

## Trafficking and signaling through the cytoskeleton: a specific mechanism

Yinon Shafrir<sup>1</sup>, Daniel ben-Avraham<sup>1</sup> and Gabor Forgacs<sup>1,2,\*</sup>

<sup>1</sup>Department of Physics, Clarkson University, Potsdam, NY 13699-5820, USA

<sup>2</sup>Department of Physics and Biology, University of Missouri, Columbia, MO 65211, USA

\*Author for correspondence (e-mail: forgacs@clarkson.edu)

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### SUMMARY

**A specific mechanism for the intracellular translocation of nonvesicle-associated proteins is proposed. This movement machinery is based on the assumption that the cytoskeleton represents an interconnected network of filamentous macromolecules, which extends over the entire cytoplasm. Diffusion along the filaments provides an efficient way for movement and with this, for signal transduction, between various intracellular compartments. We calculate the First Passage Time (FPT), the average time it takes a signaling molecule, diffusing along the cytoskeleton, to arrive from the cell surface to the nucleus for the first time. We compare our results with the FPT of free diffusion and of diffusion in the permeating cytoplasm. The latter is hindered by intracellular organelles and the cytoskeleton itself. We find**

**that for filament concentrations even below physiological values, the FPT along cytoskeletal filaments converges to that for free diffusion. When filaments are considered as obstacles, the FPT grows steadily with filament concentration. At realistic filament concentrations the FPT is insensitive to local modifications in the cytoskeletal network, including bundle formation. We conclude that diffusion along cytoskeletal tracks is a reliable alternative to other established ways of intracellular trafficking and signaling, and therefore provides an additional level of cell function regulation.**

Key words: Cytoskeleton, Percolation, Signaling, Diffusion, First passage time

### INTRODUCTION

The normal functioning of a cell requires constant interaction with its environment. Signals from neighboring cells or the extracellular matrix are continuously perceived by membrane-bound receptors. A change in their state, due to either biochemical or physical modifications, typically leads to a cascade of signaling events. These may involve further chemical changes (e.g. phosphorylation of kinases) or physical processes (e.g. diffusion). Despite great efforts, at present very little is known about how a specific signal may be transmitted in vivo from the cell surface to a particular cellular compartment. The majority of documented biochemical signaling pathways are only partially mapped; usually events at the beginning and the end of the process are known, with question marks representing the rest (Lagna et al., 1996; Macias-Silva et al., 1996). The difficulty stems from the fact that often numerous signaling molecules have to arrange reversibly and precisely to carry the information (Isenberg, 1996; Pawson, 1995; Rosales et al., 1995). The questions of how these molecules find each other, or how they know what is the correct sequence of their reactions, are not commonly raised.

The notion that physical mechanisms may play an important role in intracellular signaling has recently emerged (Forgacs, 1995; Ingber, 1997; Janmey, 1998; Maniotis et al., 1997). Examples of combined mechanochemical or mechano-electrical signaling pathways abound. Sensory hair

cells change their shape upon variation in sound intensity and linear or angular acceleration in the surrounding environment (Hudspeth, 1997). These changes induce electrical signals, which propagate along nerve cells until they arrive at the central nervous system. The ubiquitous calcium ion can signal differentially, by changing the amplitude (AM mode) or the frequency (FM mode) of its wave propagation (Dolmetsch et al., 1997). Gene expression has been linked to stress through specific signaling pathways (Li et al., 1996a,b; Shyy et al., 1995; Wang et al., 1995). Nicklas and coworkers (1995) gave strong evidence that tension causes the dephosphorylation of kinetochore proteins. They demonstrated that in the absence of tension on the kinetochore of a single misaligned chromosome, anaphase is delayed. When tension is reestablished by micromanipulation, the cell enters anaphase and cell division can proceed; tension is a checkpoint signal. When the same proteins are phosphorylated (no tension), the cell stalls at metaphase. These findings indicate that in the course of signal transduction, physical processes affect biochemical reactions directly at the molecular level.

Even though the above examples clearly demonstrate the importance of physical mechanisms, the specific way that such mechanisms may participate in intracellular signaling remains elusive. The present work makes an attempt in this direction. In order to be an effective information carrier, a typical signaling molecule has to translocate from one part of the cell to another, before it can react with its downstream partner or reach its target. Movement may proceed via diffusion.

However, the crowded intracellular environment may compromise the efficiency of free, random diffusion (Jones and Luby-Phelps, 1996; Luby-Phelps et al., 1986; Ogston et al., 1973). Saxton (1994) has demonstrated that diffusion may become anomalous, when these obstacles are taken into consideration: the diffusion time is no longer proportional to the mean square displacement, but to a larger power of distance, and is considerably longer. Using the method of fluorescence recovery after photobleaching, Seksek et al. (1997) have quantified the translational diffusion of microinjected dextrans and ficolls. Their findings are consistent with anomalous diffusion for molecular sizes above 500 kDa. For smaller solute molecules the crowdedness of the cytoplasm does not seem to impede movement in the cells studied by these authors. When the signaling pathway involves a cascade of protein reactions, the movement of molecules has to be targeted, leading to further decrease in the effective diffusion rate (Berg and von Hippel, 1985). For directed intracellular translocation, vesicle-associated proteins may employ molecular motors (Presley et al., 1998). Although motor-driven transport is reliable, it is slow and requires the synchronized assembly and disassembly of the motor-cargo complex and a continuous input of energy. Very little is known about how nonvesicle-associated signaling proteins move through the cytoplasm.

We propose that diffusion along the cytoskeleton, using its fibers as guiding tracks, is an efficient mechanism for intracellular translocation of signaling molecules. Numerous signaling molecules are known to be anchored to the cytoskeleton (Mochly-Rosen, 1995; Janmey, 1998). For example, phosphofructokinase binds to, and is inhibited by, tubulin and microtubules (Lehotzky et al., 1993), v-Src, c-Src and some protein kinase C isoforms bind to the actin cytoskeleton (Grondin et al., 1991; Zalewski et al., 1991) and tyrosine kinase Yes colocalizes with vimentin intermediate filaments (Ciesielski-Treska et al., 1995).

Cytoskeletal filamentous macromolecules have net negative charge (Tang and Janmey, 1996; Tang et al., 1997). Signaling molecules are complex proteins. Several of the amino acids in them have net positive charge (e.g. arginine, lysine). Electrostatic interactions could thus restrict the motion of signaling molecules to the vicinity of the filaments and provide the conditions for their diffusion along the cytoskeleton. As we show, such a mechanism allows for first-principle calculations of the relevant diffusion constants (Kramers, 1940). Diffusion on the cytoskeleton may have several advantages over diffusion in the surrounding cytoplasm. The three major classes of cytoskeletal filaments (actin, microtubule and intermediate filaments) form an interconnected network, which spans the entire cytoplasm and provides numerous paths for reaching specific intracellular targets. These paths, by their very nature, avoid all obstacles formed by the various organelles. The typical diffusion time from the surface to the nucleus or other compartments is considerably shortened, since the cytoskeletal filaments constrain the molecules into more focused trajectories. Furthermore, the speed of signal transduction could be actively controlled by the cell, by altering the structure of its cytoskeleton. These suggestions are consistent with recent experimental findings (Galigniana et al., 1998; Kamimura et al., 1995).

In the present work we concentrate on the First Passage

Time (FPT): how long on average it takes a molecule, diffusing along the cytoskeleton, and starting at the cell's surface, to arrive at the nucleus for the first time. Suppose that a particle diffuses for some time  $t$ , and the root mean square displacement covered during that time is  $r$ . Then, one says that  $t$  is the diffusion time needed to cover the distance  $r$ . The FPT and the diffusion time are clearly related, but are actually distinct quantities: the former is the average time required to diffuse through a given distance towards a specified target, while the latter is the (fixed) time required to isotropically span a certain average distance. In our case the distance and target are fixed, and so the FPT is the appropriate measure.

