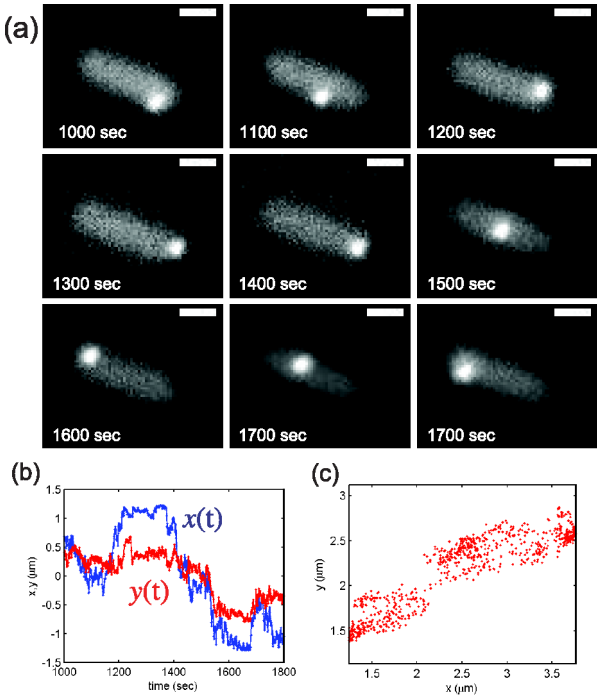

FIG. 1 (color online). Motion of a tagged RNA molecule inside an *E. coli* cell [see also movie in [13]]. Cells were grown and treated as described previously [10–12]. Cells were imaged with a Nikon Eclipse inverted epifluorescence microscope, under a 60X objective. Time-lapse movies were taken with a Cascade:512B (Roper Scientific) high sensitivity camera for 30 minutes at 1 frame/second, 200 msec exposure time. Each camera pixel covers a square of size $\sim 67 \times 67$ nm^2, thus oversampling the optical resolution limit (Airy radius ~ 200 nm). (a) A series of epifluorescent images of the cell. Images are 100 sec apart (scale bar $= 1$ μm.) (b) A plot of the x and y coordinates (axis chosen arbitrarily) of particle position during the time covered in panel A. (c) A two-dimensional plot of the particle trajectory (same data as in panels A and B).

