Active diffusion: The erratic dance of chromosomal loci

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Since the early days of microscopes and such pioneering discoveries as that of bacteria by van Leeuwenhoek (1), scientists have been fascinated by the microscopic world of living systems. Nearly 200 years ago, the botanist Brown (2) made observations of the erratic motion of small particles in pollen that were to have implications far beyond botany and biology. In fact, taken together with the theoretical understanding provided in 1905 by Sutherland (3) and Einstein (4) and the careful experiments by Perrin (5), Brownian motion can be credited with no less than the conclusive establishment of the atomic nature of matter. Ironically, in the meantime, random motion received comparatively little attention in biology, because more effort was naturally focused on directed motion and processes in living systems (e.g., intracellular transport). This lack of attention began to change with the growing evidence of random athermal motion known as active diffusion, especially in the cytoplasm of eukaryotic cells. In PNAS, the work by Weber et al. (6) demonstrates active fluctuating motion at an even smaller scale in bacteria and within the nucleus of yeast cells. This motion is shown to be nonthermal in origin, providing yet more evidence for an emerging and distinct class of microscopic motion in living systems—neither directed nor thermal.

The fundamental insight due to Sutherland (3) and Einstein (4) was that Brownian motion is a manifestation of the thermal agitation that is ever present in systems in equilibrium at any temperature above absolute zero. This insight is most succinctly captured in the Stokes–Einstein formula (Eq. **1**)

$$D = \frac{k_B T}{6\pi\eta a}, \qquad [1]$$

which directly relates the diffusion coefficient D of a particle of size a to the temperature T and the Stokes drag coefficient $6\pi\eta a$, which represents the ratio of the drag force to the velocity of the particle moving through a liquid of viscosity η. In simple liquids, diffusion is characterized by the mean square displacement (MSD) after a time interval τ that grows proportional to $D\tau$. In more complex, viscoelastic materials, thermal motion is subdiffusive, which is illustrated in Fig. 1, with the MSD growing as τ^α with the exponent $\alpha < 1$. Nevertheless, the amplitude of this motion is still proportional to temperature and varies inversely with the viscoelastic resistance of the medium.

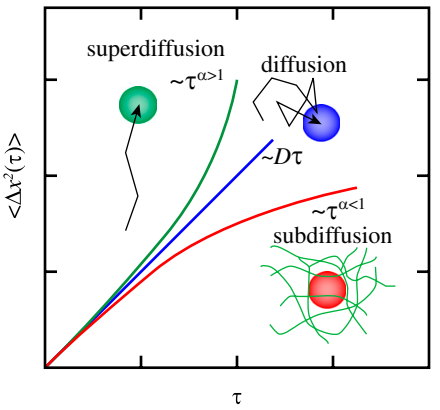

Fig. 1. Thermal diffusion (blue line) of a particle in a liquid is characterized by an MSD given by $<\Delta x^2(\tau)> = 2D\tau$, where the displacement $\Delta x(\tau) = x(t+\tau) - x(t)$ along one axis is measured over a time interval τ. In equilibrium, this linear dependence on τ is only expected for motion in simple liquids. In viscoelastic materials, such as polymer solutions, subdiffusive motion (red) is expected in equilibrium. By contrast, superdiffusive motion (green) often indicates partially or fully directed motion (e.g., for transport along a substrate) (11).

Thus, diffusion can be viewed, at least superficially, as a balance between a thermal driving force tending to increase motion and the viscous or viscoelastic resistance of the medium. At a deeper level, however, things are more subtle: the thermal agitation and the resistance of a material are not independent of each other. The motion is determined by the ratio of the driving force f to the resistance η. Thus, one might expect the MSD, being quadratic in the particle motion, to vary inversely with square of the resistance as f^2/η^2. The Stokes–Einstein formula tells us that the thermal force cannot be a function of temperature alone, but it must also depend on the material properties of the medium. Specifically, for liquids, the mean square of the thermal force must be proportional to the product $T\eta$.

The thermal forces are, thus, inextricably linked to the material properties of the medium. This makes it especially challenging to disentangle these two contributions to microscopic motion, which the work by Weber et al. (6) sets out to do. Although it is easy to identify coherent, directed motion as active, it can be difficult to identify whether nondirected motion is thermal or not. All too often, it has simply been assumed that intracellular motion that is not directed must instead be thermal Brownian motion.