At physiological conditions the concentration of cytoskeletal filaments is above the percolation threshold (the point where they form an interconnected network for the first time; Forgacs, 1995). We demonstrate that in this regime further increase in filament concentration results in decrease of the FPT along the cytoskeleton, which ultimately approaches the limit of the FPT in free diffusion. This conclusion indicates that, although under physiological conditions fast free diffusion of signaling molecules all the way from the cell surface to the nucleus is unlikely (see below), movement along the cytoskeleton is feasible and may be just as fast.

## MATERIALS AND METHODS

### Percolation model of the cytoskeleton

The concept of percolation, widely utilized in physical and engineering sciences (Sahimi, 1994), refers to the transition when by increasing the concentration of certain structural elements of a given system (e.g. the concentration of F-actin in the cytoskeleton) an interconnected network of these elements is formed, which extends from one end of the system to the other (see Fig. 1). The percolation transition is accompanied by important changes in the topological characteristics (connectivity, fractal dimension, etc.) and physical properties (viscosity, elasticity, conductivity, etc.) of the system in question (de Gennes, 1976a,b; Bouchaud et al., 1986; Clerk et al., 1990; Peyrelasse and Boned, 1990; Hamilton et al., 1998). Important to our case is the fact that a percolation network of filaments is capable, due to its interconnectedness, of transmitting information across the cell.

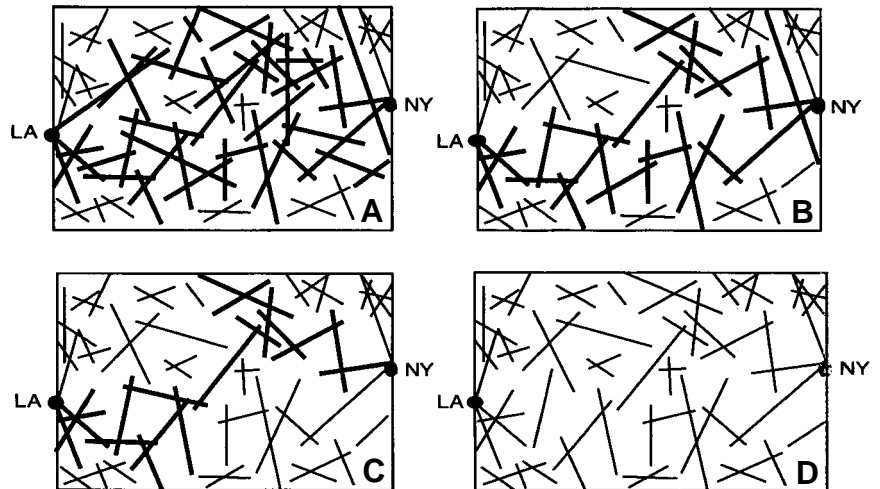
We hypothesize that the structure of the cytoskeletal mesh can be modeled and analyzed in terms of percolation theory. This has already been done for the case of *in vitro* actin networks, when it was shown that F-actin networks do indeed behave as percolation clusters (Sackmann, 1994; Tempel et al., 1996).

An attractive feature of a percolation network is that it is globally random. The final configuration achieves a state of maximum entropy, subject to the constraints of excluded volume interactions between filaments. [The latter give rise to local ordering of the filaments, seen in electron micrographs of the cell (Heuser and Kirschner, 1980; Small et al., 1994).] Thus, according to the percolation postulate there is no (free) energy expenditure associated with the global organization of the cytoskeleton - an obvious biological advantage over all other possible arrangements. In reality, energy is needed to disrupt and reestablish the chemical interconnections, i.e. to rebuild the filaments, but not for the establishment of any organized pattern.

### Model cytoskeleton

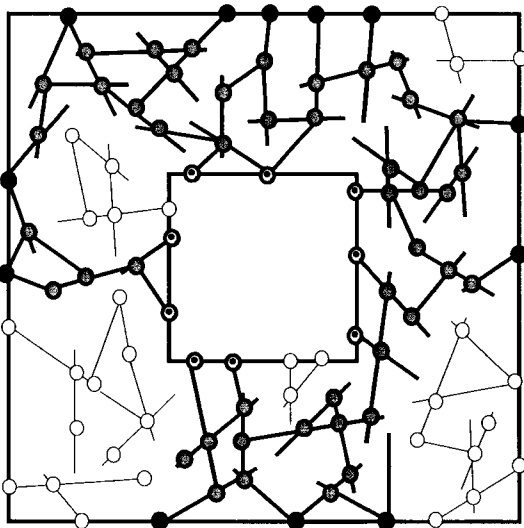
As a model for the cell we use a cube of side 5  $\mu\text{m}$ , centered inside another cube of side 10  $\mu\text{m}$ . The former cube represents the nucleus. Our model cytoskeleton consists of rigid rods. The rods may represent various cytoskeletal filaments if their diameter is chosen

**Fig. 1.** Illustration of the concept of a percolation transition. (A) The intact telephone network connecting Los Angeles (LA) and New York (NY) (prior to the age of cellular phones). This network consists of a multitude of cables, or optical fibers of (varying) finite lengths, is interconnected, and has the appearance of being random. (Heavy lines belong to the percolating network.) It also has redundancy; the signal from LA can arrive in NY in a number of ways. (B,C) If this network is subject to a series of disasters, with more and more of the links randomly destroyed, connection between the two cities would still be maintained up to a critical number of finite elements (although the time for the signal to arrive will typically increase). (D) Telephone service is definitely interrupted below the 'percolation threshold', when an interconnected cluster of connections extending from LA to NY no longer exists, although many finite clusters of wires may still be present.



appropriately. In the present work we use rods of diameter 10 nm with length between 0.5-1.5  $\mu\text{m}$ . Each rod has six parameters that describe its location in space: Cartesian coordinates for one of the endpoints, the rod's length and two angles, determining its orientation. The random network of rods is generated in a computer, using independent random numbers for each of the six parameters.

While constructing the network, the following rules are observed. Two rods cannot penetrate each other. Once two filaments are within minimal distance of one another (soft core=0.2  $\mu\text{m}$ ) a node is formed at the point of shortest distance and the two rods are said to be connected. Rods are restricted to the space between the nucleus and the cell surface. Special nodes are reserved for points where a rod reaches the surface of the nucleus (exit points) or the cell surface (entry point) with one of its ends (see Fig. 2). The FPT is calculated along a network constructed with the above rules.



**Fig. 2.** Two-dimensional projection of the model cell with a schematic representation of the model cytoskeleton. Filaments that interconnect from the cell surface to the nucleus are drawn with heavy lines. Those which do not form percolation paths are denoted by thin lines. Connected entry and exit nodes and nodes along percolating clusters are shown, respectively, as filled, dotted and shaded circles. All other nodes are represented by empty circles.

### Kramer's model of diffusion along cytoskeletal filaments

In order to estimate the diffusion constant of a signaling molecule along the cytoskeleton we follow the classical treatment of Kramers (1940). In our model a periodic one-dimensional field of force with barriers and valleys restricts the particle (signaling molecule) to the vicinity of a filament. In addition, the particle is also subject to irregular forces due to the surrounding medium (i.e. cytoplasm). It is these random forces that allow the particle to overcome the potential barriers separating valleys, and with this perform Brownian motion along filaments. Kramers' formalism provides an expression for the rate of movement from one valley to the other, from which the diffusion constant can readily be calculated. Some details of the calculations are given in Appendix A.