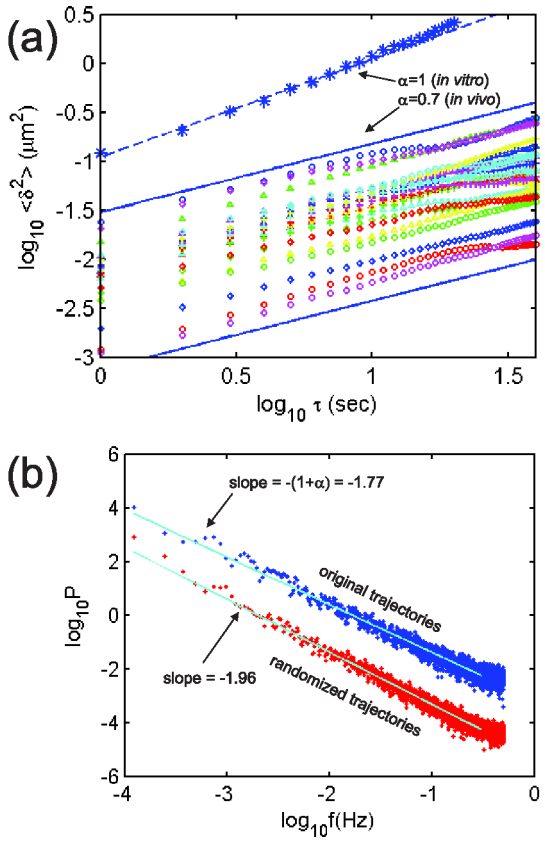

FIG. 2 (color online). Subdiffusive motion of RNA molecules in the cell. Movies were read into Matlab software (Mathworks). The fluorescent particles were automatically recognized and followed, to yield a time series of particle coordinates $\mathbf{r}(t) = (x(t), y(t))$ for each RNA molecule, where $t = \{0, \Delta T, 2\Delta T, 3\Delta T \ldots\}$ is discretized by the camera framing interval ΔT. This vector was used to calculate the mean square displacement as a function of time interval: $\langle \delta^2(\tau) \rangle$, where $\delta = |\mathbf{r}(t + \tau) - \mathbf{r}(t)|$ and averaging is performed over all pairs of time points (t_1, t_2) obeying $|t_1 - t_2| = \tau$, thus τ is also discretized by ΔT. (a) The mean squared displacement $\langle \delta^2 \rangle$ of the molecule is plotted as a function of the time-interval between measurements τ. Different markers and colors denote different trajectories (total of 23 trajectories from 3 different experiments). Solid lines = slope 0.7. Deviations from the 0.7 slope at longer times are due to the effect of limited cell size, and the averaging over a smaller number of position pairs. Also shown in the figure is a typical plot of $\langle \delta^2(\tau) \rangle$ for an RNA particle diffusing in 70% glycerol. In this case the motion is normal diffusion ($\alpha = 1.04 \pm 0.03$, 4 trajectories), as demonstrated by the dashed line with slope 1. (b) Power spectrum $P(f)$ of RNA trajectories. The complete set of $x(t)$ and $y(t)$ trajectories were concatenated, and the power spectral density of the combined trajectory was calculated [see [12,13]]. Blue dots = measured $P(f)$; solid line = linear fit yielding slope -1.77 ± 0.03. A calculation using only the $x(t)$ and $y(t)$ coordinates separately gave similar results. As an additional test for the validity of the spectral density calculation, the trajectory steps $[\Delta x(t), \Delta y(t)]$ were randomly permutated and then reintegrated [27]. The resulting new trajectory should exhibit a random walk behavior, with $P(f) \sim f^{-2}$ [28]. The calculated spectral density (red dots) is in agreement with this prediction, yielding a slope of -1.96 ± 0.04.

acts with the random medium in which it is moving. Whereas a particle moving in a uniform medium, whatever the viscosity, constantly makes small jumps due to thermal energy, some types of random media can "trap" the particle in one location for varying and widely distributed periods, allowing only infrequent "jumps" between locations and leading to the observed subdiffusion on the relevant time scale of particle/obstacle interactions [7,8]. This trapping can be geometrical—as in a percolation cluster, whose fractal geometry often causes the particle to get stuck in cul de sacs—or it can have a temporal origin, with the particle constantly binding to obstacles with a broad distribution of binding times.

Formally, the broad distribution of "cage times" t_c leads to anomalous $\delta^2(\tau)$ behavior. A power-law distribution $P(t_c) \sim t_c^{-\nu}$ with $2 > \nu > 1$ leads to subdiffusion with an exponent $\alpha = \nu - 1$ [8,15]. The distribution of cage times has been measured directly for tracer particles moving in actin networks *in vitro* [15]. Another formulation ascribes the motion to thermal fluctuations opposed by a time

dependent friction [16]. Both formulations offer an additional way of characterizing subdiffusive motion, by measuring the power spectrum of the particle's motion: $P(f) = |X(f)|^2$, where $X(f)$ is the Fourier transform of the particle position $x(t)$ [and similarly for $y(t)$]. The power spectrum is equal to the Fourier transform of the autocorrelation function, and thus characterizes the particle's ''memory'' of its previous position. Under these assumptions the power spectrum should obey a power law: $P(f) \sim f^{-(1+\alpha)}$, where α is the subdiffusive exponent. This behavior is indeed observed, yielding an additional estimate of α in our system of 0.77 ± 0.03 [Fig. 2(b)].

Each trajectory can be characterized by the generalized diffusion coefficient Γ and the subdiffusion exponent α. The changes in these parameters under different conditions are summarized in Table 1 (supplementary material) [12]. While α is an indicator of the basic interaction between the moving particle and its surroundings, Γ is a more cell-specific parameter, expected to depend on the details of the particular system, such as cell size, RNA and protein levels, and so on. We found that Γ varies considerably (standard deviation/mean >3, with values in the range $<10^{-3}$ to $>10^{-2}$ $\mu m^2/sec^{0.7}$; 70 trajectories, 3 experiments), whereas α is more robust (standard deviation/mean <0.5; same data set). There are additional trends, although the overall variance makes their significance rather weak: RNA molecules lacking a ribosome-binding site (RBS) move faster (higher Γ) than molecules with an RBS sequence, and RNA molecules move faster in cells growing more slowly. Slowly growing cells are known to have smaller numbers of ribosomes, while having a higher concentration of proteins and DNA [17]. These findings might mean that the movement of RNA molecules is coupled to, and impeded by, the $\sim 10^4$ ribosomes in the cell.

The more universal (and arguably more significant) exponent α, despite some variation between experiments, shows a surprising constancy over a wide range of system parameters. These include features of the tagged RNA molecules: presence or absence of RBS sequence and length of the tagged RNA molecule; growth conditions: growth rate and growth in the presence of Tetracycline, Chloramphrenicol, and Nalidixic acid; and RNA movement in strains deficient in cytoskeletal elements: MreB, the prokaryotic actinlike protein, and FtsZ, the tubulin homologue [12].

