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Cytoplasmic Transport: Bacteria Turn to Glass Unless Kicked

Bacteria lack the cytoskeleton and motors of eukaryotic cells, and their cytoplasm has been considered to be purely fluid. New data show that bacterial cytoplasm can solidify to resemble a soft glass, unless enzymatic activity creates motions to fluidize it.

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A standard explanation for why bacteria — unlike eukaryotic cells — do not express molecular motors, like myosin, kinesin, or dynein, or have three-dimensional cytoskeletons is that they do not need them. The bacterial cell wall determines their shape, and the interior volume of bacteria ($\sim$1 μm^3) is so small that thermally-driven transport of molecules is fast enough not to be the rate-limiting step of reactions needed to maintain viability. Only as cells evolved to become larger and more irregularly shaped did they need directed transport to overcome the slow rate and dilution associated with molecules diffusing from their source to their destinations many microns or even meters away, as in motor neurons. Directed transport required not only motors but also cytoskeletal filaments as tracks, and, as cells developed cytoskeletons, the viscoelasticity of the cell interior became much more complex than that of a solution of proteins and nucleic acids. Many studies have modeled the eukaryotic cell interior as a polymer gel or a soft colloidal glass rather than a liquid, but

the bacterial cytoplasm was until recently thought to lack the complex gel–sol transitions of eukaryotic cells. A report by Parry *et al.* [1] now shows that this simple model is not adequate. The baseline state of bacterial cytoplasm shows complex properties reminiscent of materials such as glasses, and the motion and fluidity of bacterial cytoplasm might depend on the constant activity of enzymes. These enzymes are not traditional motors, but they can jostle the cytoplasm more vigorously than thermal fluctuations alone [2].

Almost from the time that scientists first examined living cells using the light microscope, they noticed the transient formation of distinct regions of cytoplasm from which diffusing or transported intracellular particles were excluded and in which no Brownian motion was observed. These solid-like regions have been described as glassy or 'hyaline' since at least the 19th century [3]. Other biologists used the term gel [4] — derived from the Latin *gelare*, to freeze — to describe these non-fluid regions of cytoplasm. The precise meanings of the terms glass and gel have been notoriously hard to define, but the physical properties of the cell interior drew the attention of

both biologists and physical chemists who made analogies between the cytoplasm and other complex fluids [5]. The idea that the cell is filled with an invisible (to contemporary microscopes) polymer network was, however, not the ubiquitous image that it now is in cell biology, and concepts of colloidal physics, foams, and molecular crowding were considered to account for the glassy appearance of the cell interior. An enormous amount has now been learned abut the molecules and assemblies that endow soft cells with variably fluid or solid properties, even if there are still many discussions about whether this living material has more in common with polymer networks, soft glasses, or some other viscoelastic materials.

The concept of cytoplasmic glassiness occurs in many different contexts in animal and plant cells. For example, a category of malignant cells termed 'glassy cells' is used in diagnosis of some cancers [6], and phase transitions similar to inorganic glass transitions, where even small molecules have restricted motion, occurs in the cytoplasm of plants that can survive desiccation [7,8]. In contrast, the cytoplasm of bacteria has generally been considered to be like a simple fluid filled with many solutes, but not so concentrated or reticulated as to form polymer networks or colloidal solids. This distinction between prokaryotic and eukaryotic cytoplasm was evident in early studies, such as in an analysis of the cytoplasmic fluidity of different pathogens in which the eukaryotic parasite *Entameoba histolytica* was

compared with the bacterium *Escherichia coli*: "In *E. coli* the ecto- and endoplasm can with difficulty be distinguished… and there is never present in this species the glass-like, perfectly hyaline appearance of the ectoplasm invariably observed in *E. histolytica*" [9].