### First passage time through a percolation network

We have developed an algorithm for computing the FPT from any entry point to any set of exit points in a random network of filaments. A random network of filaments is characterized by junctions or nodes (where filaments contact each other, possibly through intervening proteins), and the lengths of the filaments in between the junctions. Our technique generates a set of linear equations for the FPT from all the junctions of the network to the set of exit points, i.e. the points where the filaments reach the nucleus. The equations are solved by standard algorithms e.g. Gauss-Seidel, or Successive Over Relaxation method; Press et al., 1986), yielding the desired FPTs. This algorithm is applied to the situation where the entry points are located in the vicinity of the cell membrane, where the signaling molecule commences its Brownian motion to the nucleus. The algorithm is detailed in Appendix B.

### Computer

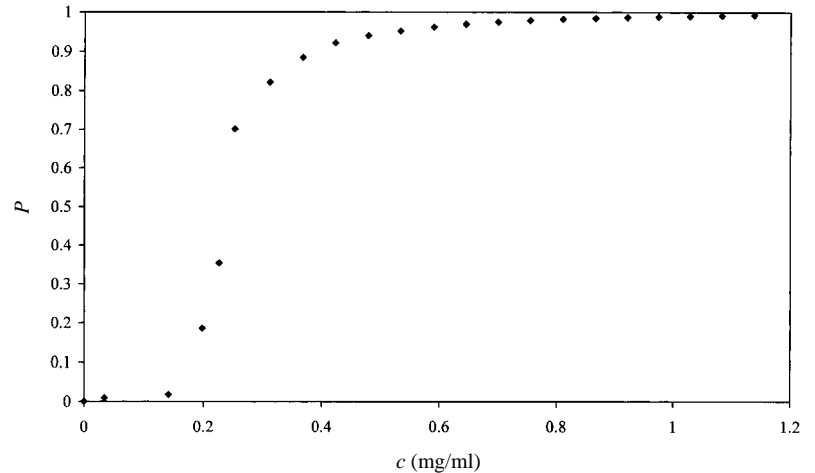
Calculations were performed on IBM Power PC Risc 6000 stations. CPU time for one run (construction of the cytoskeleton and the calculation of FPT for a given filament configuration) varied between 40 minutes and 10 hours, depending on the filament concentration.

## RESULTS

### The cytoskeleton as a percolation network

According to our model, a molecule can carry a signal from the cell surface to the nucleus if it can diffuse along an interconnected network of cytoskeletal filaments. There are three major classes of such filaments: actin, microtubules and

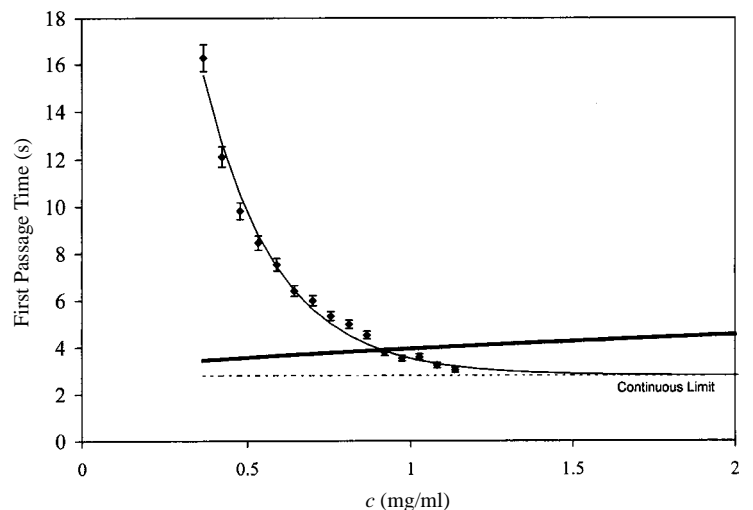
**Fig. 3.** The probability  $P$  that a given rod or filament within our model cell belongs to a percolation network as the function of filament concentration  $c$ . The length of filaments varies between 0.5-1.5  $\mu\text{m}$ , and their diameter is 10 nm. Filaments cannot penetrate each other. Two filaments are interconnected and form a node if they are within each other's soft core. The soft core is the diameter of an imaginary cylinder, which is coaxial with the rods. The value of the soft core in the simulations is 200 nm, the linear size of typical cross-linking proteins (i.e. alpha actinin, filamin, plectin). Percolation occurs at filament concentrations above 0.2 mg/ml. Each point on the graph represents an average of 30-250 runs, depending on the value of  $c$ .



intermediate filaments. The proportion of these filaments depends on cell type, with actin typically being the most abundant. In the present work we consider only one type of filament. If these are identified with microfilaments, then nodes model actin-crosslinking molecules (e.g. filamin).

For low values of filament concentration  $c$ , the network is disconnected. For very large concentrations, there emerge numerous uninterrupted paths connecting entry and exit points. In between these two extreme situations, there is a critical percolation concentration  $c_{cr}$ , at which the cell's surface and its nucleus are connected for the first time (see Fig. 1). The probability  $P$  that a given subunit (i.e. filament in our case) belongs to the globally interconnected cluster, is a customary measure of how close the system is to the percolation transition. Clearly, this probability is strictly zero below the transition, where there is not a single connected path, and greater than zero above it. Fig. 3 shows the dependence of  $P$  upon  $c$  in our simulations. Note that in a finite system like ours there is always a finite probability that the randomly generated rods interconnect to form a path from the cell surface to the nucleus. The larger the size of the system, the smaller  $P$  becomes at subcritical (small) concentrations. (In the purely theoretical 'thermodynamic' limit of infinite size,  $P$  is indeed zero below a critical concentration; Sahimi, 1994.) As a consequence, in a real cell there could, in principle, always be an interconnected network of filaments extending from the cell membrane to the nucleus. However, at low filament concentrations such networks are not reliable transmitters of information.

**Fig. 4.** First Passage Time (FPT) along a random filament network (line through data points), as a function of filament concentration  $c$ . Filaments have the same properties as in Fig. 3. Error bars indicate that fluctuations in the FPT decrease rapidly with  $c$ . The value of the FPT along the discrete model cytoskeleton converges to that in the continuous cytoplasm without obstacles (broken line). (In these simulations we used  $D_1/D_3=0.93$ , and  $D_3=10^{-7}$   $\text{cm}^2/\text{second}$ ; see Appendix A). The prediction of Ogston's theory (Ogston et al., 1973) for the FPT in the cytoplasm, where the filaments act as obstacles, is shown with the heavy line. For higher concentrations, the increase along that line is exponential. Inclusion of other cytoplasmic organelles would further hinder continuous diffusion.

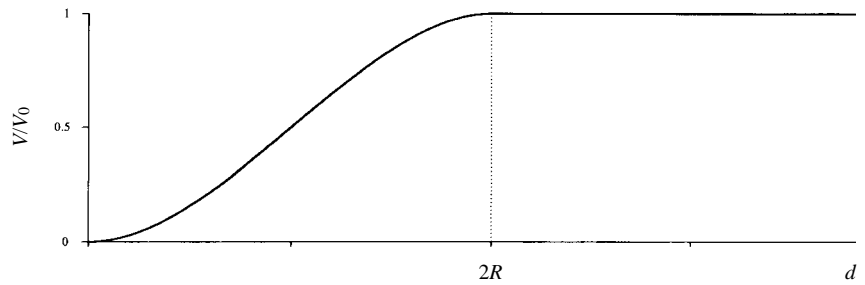


As can be seen from Fig. 3, percolation sets in at filament concentrations above 0.2 mg/ml, which is considerably below the physiological values. (In vivo F-actin concentration may reach 20 mg/ml, depending on cell type.) Thus, even in the event that many filaments are locked into bundles and stress fibers, and therefore fail to be distributed randomly and uniformly (see below), plenty still remain to form an efficient network. This result shows the advantage of employing the cytoskeleton for signal transduction: even if the number of filaments is drastically decreased, as long as they form a percolating network, with redundant connections between the cell surface and the nucleus, vital signals can reach their destination.