What causes this robustness of the subdiffusive motion? Possible insight comes from a recent *in vitro* study [9] in which the authors used fluorescence correlation spectroscopy (FCS) to study the motion of various tracer proteins in a concentrated solution of polymers. They found the motion to be subdiffusive, with the exponent α obeying $\alpha = \alpha_l + (1 - \alpha_l)e^{-\phi/\phi_0}$, where $\alpha_l = 0.74 \pm 0.02$ is the asymptotic value reached at high obstacle density (as measured by the excluded volume that obstacles occupy, ϕ). The threshold density ϕ_0, which determines how fast α will approach the limiting value, depends on the size of the obstacles and the tracer particle, and on their characteristics—e.g., globular or chainlike [9]. Once the obstacle density ϕ is sufficiently higher than ϕ_0, α is stable at $\alpha \approx \alpha_l$, and does not vary with the system parameters.

Figure 3 in the supplementary material [12] summarizes our results in the context of these findings. In our system, we found that α is stable and close to the asymptotic *in vitro* value [9] [$\alpha \approx 0.74$, using the combined estimates of $\langle \delta^2(\tau) \rangle$ and $P(f)$], suggesting that ϕ is higher than ϕ_0, and likely $\phi \gg \phi_0$. Since ϕ_0 depends on the obstacles as well as on the tracer particle, it is likely that smaller particles in the cytoplasm—most proteins included—will have a higher critical density value, ϕ_0, and therefore might have a higher α value at cytoplasmic conditions, perhaps up to $\alpha \approx 1$, i.e., normal diffusion [this will also reconcile our findings with the apparent observations of cytoplasmic protein diffusion by Elowitz *et al.* [5]]. But that is not necessarily the case. With regards to larger moving particles, such as mRNA molecules or ribosomes, our results suggest that they are likely to experience a medium whose obstacle density is much above criticality, and their motion is therefore subdiffusive.

As for the consequences of subdiffusive motion for cellular reactions, we note that a molecule which moves in a subdiffusive manner will not only take longer to meet its target, but will also stay in the vicinity of the target for a longer time: the typical time t for a subdiffusing particle to travel a distance l scales as $t \sim l^{2/\alpha}$, compared to $t \sim l^2$ for a ''Brownian'' particle. Following the line of reasoning in [4], one can show that a subdiffusive molecule initially at some distance r from a target of size a has a probability p of finding it which is $\approx (a/r)^{3-2/\alpha}$. Note that p increases for lower values of α, and the chances of escape $1\text{-}p$ (i.e., failing to reach the target) approach zero for $\alpha \to 2/3$. The price paid for the lower escape rate is a lower association rate constant, $k = \Gamma^{1/\alpha}/a^{2/\alpha-3}$.

We mention here one instance of gene expression in bacteria that might be critically affected by these features of subdiffusive motion: how do transcription factors find their DNA target? In a typical scenario, a DNA-binding protein present in a small copy number in the cell [usually a few to a hundred [18]] needs to bind to a specific site in the bacterial genome, in order to up- or down-regulate the activity of a gene located downstream of that site [17]. Theoretical calculations show that simple three-dimensional diffusion will yield target-finding times which seem much longer than those observed [1,4], and therefore it is often postulated that 3D diffusion is combined with 1D motion along nontarget areas of the DNA. This formulation of the problem ignores the fact that many transcription factors are transcribed (and presumably translated) from a genomic locus very close to their DNA target [19]. Phage λ repressor [20] and the Lac repressor [21] coding regions are both located immediately adjacent to their prospective binding targets, and therefore are probably produced, at most, a few

tens of nm from where they bind. The problem then becomes the complementary one: the transcription factor has to find its DNA target before the two species drift apart. Subdiffusion changes the situation drastically, perhaps tipping the balance between the protein ''running away'' from the DNA and the protein finding its target in time.

In recent years evidence has accumulated for anomalous diffusion in various eukaryotic systems [16,22,23]. However, the eukaryotic cytoplasm is extremely complex: containing different organelles, an elaborate cytoskeleton, and various mechanisms for active transport of molecules in the cell [24]. As might be expected in light of this complexity, the complete spectrum of cellular movements (as manifested by the exponent α) has been observed, sometimes in the context of a single experiment: subdiffusion with a large range of α values [23], normal diffusion ($\alpha = 1$), and finally superdiffusive motion ($\alpha > 1$) suggesting the occurrence of active transport [16].