Parry *et al.* [1], now armed with higher precision imaging methods and motivated by a recent flurry of activity in soft matter and biophysics that describes the cytoplasm as an active material far from equilibrium, take a new look at diffusion in the bacterium. They find some striking effects that depend on the size of the diffusing species and the metabolic state of the cell. By tracking the motions of individual protein assemblies and other particles of different sizes, the authors show that small molecules indeed move in a manner consistent with diffusion, but, when chemical energy stores are depleted by addition of compounds like dinitrophenol that prevent the generation of ATP, larger solutes appear to be trapped in cages from which they can only infrequently escape. Even when they can move in a limited manner, they do so at different rates in different regions of the bacterial interior. The need for energy in order to observe diffusive motion does not involve traditional molecular motors or the movement of chromatin driven by DNA-remodeling enzymes. Parry *et al.* [1] conclude that the constrained and location-dependent motions of large solutes in energy-depleted but otherwise intact bacteria reveal that the basal state of the bacterial cytoplasm is best described as having glass-like properties, which the metabolic energy of the cell can fluidize.

As evidence for glassy behavior, Parry *et al.* [1] show three characteristics also found in more standard glasses: non-Gaussian fluctuations; nonergodic behavior; and dynamic heterogeneity. Of these, ergodicity is a particularly fascinating idea introduced by Boltzmann approximately 150 years ago, in an effort to reconcile the second law of thermodynamics with mechanics and the atomic theory of matter. As examples of ergodicity, most materials are locally homogeneous, when viewed over sufficiently long time periods. For instance, in a simple liquid, different regions of the fluid will appear to be different on the scale of nanometers,

although these differences will vanish over the course of time. Different regions of the fluid can be thought of as different samples, and these will have the same average properties over time: the sample or *ensemble average* is the same as the *time average* of a single sample. This is the essence of ergodicity.

As appealing as the ergodic hypothesis may be, however, it is not always true in practice. When cooled down, some liquids will undergo a transition to a glass, in which differences from point to point in the sample become frozen, and these differences do not vanish over time (even on geological or astrophysical time scales!), as shown in Figure 1. Parry *et al.* [1] observe such a violation of ergodicity in the bacterial cytoplasm, along with the closely associated property of dynamic heterogeneity: given that not all regions of a sample have the same average properties, one can observe significant spatial variation of the local dynamics, e.g., of particles within the medium. In addition to their findings of non-Gaussian fluctuations, nonergodicity and dynamical heterogeneities, Parry *et al.* [1] also find that metabolic processes significantly enhance motion in bacteria and can even appear to 'uncage' otherwise constrained or confined particles. Specifically, the authors find that when enzymes are turned off in response to chemical signals, like nutrient deprivation, motion in the cytoplasm is strongly suppressed. They interpret the effect of metabolism as a sort of fluidization of the otherwise solid-like cytoplasm by enzymatic activity. Interestingly, studies of plant cell cytoplasm show the converse relationship: when cytoplasmic fluidity is lost by removal of water in desiccation-tolerant plants, enzymatic activity ceases even without chemical modification of the enzyme [10].

While the suggested analogy between the bacterial cytoplasm and glassy systems is a very interesting one, other interpretations are possible. Polymer and colloidal gels also exhibit nonergodicity and dynamical heterogeneity [11], while molecular motor activity is known to lead to non-Gaussian effects in biopolymer systems [12]. Nevertheless, with the results reported by Parry *et al.* [1], bacteria join eukaryotic cells as having cytoplasms that cannot be
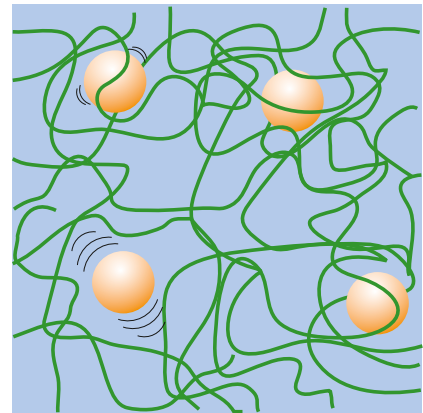

Figure 1. Constrained particle motion in a gel.
The motion of particles embedded in an inhomogeneous polymer gel show dynamical heterogeneity, with highly constrained motion in denser regions (upper left and right) and freer motion in regions of lower concentration (lower left). In a cross-linked gel, these heterogeneities persist in time, thereby breaking ergodicity.

characterized as solutions of freely diffusing molecules. These findings have implications for how rate constants and biochemical reaction pathways that are defined in dilute solution *in vitro* can be applied to the intracellular setting. As was recognized by earlier generations of cell biologists, the concepts and methods of physical chemistry have a deep, yet only partly appreciated, relevance to biological function.