#### FPT as a function of filament concentration

The dependence of the FPT on filament concentration is summarized in Fig. 4. As could be expected, the FPT is large for small filament concentrations, when very few interconnected paths between the cell surface and the nucleus exist, but decreases rapidly with increasing  $c$ . Provided  $D_1$ , the (quasi one-dimensional) diffusion constant along the filaments is comparable with that for (three-dimensional) free diffusion,  $D_3$  (see Appendix A), the FPT along the cytoskeleton and in the permeating cytoplasm are close in

**Fig. 5.** Inaccessible volume  $V$  for the diffusive motion of a small molecule as a function of  $d$ , the distance from a filament. Obstacles are represented by spheres of radius  $R$ .  $V$  is normalized by the inaccessible volume  $V_0$  far away from the filament (in the continuum). If the molecule diffuses along a filament instead of diffusing in the continuous cytoplasm, it encounters a smaller fraction of obstacles.



value for filament concentrations as low as 1.5 mg/ml. We calculated the FPT for free diffusion by solving the appropriate differential equation (Dynkins' equation; Dynkins, 1961) on a fine grid, constructed in the space between the nucleus and the cell membrane. Note that diffusion along a discrete set of filaments imbedded in a continuum can never be faster than diffusion in the continuum itself, provided the corresponding diffusion constants are equal. The fact that we find diffusion along the filaments for physiological concentrations to be as fast as in the cytoplasm, is itself surprising. The true advantage of our mechanism, however, is more evident when obstacles due to various organelles in the cell are considered.

In Fig. 4 we also compare our results with the findings of Jones and Luby-Phelps (1996), as analyzed in terms of Ogston's theory (Ogston et al., 1973). These investigators consider the cytoskeletal filaments as obstacles hindering intracellular diffusion, rather than tracks that can facilitate it. Note that with increasing  $c$  the FPT along the filaments decreases to a minimum, while the FPT in the permeating cytoplasm rises steadily. (At the physiologically relevant concentration of 5 mg/ml, Ogston's theory leads to an FPT of 64 seconds, which is more than 20 times our value.) The effect of obstacles is even more dramatic if organelles other than the cytoskeletal filaments are considered. Such obstacles represent a much larger excluded volume than the thin filaments of the cytoskeleton.

Clearly, a large molecular complex moving inside the cell would encounter obstacles whether it freely diffuses or glides along cytoskeletal filaments. In the latter case, however, the filaments themselves, by definition, do not represent barriers to motion. In addition, if the molecule approaches an obstacle, due to the interconnected nature of the cytoskeleton, it can avoid it by simply shifting from one filament to another. Such a shift means that the molecule is forced to change the direction of its motion. This may also take place spontaneously, each time the molecule arrives at a

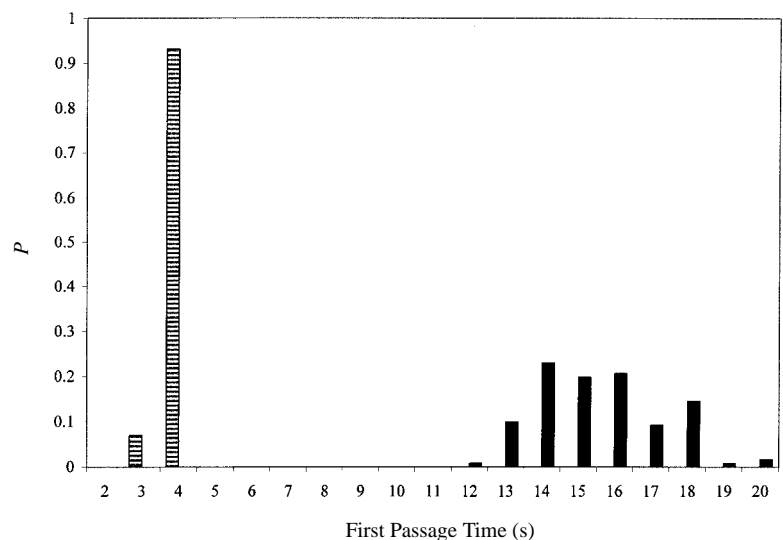
junction. In this sense the filaments themselves seem to represent obstacles. However, a change in the direction of motion is incorporated in diffusive transport and is already part of our formalism.

Furthermore, the motion of a messenger moving along a filament is hindered less (than in the cytoplasm) because of the depletion in the number of obstacles in the vicinity of a filament. We demonstrate this purely excluded volume effect in Fig. 5. We represent obstacles as spheres of radius  $R$ . Our calculation is based on the plausible assumption that the center of such obstacles cannot be within a distance  $R$  of a filament. Assuming the size of the diffusing molecule is considerably smaller than the typical size of an obstacle (e.g. lysosome, mitochondrion, etc.), we can calculate the fraction of inaccessible volume for motion at a distance  $d$  away from a filament. Beyond  $d=2R$ , the presence of the filament is immaterial; the diffusing particle encounters the excluded volume characteristic for the continuum. The region  $d<2R$  is, however effectively depleted in spheres of radius  $R$ ; motion along a filament is practically unimpeded.

### Sensitivity of FPT to the local structure of the cytoskeleton

The individual data points in Fig. 4 represent the average value of FPT, obtained by performing a number of runs (typically 30-250) at any given value of  $c$ . For very low  $c$  there are large fluctuations in the FPT, since the various network configurations generated by the computer may differ drastically. (It is possible, for example, that in some configurations there exists a connected path from the cell

**Fig. 6.** Sensitivity of fluctuations in the FPT to filament concentration. For the low concentration of 0.36 mg/ml (filled bars), the probability distribution  $P$  (i.e. normalized histogram) to find a given FPT is flat and the spread in its values is large. This is due to large differences in the various network configurations, or the sensitivity to local structure of the cytoskeleton. At  $c=1.03$  mg/ml (hatched bars),  $P$  is much sharper and the FPT is practically insensitive to the local structure of the cytoskeleton. The total number of runs at any given concentration varied between 30 and 250, with fewer runs at higher  $c$ .



surface to the nucleus, whereas in others there is none and the FPT is infinite.) Fig. 6 illustrates the sensitivity of FPT to the configuration of the filament network. Due to the large fluctuations at very low  $c$ , the network is not a reliable information carrier. However, with increasing  $c$  fluctuations decrease rapidly. This indicates that already for relatively low filament concentration, considerably below the typical values found in living cells, the information-carrying ability of the cytoskeleton becomes practically independent of its local structure – a property necessary for reliable signal transduction.

Similar results are obtained if the local structure of the cytoskeleton is modified by changing the length distribution of filaments, as shown in Fig. 7A.