Bacterial cells offer two main advantages when trying to investigate the sources of cytoplasmic subdiffusion. First, we are dealing with a much simpler system. No organelles are present [besides the bacterial nucleoid [17] which the moving particles seem to avoid, data not shown]; there are no known active transport mechanisms for RNA and proteins [the only known ones relate to chromosome and plasmid movement during cell division [2]], and a very limited cytoskeleton compared to the cell-encompassing network of eukaryotes. The fact the α remains little changed in mutants deficient in the cytoskeletal proteins MreB and FtsZ suggests that the bacterial cytoskeleton is not the prime mover in subdiffusion. In the case of movement inside eukaryotic cells, the cytoskeleton might play a more critical role [22,25,26].

A second advantage is our use of direct tracking of the individual particles. This technique allows us to measure directly the trajectory of the RNA molecule [$\boldsymbol{r}(t)$] and unambiguously detect its subdiffusive nature. This is in contrast to other techniques used for studying cytoplasmic mobility—FRAP (fluorescence recovery after photobleaching) [5] and FCS [9,23]—where analysis is more model dependent and therefore open to multiple interpretations.

In sum, we conclude that subdiffusion in the bacterial cell is independent of the main cytoskeletal elements, and is more likely explained by the extremely crowded environment of the cytoplasm. These new findings regarding cellular subdiffusion and its characteristics demand a rethinking of the kinetics of intracellular kinetics in bacteria, and perhaps in larger, eukaryotic cells as well.

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[1] P. H. von Hippel and O. G. Berg, J. Biol. Chem. **264**, 675 (1989).
[2] J. Errington, Nat. Cell Biol. **5**, 175 (2003).
[3] N. Shimamoto, J. Biol. Chem. **274**, 15 293 (1999).
[4] S. E. Halford and J. F. Marko, Nucleic Acids Res. **32**, 3040 (2004).
[5] M. B. Elowitz *et al.*, J. Bacteriol. **181**, 197 (1999).
[6] J. Deich *et al.*, Proc. Natl. Acad. Sci. U.S.A. **101**, 15 921 (2004).
[7] S. Havlin and D. Ben-Avraham, Adv. Phys. **51**, 187 (2002).
[8] R. Metzler and J. Klafter, Phys. Rep. **339**, 1 (2000).
[9] D. S. Banks and C. Fradin, Biophys. J **89**, 2960 (2005).
[10] I. Golding and E. C. Cox, Proc. Natl. Acad. Sci. U.S.A. **101**, 11 310 (2004).
[11] I. Golding *et al.*, Cell **123**, 1025 (2005).
[12] See EPAPS Document No. E-PRLTAO-96-014610 for a more detailed description of materials and methods; Table I and Figure 3. For more information on EPAPS, see http://www.aip.org/pubservs/epaps.html.
[13] See EPAPS Document No. E-PRLTAO-96-014610 for movie. For more information on EPAPS, see http://www.aip.org/pubservs/epaps.html.
[14] H. C. Berg, *Random Walks in Biology* (Princeton University Press, Princeton, NJ, 1993), p. 152.
[15] I. Y. Wong *et al.*, Phys. Rev. Lett. **92**, 178101 (2004).
[16] A. Caspi, R. Granek, and M. Elbaum, Phys. Rev. E **66**, 011916 (2002).
[17] F. C. Neidhardt, J. L. Ingraham, and M. Schaechter, *Physiology of the Bacterial Cell: A Molecular Approach* (Sinauer Associates, Sunderland, MA, 1990).
[18] P. Guptasarma, Bioessays **17**, 987 (1995).
[19] P. B. Warren and P. R. ten Wolde, J. Mol. Biol. **342**, 1379 (2004).
[20] M. Ptashne, *A Genetic Switch: Phage Lambda and Higher Organisms* (Cell Press: Blackwell Scientific Publications, Cambridge, MA, 1992).
[21] J. R. Beckwith, D. Zipser, and Cold Spring Harbor Laboratory, *The Lactose Operon* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1970).
[22] I. M. Tolic-Norrelykke *et al.*, Phys. Rev. Lett. **93**, 078102 (2004).
[23] M. Weiss *et al.*, Biophys. J. **87**, 3518 (2004).
[24] B. Alberts, *Molecular Biology of the Cell* (Garland Science, New York, 2002).
[25] M. T. Valentine *et al.*, Biophys. J. **88**, 680 (2005).
[26] H. Salman *et al.*, Biophys. J. **89**, 2134 (2005).
[27] R. Segev *et al.*, Phys. Rev. Lett. **88**, 118102 (2002).
[28] R. N. Mantegna, H. E. Stanley, and ebrary, Inc., *An Introduction to Econophysics Correlations and Complexity in Finance* (Cambridge University Press, Cambridge, England, 2000).