

Minireview

Chaotic actin

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Abstract

Living cells organize their internal contents by coordinating the chaotic thermal motion of protein molecules. A recent study, combining experiment with stochastic simulation, shows how this might be achieved in the case of actin polymerization.

As the big battalions of post-genomic research raise their sights from the nucleus to the rest of the cell, they will encounter a much wilder terrain. The further you get from DNA, the more that Demon Chaos reigns. Nucleotide sequences are copied from cell to cell with machine-like precision, so we can usefully speak of 'information content', 'coding', or 'blueprints'. But out in the hinterland of the protein-rich cytoplasm, instructions coming from DNA are blurred by distance - like the dog-whistles of a shepherd trying to control a large and unruly flock of sheep. Genome studies may tell us what all the components of a cell are, and give insight into their structure. But DNA sequences alone can never tell us how to manufacture hands, eyes and brains when the building blocks are dancing with manic ferocity due to thermal motion.

An issue of *Science* last year (3 December 1999) featured mechanisms used by living cells to perform quality control in basic cellular processes such as DNA replication and protein synthesis - 'quality control' here being business-speak for chaos management. Chaperones, proteasomes, and base-excision repair complexes have all evolved so as to ensure that the genome operates with high accuracy. These molecular machines are diverse in design but have in common the fact that they consume ATP molecules and use the energy that is provided to create order. How this coupling is achieved is especially transparent in the elegant strategy known as kinetic proofreading, first posited by John Hopfield in the mid-1970s [1]. His idea was that an enzyme reaction can be made more selective if a relatively slow energy-utilizing step is introduced between substrate

binding and catalysis. Substrates that bind weakly to the enzyme have a higher off-rate and consequently only rarely remain associated long enough to react. Kinetic proofreading is now thought to operate in many basic genetic processes, such as protein translation and DNA untangling, and it might also be important in the cytoplasm - for example in the action of small GTP-binding proteins.

We know much less about how order is created on a larger scale. Cell polarity, embryonic patterns, left-right asymmetry of the body, and even stereotypical behavior, are all dictated by genes. How genetic information can operate through a fog of thermal motion to produce such patterns is for the most part a mystery. But we can be fairly sure that the cytoskeleton will be an important target. It is clear, for example, that molecular motors moving along cytoskeletal filaments operate close to chaos. Their movements are partly diffusive in origin, and although motors generate chemical energy by hydrolysing ATP, part of this energy is used to ensure that their movements are unidirectional. Another interface between stochasticity and the cytoskeleton is in the polymerization of microtubules, which Mitchison and Kirschner [2] showed in the 1980s to occur in an irregular, hesitant manner known as dynamic instability. Microtubules grow by the end-addition of tubulin dimers associated with GTP and shrink by releasing tubulin-GDP from these same ends, the nucleotide having been slowly hydrolysed while in the polymer. This curious and seemingly wasteful behavior is believed to make microtubule polymerization sensitive to external conditions. The delay caused by GTP hydrolysis works in a manner not unlike that of kinetic proofreading to

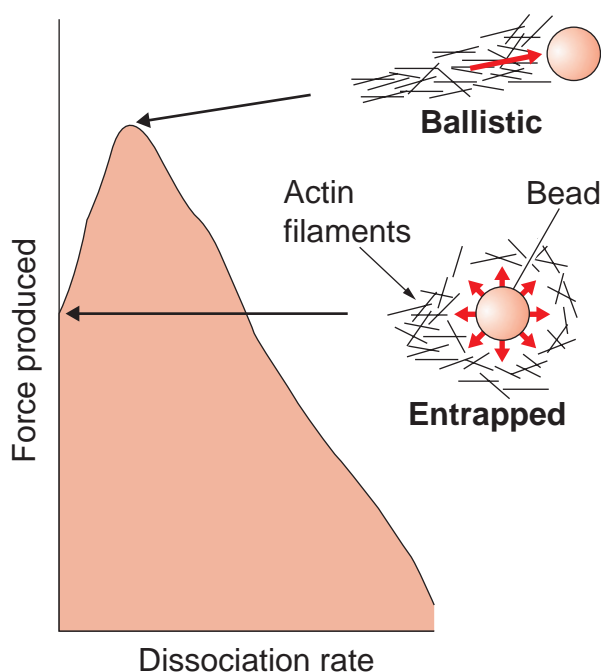


Figure 1
Cooperative action of polymerizing actin filaments. Calculations of the force exerted by actin filaments on a nucleating bead show its dependence on the dissociation rate of actin monomers. At low dissociation rates, the bead experiences a very small force and remains trapped in its cloud of cross-linked actin. At an optimal value of dissociation rate, the forces are sufficient to drive the bead through its actin cloud and to rocket through the cytoplasm (data from [3]).

permit microtubule ends to explore their surroundings. Dynamic instability helps to explain, for example, how mitotic spindles find the center of a cell, and how cells become polarized by external signals.