### The effect of bundling

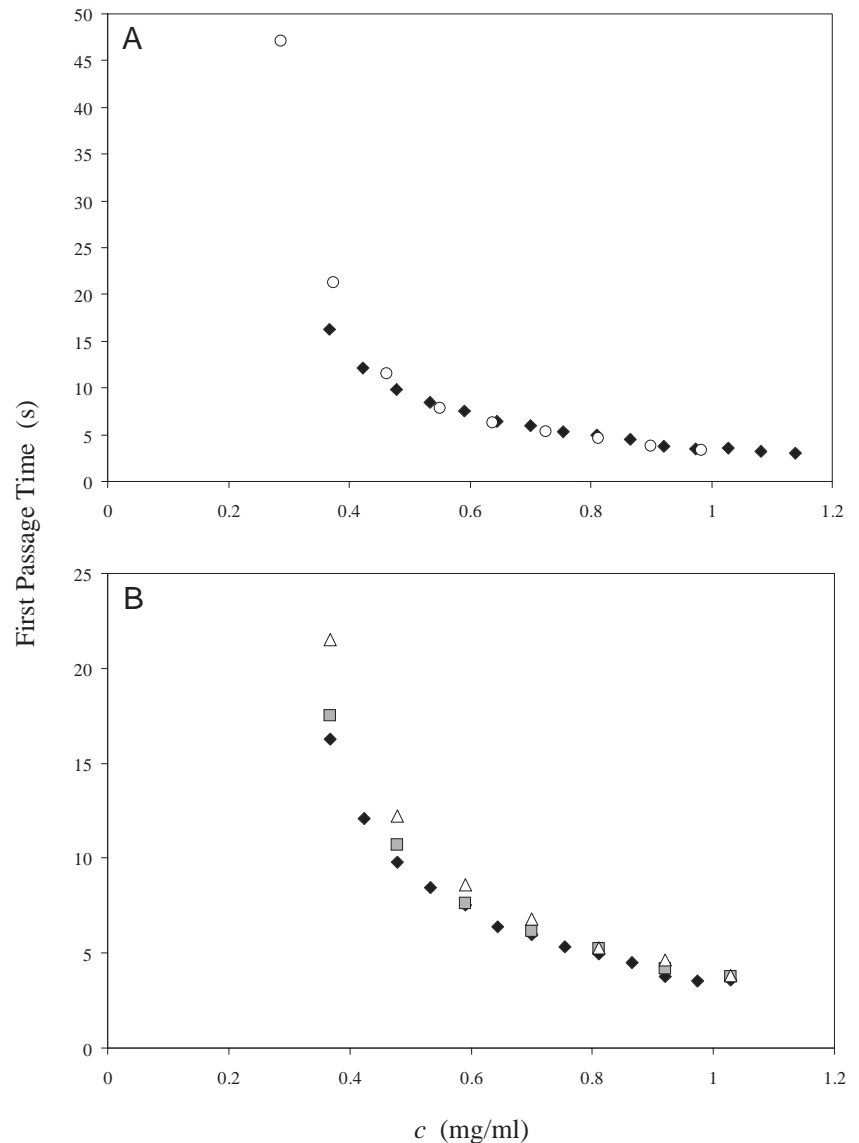
Actin filaments often form ordered structures, like stress fibers and bundles. If all the filaments were part of such structures, they could not propagate signals as postulated by the percolation model. Here we investigate the effect of local bundling in a cell whose cytoskeleton is intermediate between fully ordered and completely random. This is accomplished by modifying the algorithm for constructing the model cytoskeleton. Once a filament has been created, another one within its soft core is preferentially oriented parallel to it. This favors the formation of bundles. The number of filaments in a given bundle depends on the probability of the bias to orient filaments in parallel, a parameter we can easily change in the simulations. In Fig. 7B we present results for the FPT for two values of the bias, as compared with the case of no bias. We see that for  $c$  approx. 1 mg/ml, the FPT is practically insensitive to bundle formation, indicating again that already at such low concentrations the network can be reliably used for signal propagation.

## DISCUSSION

The role of the cytoskeleton in intracellular signaling has been demonstrated in numerous studies (for a review see Janmey, 1998). Gene expression can be drastically altered by either depolymerization of the cytoskeletal filaments

or by changing the global structure of the network. The assembly may control gene expression in both agonistic and antagonistic manners (Oren et al., 1999). The cytoskeleton presents temporary docking sites for numerous signaling molecules and by this regulates communication between different cellular compartments (Nebe et al., 1997; Janmey, 1998). Using the green fluorescent protein method Galigniana et al. (1998) showed that protein translocation through the cytoplasm may proceed along cytoskeletal tracks without the apparent involvement of motor molecules. Such movement assumes that the cytoskeleton provides uninterrupted paths between the initial and final points of motion. This is possible only if the cytoskeleton forms an interconnected network, apart from the rare case when motion from the cell surface to the nucleus involves a single filament. The most general of interconnected assemblies are percolation clusters.

In this work we propose a specific way of intracellular communication, utilizing the cytoskeleton formed by the interconnected network of actin, microtubules and intermediate filaments (Pollard et al., 1984; Svitkina et al., 1996). We have



**Fig. 7.** (A) Sensitivity of the FPT to filament length. Filament length varies uniformly between 0.5-1.0  $\mu\text{m}$  (open circles) or 0.5-1.5  $\mu\text{m}$  (diamonds). Away from the percolation transition at  $c$  approx. 0.2-0.3 mg/ml the FPT is insensitive to filament length. Choosing a narrower or broader distribution does not significantly change this conclusion. (B) The effect of local bundling on the FPT. Results for the FPT at 20% (squares) and 40% (triangles) bias to orient filaments within each other's soft core in parallel are compared to the case of no bias (diamonds).

suggested that molecules may diffuse along the filaments of the cytoskeletal mesh in a quasi one-dimensional manner, and with this effectively carry intracellular signals. In particular, we have studied the FPT, the first passage time for a given molecule to reach the nucleus starting from the cell surface. We have demonstrated that even for filament concentrations below physiological values the FPT is insensitive to the local structure of the network, including the bundling of filaments.

Our analysis indicates that when the hindering effect of intracellular organelles is considered, the proposed movement machinery, when available, is more efficient than free diffusion in the permeating cytoplasm (see Figs 4 and 5).

Our study, by its nature, is restrictive. Due to finite computer time we cannot reach true physiological filament concentrations. The results shown in Fig. 4, however, suggest that, as far as the FPT is concerned, we have already reached saturation at the highest  $c$  considered.

We follow a particular signaling pathway, the motion of a single molecule. A general signaling pathway may involve numerous kinases and phosphatases. Some of these molecules may diffuse also in the cytoplasm, find reaction partners immobilized on the cytoskeleton, and then continue along the tracks.

We use a static network, whereas real cytoskeletons are strongly dynamic assemblies, with continuous polymerization and depolymerization of filaments; however, during most of the cell cycle, under physiological conditions, in non-moving cells, only local changes take place in the structure of the cytoskeleton. Above the percolation limit such changes would not alter the interconnected nature of the network, and as demonstrated here, would not modify the value of FPT. Our argument fails if the residence time of the diffusant on a single filament is shorter than the lifetime of the filament. As found by McGrath et al. (1998), for physiological concentrations, the typical turnover time of a cytoskeletal actin filament is around 6 minutes. Our value for the FPT, which incorporates motion along several filaments (on average at least three, to span the distance from the cell membrane to the nucleus), is about 3 seconds (using  $D=10^{-7}$  cm<sup>2</sup>/second, a value similar to that found by McGrath et al., 1998), which is considerably shorter than the turnover time of even a single filament. (Since the FPT is proportional to the diffusion constant, even a tenfold decrease in  $D$  does not modify this conclusion.)

We model cytoskeletal filaments as rigid rods, instead of considering them as semiflexible. In this work we consider actin-like filaments with lengths of the order 1  $\mu$ m, which is typical for living cells. The persistence length of actin is of the order 10  $\mu$ m (Gittes et al., 1993; Ott et al., 1993; Isambert et al., 1995), thus under physiological conditions actin filaments behave mostly as rigid rods.

Our pathway starts at the cell surface and terminates at the nuclear wall. The question of how a potential transcriptional activator may penetrate the nuclear wall is beyond our analysis. (Depending on its size it may either diffuse through the nuclear pore opening or be actively transported through it.)

The concentration dependence of FPT, illustrated in Fig. 4, gives a prediction which, in principle, can be tested experimentally. Using the green fluorescent protein method in combination with cytoskeleton disrupting agents, the degree of filament interconnectedness can be differentially modified.

Following the translocation of a signaling molecule that uses cytoskeletal tracks, the time it takes to arrive from the cell surface to the nucleus can be assessed and compared with the results shown in Fig. 4. Some experimental work along these lines has already been carried out. Galigniana et al. (1998) have shown that the hormone-dependent movement of the non-vesicle associated steroid glucocorticoid receptor through the cytoplasm utilizes the cytoskeleton. Kamimura et al. (1995) have demonstrated that an intact microtubule network is necessary for the modulation of gene activity by intracellular non-vesicle associated vitamin D receptors.