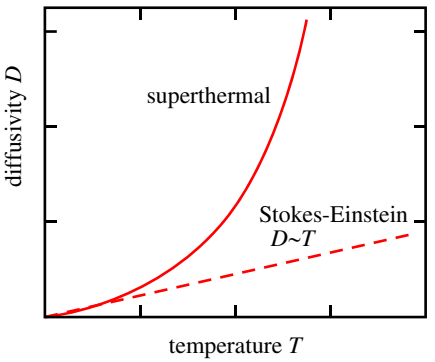

Fig. 2. By modulating the temperature, the work by Weber et al. (6) finds a much stronger, superthermal dependence of the effective diffusivity than expected from the Stokes–Einstein relation. Their results are consistent with an underlying activated process that drives the fluctuations.

The most direct way to clearly identify nonequilibrium or athermal motion is to independently measure both the mechanical or viscous resistance of the material as well as the fluctuating motion (7). The combination of these measurements can allow one to quantify possible fluctuations in excess of what is expected due to thermal agitation. So far, such a direct approach has been limited to in vitro systems, whereas results in vivo have remained indirect (8, 9). In PNAS, the work by Weber et al. (6) approaches this problem, instead, through a combination of temperature modulation and inhibition of various possible molecular mechanisms driving athermal motion. Most dramatically, the authors (6) show that the chromosomal jiggling is superthermal, characterized by much stronger temperature dependence than predicted by the Stokes–Einstein relation (Fig. 2), in both the cytoplasm of *Escherichia coli* bacteria and the nucleus of *Saccharomyces cerevisiae* yeast. Their results suggest an activated process driving the fluctuating motion.

The work by Weber et al. (6) goes on to probe the origins of the athermal motion using a variety of biochemical interventions aimed at suppressing specific enzymatic activity. Although they do not identify a unique cause for the

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chromosomal dance, the authors convincingly show that it depends on ATP; on depletion of ATP, the MSD is found to have substantially reduced amplitude. Because the activity depends on ATP, it is natural to assume that depletion of ATP should leave the system in a more thermal, equilibrium state. Interestingly, however, ATP depletion is not seen to change the subdiffusive character of the motion: specifically, the exponent α is ~0.4 both before and after ATP depletion. This finding is very surprising in view of the interdependence of the thermal driving forces and the material resistance noted above. The amplitude of the fluctuating thermal forces must depend on the material resistance, which becomes time-dependent in viscoelastic materials. Weber et al. (6) suggest that the ATP-dependent fluctuations must have thermal-like time dependence to account for the lack of variation of the subdiffusive exponent on ATP depletion.

Alternatively, it is also possible that the ATP depletion only partially suppresses the active processes driving the jiggling. Then, the observed exponent $\alpha = 0.4$ can be understood by a variation of models for active diffusion in eukaryotic cells (8, 9). If the active processes driving the motion have a short correlation time, they could be characterized by a white-noise spectrum. Then, subdiffusive behavior with $\alpha = 0.4$ would be consistent with such activity in a viscoelastic environment characterized by a frequency-dependent shear modulus $G \sim \omega^{0.7}$, close to the Zimm behavior expected for flexible polymer solutions (10).

Although they do not identify a unique cause for the chromosomal dance, the authors convincingly show that it depends on ATP.

Whatever the microscopic origin of the chromosomal jiggling, the fact that it is of larger amplitude than thermal Brownian motion has important implications for cells. This extra agitation may significantly speed up diffusion-limited reactions and processes, both in bacteria and within eukaryotic nuclei. This work should also serve as an important cautionary note for the use of thermal diffusion analysis of intracellular fluctuations, even when nondirected motion is seen.

Of course, major challenges remain for our microscopic understanding of activity in living systems. Here, a very promising avenue of research would be the development of in vitro, reconstituted model systems, which have proven very fruitful for understanding cytoskeletal activity. An interesting technical challenge would be the direct measurement of the micromechanical chromosomal environment in vivo. This measurement would permit, for instance, a direct test of the intriguing hypothesis of ATP-dependent activity that resembles thermal fluctuations (6). In any case, long after the work of Brown, it seems there is renewed interest in random motion in living systems and, in particular, in addressing the question of the vitality of such motion, with which Brown originally grappled (2).

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