A recent study has now uncovered what could be a third strategy of chaos management, operating in this case on actin filaments [3]. The experimental system was derived from the pathogenic bacterium *Listeria*, which spreads through the cells it infects by inducing polymerization of host actin. Filaments are nucleated at one end of the bacterium and then cross-linked by proteins obligingly supplied by the infected cell. The 'comet tail' of actin that is produced then drives the bacterium through the cytoplasm (and into a neighboring cell). A highly simplified system based on this phenomenon was set up in which the bacterial protein ActA, which catalyses actin polymerization on the bacterium, was coupled to the surface of spherical polystyrene beads, 0.5 μm in diameter. The beads were suspended in a cytoplasmic extract obtained from frog eggs, to which fluorescently labeled actin had been added. Because of the ActA on its surface, each bead developed a symmetrical cloud of actin filaments which then became progressively cross-linked by

factors in the cytoplasm. As the cloud became more structured, the Brownian movement of the beads became increasingly restricted and short-range. But occasionally - like a rabbit breaking free of a snare - an individual bead would abruptly quit its shell of actin and migrate rapidly through the cytoplasm, moving in an unprecedented and apparently random direction. As the now ballistic bead moved, it produced a trail of cross-linked actin filaments, closely mimicking in this regard the behavior of living bacteria.

How can we explain this strange behavior? Van Oudenaarden and Theriot [3] propose the following explanation, which they support by computer simulations. The bead is pushed, they assert, by the polymerizing ends of actin filaments, which, as they grow longer and become increasingly cross-linked, press ever more closely onto the bead surface. The rising pressure restricts access of actin monomers to the ends of actin filaments until growth becomes possible only if and when the filament moves transiently away from the bead by thermally driven flexing. With each addition of a new monomer, moreover, the filament exerts a slightly greater pressure against the bead, a mechanism described theoretically by Mogilner and Oster [4] in 1996, and dubbed by them an elastic Brownian ratchet. The crucial question of the present study, however, is how the Brownian ratchets of filaments around the bead surface can work in concert so as to break symmetry and allow the bead to escape. The proposed answer is that the polymerization of individual actin filaments is coordinated by the slight movements of the bead. A small push to the bead caused by the addition of an actin molecule to one filament makes the addition of a second actin to a filament close by slightly easier - that is, neighboring filaments tend to grow together. Conversely, and for the same reason, monomer addition to actin filaments on opposite sides of a bead work against each other, the one tending (very slightly) to inhibit the other. Stochastic simulations with 50 or so symmetrically arranged actin filaments show that, if conditions are just right, groups of filaments will grow cooperatively and exert enough force to drive the bead out of its shell and through the cytoplasm (Figure 1).

What magical conditions allow the bead to break out of its symmetric cage? One important parameter that emerges from the theoretical analysis is the rate of dissociation of actin monomers from the filament ends. If this is too slow, the bead remains trapped in its cocoon of actin and its movements become increasingly restricted. Too great, and the bead fails to develop a dense actin network, and continues its Brownian movements unimpeded. Only in a critical range of off rates is cooperation between actin filament possible so that the bead breaks symmetry and moves rapidly through the cytoplasm. But how does it happen that the artificial system has just the right values of dissociation rates? The clever part of the theory is that off rates actually change with time - increasing with the pressure developed between the actin shell and the bead. So the system arrives at the correct

range of off rates not through magic or divine providence but as an inescapable consequence of mechanics. We can also see from this argument why there has to be an incubation time before a bead can escape – the cloud of actin needs time to develop sufficient compressive tensions and hence reach the correct off rate.

Although these experiments concern movements in a reduced and highly simplified system, they are likely to have wide biological ramifications, given that actin polymerization drives many important cell movements, such as locomotion over surfaces [5]. From an even wider standpoint, they also flag the essential contribution statistical mechanics will make to post-genomic biology. As we learn more about the molecules of the cell and their atomic structure - and as techniques are developed that reveal individual molecules at work - so the underlying uncertainty of living processes comes to the fore. Cells have evolved strategies such as kinetic proofreading, dynamic instability and Brownian ratchets as part of a delicate balancing act. They have to tread the line between too much thermal energy, which can disrupt their delicate internal organization, and too little thermal energy, which means they are dead. Understanding this balancing act is a major challenge for the new biology - and the potent mix of experiment and computer simulation used in the study by Van Oudenaarden and Theriot is likely to be one of our best resources.

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