A number of findings arrived at in this work could, in principle, be explained by the use of molecular motors. Indeed, recent work has demonstrated instances of motor-driven transport of supramolecular complexes not in vesicles. Such transport has been shown for flagellar protein subassemblies (Cole et al., 1998) and intermediate filament subassemblies (Pralhad et al., 1999). Since motor-driven transport is powered by energy producing ATP hydrolysis, molecular motors could push impeding obstacles aside. Furthermore, by the use of molecular motors of opposite polarity, such transport could effectively mimic diffusion. Such a mechanism has been suggested by Rodionov et al. (1998) and Rogers and Gelfand (1998) for the uniform distribution of melanosome pigment granules in melanophores. This diffusion, however, due to differences in motors, would most likely be biased. Effective biased diffusion has also been observed for motion due to a single motor (Okada and Hirokawa, 1999).

The main advantage of the mechanism proposed in this work in comparison with motor-driven transport is that no extra expenditure of energy is required. Energy production could also be a speed-limiting factor. Indeed, effective diffusion constants reported for motor driven transport are of the order of  $10^{-10}$  cm<sup>2</sup>/second (Okada and Hirokawa, 1999; Chen, 2000), whereas molecules of diameter of the order 100 nm could execute Brownian motion along filaments with a diffusion constant of  $10^{-7}$ - $10^{-8}$  cm<sup>2</sup>/second (McGrath et al., 1998).

In this work we considered only unbiased diffusion. In the case of bias the following competing factors would have to be considered. There would exist sets of filaments that are all biased into the same junction. Such a junction acts as a trap; once the diffuser is in such a junction, it has tremendous trouble getting out. In percolation networks, there exist dangling ends where the bias is away from the conducting network (backbone), and into the dangling end. These too act as effective traps. These factors would lead to the diffuser being mired in various spots to which it can get faster than by unbiased diffusion. Since the trapping spots are evenly distributed in a random network, this might be an efficient mechanism to quickly disperse an agent (such as melanophore pigment granules) throughout the cell (Rodionov et al., 1998).

In a real cell there could be filaments that are comparable in their length with the size of the cell. Thus there is a finite probability of finding 'hot' paths of just a few (long) filaments, such that are all biased towards the nucleus. This would dramatically reduce the FPT of the lucky messengers that happen to be upon such paths (but most other messengers will be hopelessly mired). The probability of such 'hot' paths is somewhat diminished by the fact that a typical motor

dissociates from a filament after 1-2 seconds, which may not be sufficient to reach the nucleus (Okada and Hirokawa, 1999).

In summary, the effect of bias on our mechanism is not easy to assess and requires further study. We expect, however, the major conclusions of the present work to remain qualitatively unchanged.

Our proposed mechanism is complementary to other well-established intracellular signaling pathways. Movement along the cytoskeleton may be advantageous for some signaling molecules (e.g. protein kinase C in NIH 3T3 cells; Schmalz et al., 1996), but disadvantageous for others (e.g. nuclear localization signal containing proteins; Schmalz et al., 1996). A signaling molecule, translocating along cytoskeletal tracks under physiological conditions, may perform free diffusion, when such tracks are not available (Galigniana et al., 1998). The main conclusion of our analysis is that the proposed mechanism is feasible and robust. Its combination and interaction with biochemical signaling pathways may provide variability and redundancy, and with this an additional level of cell function regulation.

## APPENDIX A

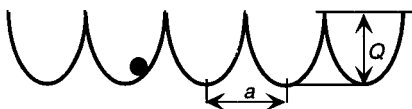
### Kramers' formalism

In his pioneering paper Kramers (1940) applied the transition state method to calculate the rate of chemical reactions. In particular, he evaluated the hopping rate of a particle, which is caught in a potential hole, and which can escape from it through the shuttling action of Brownian motion.

Consider the periodic potential, shown in Fig. 8, which restricts the motion of a particle to a one-dimensional chain. Within one period of width  $a$ , the potential is a parabola. Such a potential represents a harmonic restoring force, i.e. the force of a spring, whose magnitude is  $F=kx$ . Here  $k$  is the spring constant and  $x$  is the displacement of the particle measured from the center of the parabola. Without Brownian forces, the particle is trapped within one of the parabolas. In the presence of Brownian forces the escape rate  $r$  is finite, and according to Kramers (1940) is given by

$$r = \frac{k}{2\mu\sqrt{\pi}} \sqrt{\frac{Q}{k_B T}} e^{-\frac{Q}{k_B T}}. \quad (\text{A1})$$

Here  $Q=ka^2/8$  is the maximum value of the potential,  $k_B$  is Boltzmann's constant,  $T$  denotes the absolute temperature, and  $\mu$  is the friction constant due to the surrounding medium (i.e. cytoplasm in the case of a signaling molecule). In terms of the viscosity, the friction constant is given by  $6\pi\eta R$ , with  $R$  being



**Fig. 8.** The periodic potential used in Kramers' analysis to obtain the hopping rate of the particle over a potential barrier due to Brownian forces. Without Brownian forces the particle (filled circle) is stuck in one of the valleys.  $a$ , period width;  $Q$ , maximum value of the potential. See Appendix A for details.

the effective radius of the particle and  $\eta$  the viscosity of the medium. The above expression is valid for  $Q > k_B T$ . The diffusion constant along the chain is related to the hopping rate through  $D_1 = ra^2/2$ . For the free diffusion of the same particle in the medium (i.e. cytoplasm), the diffusion constant is given by  $D_3 = k_B T/\mu$  (Hobbie, 1997). In the present work we have used  $D_3 = 10^{-7}$  cm<sup>2</sup>/second. Finally, for the ratio of the one-dimensional and three-dimensional diffusion constants, one obtains

$$\frac{D_1}{D_3} = \frac{4}{\sqrt{\pi}} \varepsilon^{3/2} e^{-\varepsilon}. \quad (\text{A2})$$

Here  $\varepsilon = Q/(k_B T)$ . The expression in (A2) has a maximum value of 0.93 at  $\varepsilon = 1.5$ . At temperature  $T = 37^\circ\text{C}$ , this corresponds to a value for  $Q$  of approx. 1 kcal/mol, which is a typical energy for electrostatic interactions or hydrogen bonding. We conclude that under physiological conditions the values of the diffusion constants for motion along cytoskeletal filaments and in the cytoplasm could be very close.

## APPENDIX B

### Algorithm for the FPT

We now detail the algorithm for computing the first passage time from an arbitrary junction or node in an interconnected network of filaments to a subset of exit points. In our case, the exit points are those where the filaments penetrate the cell's nucleus. Junctions are points where two or more filaments are bound together through linking proteins, or points where filaments connect to the cell's surface, i.e. the entry points. Even though we are interested in the passage time from only the entry points, we must solve for all other junctions as well before the answer is available.

A junction may be connected to other junctions through intervening filaments, as well as to dangling ends. The dangling ends are essentially dead-ends: the diffusing particle may venture into them, but it must return to the junction and exit the dangling end before it can resume its sojourn to the exit points.