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Pheromones: The Scent of a Male

There has been an enduring fascination with discovering biological odours which can evoke behavioral and physiological responses in mammals. New findings in goats have now identified a key molecule involved in the effect male odours have on female reproductive cycles.

Keith M. Kendrick

Despite extensive evidence for biologically active pheromones in invertebrate species the search for them in mammals has met with less success. The term 'pheromone' was first introduced over 50 years ago [1] to describe airborne communication molecules by which individual animals could evoke robust and specific physiological/behavioral effects in recipient individuals of the same species. Pheromones can either have a 'releaser' function by promoting rapid behavioral effects, such as sexual or aggression responses, or act as 'primers' by facilitating longer term physiological changes, which may also ultimately influence behavior.

While there is reasonable evidence that biological odours can influence both physiology and behavior in a number of mammalian species, it is clear that their effects are more variable and generally less specific than in invertebrates [2,3]. As such their actions do not really conform to the strict definition of pheromones, particularly in terms of being single molecules having both species and functional specificity and whose effects are not influenced by learning. Nevertheless, the term has been adopted widely in mammalian olfactory research despite this caveat. In mammals the main focus has been on identifying pheromones influencing reproductive physiology and behavior, notably the control of puberty, ovulation and sexual attraction and receptivity. Putative signaling pheromones have also been localized to a variety of different biological sources such as urine, tears, skin glands, wool, saliva and vaginal secretions. In mice, for example, the major urinary protein pheromones in male urine, 2-sec-butyl-4,5-dihydrothiazole, 3,4-dehydro-exo-brevicomin, α- and ß-farnesene, and 6-hydroxy-6-methyl-3-heptanone from the preputial gland all appear to have an influence on oestrus synchronization and acceleration of puberty in females [4]. The peptide exocrine gland-secreting peptide 1 (ESP1) released in the tear fluid of male mice also enhances female sexual receptivity [5]. In hamsters, female vaginal secretions contain dimethyl disulfide which in association with a carrier molecule, aphrodisin, facilitates male sexual responses [6]. In pigs, androstenone (5α-androst-16-en-3-one) and androstenol (5α-androst-16-en-3-ol) in boar saliva facilitate sexual receptivity in females [7].

One of the areas where identification of pheromones has proved elusive has been in relation to the impact that male odours have on female reproductive cycles in seasonally breeding species such as sheep and goats. Odours from the wool of rams and from the head and sebaceous glands of bucks can stimulate ovulatory cycles in seasonally anoestrus females and thereby prolong the breeding season. This has become known as the 'male effect' and is testosterone dependent. In fact this primer pheromone effect is not restricted to restoring ovulatory cycles but can also influence their duration and synchronicity (see [8]). In keeping with a less rigorous definition of pheromone specificity in mammals relevant odours from the two species can influence each other to some extent. Also other non-olfactory sociosexual stimuli from the male, such as sexual vigour, are influential. Further, the number of females already ovulating modulates the strength of the male effect [8] so female-produced pheromones may act synergistically with those produced by males to induce/synchronize ovulation.

A key problem which has hindered identification of the 'male-effect pheromone', and indeed other mammalian pheromones, has been the lack of robust biomarkers to test the efficacy of the many different potential candidate molecules. In this issue of *Current Biology*, Murata *et al.* [9] have utilized such a biomarker to identify a key novel odourant molecule from the skin and sebaceous glands of the male goat responsible for influencing female reproductive cycles. This group has previously established this biomarker using recordings of the electrical activity from the hypothalamic region containing the small population of gonadotrophin releasing hormone (GnRH) neurons responsible for stimulating pulsatile release of luteinizing hormone (LH) from the anterior pituitary and subsequently ovarian function [10,11].

By a systematic exploration of different extracts from samples collected from the head of the male using gas chromatography and mass spectroscopy they have identified a small number of ethyl-branched aldehydes and ketones which reliably increased electrophysiological activity. In particular, 4-ethyloctanol proved to be the most influential single molecule, although a cocktail of 18 compounds including this was the most effective, suggesting that a number of other components also contribute. The authors have yet to demonstrate directly whether 4-ethyloctanol alone