Let  $T_i$  be the first passage time from junction  $i$  to the subset of exit points. Let junction  $i$  be connected to junctions  $j_1, j_2, \dots, j_n$ . The first passage time from these junctions is  $T_{j_1}, T_{j_2}, \dots, T_{j_n}$ . Let the junction  $jk$  be connected to the junction  $i$  through a filament of length  $N_k$  ( $k=1, 2, \dots, n$ ). Finally, let the junction  $i$  be connected to dangling ends  $d_1, d_2, \dots, d_m$ , of 'mass'  $N_{d_1}, N_{d_2}, \dots, N_{d_m}$ . The mass of a dangling end is simply the length of all its constituent filaments (see Fig. 8A). The equation for  $T_i$  is:

$$\left( \frac{1}{N_{j_1}} + \frac{1}{N_{j_2}} + \dots + \frac{1}{N_{j_n}} \right) T_i - \frac{1}{N_{j_1}} T_{j_1} - \frac{1}{N_{j_2}} T_{j_2} - \dots - \frac{1}{N_{j_n}} T_{j_n} = N_{j_1} + N_{j_2} + \dots + N_{j_n} + 2(N_{d_1} + N_{d_2} + \dots + N_{d_m}). \quad (\text{B1})$$

If any of the  $jk$  neighboring junctions is an exit point, then we simply put  $T_{jk} = 0$ , for it takes zero time to arrive at a point if you are already there! By writing an equation similar to (B1) for every junction in the network, one obtains a system of linear equations, which are then solved for the  $T_i$  values. The solution can be obtained through any of several available numerical

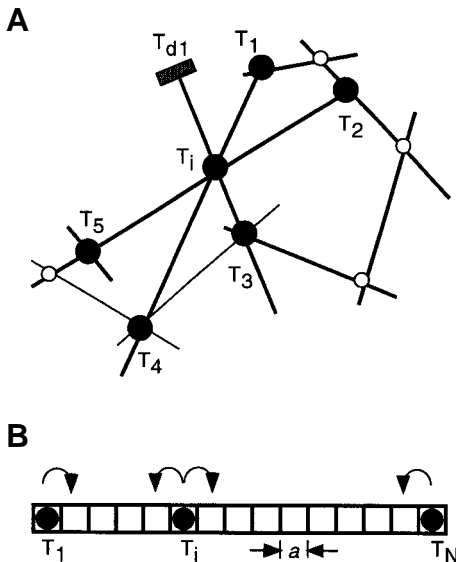
techniques. We find that the SOR (Successive Over Relaxation) variation of the Gauss-Seidel method is most appropriate (Vetterling, 1992).

To prove equation (B1), we subdivide the filaments into discrete units (similar to the tick marks on a ruler), and we assume that the diffusing particle hops from one tick mark to another. This is known as the random walk model. Suppose that the distance between adjacent tick marks is  $a$ . The diffusion limit is then recovered by letting  $a \rightarrow 0$ . Consider first a single filament of length  $L = Na$  (Fig. 8B). Let the first passage time from site (tick mark)  $i$  to the exit point be  $T_i$ . If the particle takes 1 time unit to step to the nearest site, then, with  $i=0$  and  $i=N$  being the endpoints of the filament

$$T_0 = 1 + T_1$$

$$T_1 = \frac{1}{2}(1 + T_2) + \frac{1}{2}(1 + T_0) ; T_i = \frac{1}{2}(1 + T_{i+1}) + \frac{1}{2}(1 + T_{i-1}) ; T_{N-1} = \frac{1}{2}(1 + T_N) + \frac{1}{2}(1 + T_{N-2}) . \quad (\text{B2})$$

The above system of equations expresses the simple fact that if the walker is at  $i$ , it must first get to either  $i+1$  or  $i-1$  before it 'exits'. Each possibility happens with probability  $1/2$ , and takes 1 unit of time to perform. The endpoint  $i=0$  (not an exit point) requires a special equation, because there is no point to its left. We also allow for the possibility that the endpoint  $N$  is not an exit point, so  $T_N$  needs not be zero (although it could be). Solving equation (B2) in terms of  $T_N$  we get:  $T_i = N^2 - i^2 + T_N$ .



**Fig. 9.** (A) Schematic representation of junction  $i$ , with its nearest neighbor junctions (filled circles) and dangling ends (rectangle). Dangling ends can be more complex than shown. A rectangle may denote a cluster of filaments, which have no other connection to the interconnected network than through the single filament indicated in the figure. Next nearest neighbor junctions (to  $i$ ) are denoted by open circles. Each filament on this figure is represented by a structure shown below. (B) The process of random walk along a single filament. Various possible positions of the walker (closed circle) and directions in which it can move are shown. In accordance with Fig. 8 within each square the walker moves under the influence of a parabolic potential.  $a$ , distance between adjacent tick marks; see Appendix B for details.

To simplify the last stages of the proof, we rewrite the solution for sites  $i=0$  and  $i=1$  in the seemingly idiosyncratic forms:

$$\frac{1}{N} T_0 = N + \frac{1}{N} T_N \quad (\text{B3})$$

and

$$T_1 = (N-1) + \frac{N-1}{N} T_0 + \frac{1}{N} T_N . \quad (\text{B4})$$

Consider now the junction  $i$  without dangling ends. If the walker is at  $i$ , it must step first to one of the  $n$  nearest sites before it can exit. Each possibility happens with probability  $1/n$  and takes 1 unit of time to carry out. Then, using equation (B4), we have

$$T_i = \frac{1}{n} \left[ 1 + (N_{j1} - 1) + \frac{N_{j1} - 1}{N_{j1}} T_i + \frac{1}{N_{j1}} T_{j1} \right] + \dots + \frac{1}{n} \left[ 1 + (N_{jn} - 1) + \frac{N_{jn} - 1}{N_{jn}} T_i + \frac{1}{N_{jn}} T_{jn} \right] . \quad (\text{B5})$$

The above equation can be rearranged to yield

$$\left( \frac{1}{N_{j1}} + \frac{1}{N_{j1}} + \dots + \frac{1}{N_{jn}} \right) T_i = \frac{1}{N_{j1}} T_{j1} + \frac{1}{N_{j2}} T_{j2} + \dots + \frac{1}{N_{jn}} T_{jn} + N_{j1} + N_{j2} + \dots + N_{jn} . \quad (\text{B6})$$

This is exactly equation (B1), when the mass of the dangling ends is zero.

We now focus on the special case where junction  $i$  has one simple dangling end in addition to the  $n$  nearest junctions (see Fig. 8a). This dangling end is at the edge of a filament of length  $N_d$ . Let the first passage time from this dangling end to the exit points be  $T_d$ . Then, the equation for junction  $i$  (see equation B6) is

$$\left( \frac{1}{N_{j1}} + \frac{1}{N_{j1}} + \dots + \frac{1}{N_{jn}} + \frac{1}{N_d} \right) T_i = \frac{1}{N_{j1}} T_{j1} + \frac{1}{N_{j2}} T_{j2} + \dots + \frac{1}{N_{jn}} T_{jn} + \frac{1}{N_d} T_d + N_{j1} + N_{j2} + \dots + N_{jn} + N_d . \quad (\text{B7})$$

Using equation B3, the equation for  $T_d$  is

$$\frac{1}{N_d} T_d = N_d + \frac{1}{N_d} T_i . \quad (\text{B8})$$

Thus, eliminating  $T_d$  from equations (B7) and (B8) and rearranging:

$$\left( \frac{1}{N_{j1}} + \frac{1}{N_{j1}} + \dots + \frac{1}{N_{jn}} \right) T_i = \frac{1}{N_{j1}} T_{j1} + \frac{1}{N_{j2}} T_{j2} + \dots + \frac{1}{N_{jn}} T_{jn} + N_{j1} + N_{j2} + \dots + N_{jn} + 2N_d . \quad (\text{B9})$$

The extension of the proof to the case of several dangling ends with more complex structure can be done recursively, to

finally arrive at equation (B1). This equation can also be obtained using the formalism by Koplík et al. (1988).

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## REFERENCES

- Berg, O. G. and von Hippel, P. H.** (1985). Diffusion controlled macromolecular interactions. *Ann. Rev. Biophys. Biophys. Chem.* **14**, 131-160.
- Bouchaud, E., Delsanti, M., Adam, M., Daoud, M. and Durand, D.** (1986). Gelation and percolation. *J. Physique (Paris)* **47**, 1273-1277.
- Chen, Y.** (2000). Theoretical formalism for kinesin motility I. Bead movement powered by single-headed kinesins. *Biophys. J.* **78**, 313-321.
- Ciesielski-Treska, J., Ulrich, G., Chasserot-Golaz, S. and Aunis, D.** (1995). Immunocytochemical localization of protein kinase Yes and Src in amoeboid microglia in culture: association of Yes kinase with vimentin intermediate filaments. *Eur. J. Cell Biol.* **68**, 369-376.
- Clerk, J. P., Giraud, G., Laugier, J. M. and Luck, J. M.** (1990). The AC electrical conductance of binary disordered systems, percolation clusters, fractals and related models. *Adv. Phys.* **39**, 191-309.
- Cole, D. G., Diener, D. R., Himelblau, A. L., Beech, P. L., Fuster, J. L. and Rosenblum, J. L.** (1998). Chlamydomonas kinesin-II-dependent intraflagellar transport (IFT): IFT particles contain proteins required for ciliary assembly in *Caenorhabditis elegans* sensory neurons. *J. Cell. Biol.* **141**, 993-1008.
- de Gennes, P. G.** (1976a). Critical dimensionality for a special percolation problem (relevant to the gelation in polymers). *J. Physique (Paris)* **30**, 1049-1054.
- de Gennes, P. G.** (1976b). On the relation between percolation theory and the elasticity of gels. *J. Physique (Paris)* **37**, L1-L2.
- Dolmetsch, R. E., Lewis, R. S., Goodnow, C. C. and Healy, J. I.** (1997). Differential activation of transcription factors induced by  $Ca^{2+}$  response amplitude and duration. *Nature* **386**, 855-858.
- Dynkin, E. B.** (1961). *Markov Processes*. Springer Berlin. 174pp.
- Forgacs, G.** (1995). On the possible role of cytoskeletal filamentous networks in intracellular signaling: an approach based on percolation. *J. Cell Sci.* **108**, 2131-2143.
- Galigiana, M. D., Scruggs, J. L., Herrington, J., Welsh, M. J., Carter-Su, C., Housley, P. R. and Pratt, W. B.** (1998). Heat shock protein 90-dependent (geldanamycin-inhibited) movement of the glucocorticoid receptor through the cytoplasm to the nucleus requires intact cytoskeleton. *Mol. Endocrinol.* **12**, 1903-1913.
- Gittes F., Mickey, B., Nettleton, J. and Howard, J.** (1993). Flexural Rigidity of Microtubules and Actin Filaments Measured from Thermal Fluctuations in Shape. *J. Cell. Biol.* **120**, 923-934.
- Grondin, P., Plantavid, M., Sultan, C., Breton, M., Mauco, G. and Chap, H.** (1991). Interaction of pp60c-src, phospholipase C, inositol-lipid and diacylglycerol kinases with the cytoskeletons of thrombin-stimulated platelets. *J. Biol. Chem.* **266**, 15705-15709.
- Hamilton, B., Jacobs, J., Hill, D. A., Pettifer, R. F., Teehan, D. and Canham, L. T.** (1998). Size-controlled percolation pathways for electrical conduction in porous silicon. *Nature* **393**, 443-445.
- Heuser, J. I. and Kirschner, M. W.** (1980). Filament organization revealed in platinum replicas of freeze-dried cytoskeletons. *J. Cell Biol.* **86**, 212-234.
- Hobbie, R. K.** (1997). In *Intermediate Physics for Medicine and Biology*. Springer, New York, pp. 87-88.
- Hudspeth, A. J.** (1997). How hearing happens? *Neuron* **19**, 947-950.
- Ingber, D. E.** (1997). Tensegrity: the architectural basis of cellular mechanotransduction. *Annu. Rev. Physiol.* **59**, 575-599.
- Isambert, H., Venier, P., Maggs, A. C., Fattoum, A., Kassab, R., Pantaloni, D., Carlier, M. F.** (1995). Flexibility of actin filaments derived from thermal fluctuations. Effect of bound nucleotide, phalloidin, and muscle regulatory proteins. *J. Biol. Chem.* **270**, 11437-11444.
- Isenberg, G.** (1996). New concepts for signal perception and transduction by the actin skeleton at cell boundaries. *Cell Dev. Biol.* **7**, 707-715.
- Janmey, P. A.** (1998). The cytoskeleton and cell signaling: component localization and mechanical coupling. *Physiol. Rev.* **78**, 763-781.
- Jones, J. D. and Luby-Phelps, K.** (1996). Tracer diffusion through F-actin: effect of filament length and cross-linking. *Biophys. J.* **71**, 2742-2750.
- Kamimura, S., Gallieni, M., Zhong, M., Beron, W., Slatopolsky, E. and Dusso, A.** (1995). Microtubules mediate cellular 25-hydroxyvitamin D3 trafficking and the genomic response to 1,25-dihydroxyvitamin D3 in normal human monocytes. *J. Biol. Chem.* **270**, 22160-22166.
- Koplík, J., Redner, S. and Wilkinson, D.** (1988). Transport and dispersion in random networks with percolation disorder. *Phys. Rev.* **A37**, 2619-2636.
- Kramers, H. A.** (1940). Brownian motion in a field of force and the diffusion model of chemical reactions. *Physica* **7**, 284-304.
- Lagna, G., Hata, A., Hemmati-Brivanlou, A. and Massague, J.** (1996). Partnership between DPC4 and SMAD proteins in TGF-beta signalling pathways. *Nature* **383**, 832-836.
- Lehotzky, A., Telegdi, M., Liliom, K. and Ovadi, J.** (1993). Interaction of phosphofructokinase with tubulin and microtubules. Quantitative evaluation of the mutual effect. *J. Biol. Chem.* **268**, 10888-10894.
- Li, S., Piotrowicz, R. S., Levin, E. G., Shyy, Y. J. and Chien, S.** (1996a). Fluid shear stress induces the phosphorylation of small heat shock proteins in vascular endothelial cells. *Am. J. Physiol.* **271**, 94-1000.
- Li, Y. S., Shyy, J. Y., Li, S., Lee, J., Su, B., Karin, M. and Chien, S.** (1996b). The Ras-JNK pathway is involved in shear-induced gene expression. *Mol. Cell Biol.* **16**, 5947-5954.
- Luby-Phelps, K., Taylor, D. L. and Lanni, F.** (1986). Probing the structure of the cytoplasm. *J. Cell Biol.* **102**, 2015-2022.
- Macias-Silva, M., Abdollah, S., Hoodless, P. A., Pirone, R., Attisano, L. and Wrana, J. L.** (1996). MADR2 is a substrate of the TGFbeta receptor and its phosphorylation is required for nuclear accumulation and signaling. *Cell* **87**, 1215-1224.
- Maniotis, A. J., Chen, C. S. and Ingber, D. E.** (1997). Demonstration of mechanical connections between integrins, cytoskeletal filaments and nucleoplasm that stabilize nuclear structure. *Proc. Natl. Acad. Sci. USA* **94**, 849-854.
- McGrath, J. L. Tardy, Y., Dewey, C. F., Meister, J. J. and Hartwig, J. H.** (1998). Simultaneous measurements of actin filament turnover, filament fraction and monomer diffusion in endothelial cells. *Biophys. J.* **75**, 2070-2078.
- Mochly-Rosen, D.** (1995). Localization of protein kinases by anchoring proteins: a theme on signal transduction. *Science* **268**, 247-251.
- Nebe, B., Bohn, W., Pommerenke, H. and Rychly, J.** (1997). Flow cytometric detection of the association between cell surface receptors and the cytoskeleton. *Cytometry* **28**, 66-73.
- Nicklas, R. B., Ward, S. C. and Gorbisky, G. J.** (1995). Kinetochores chemistry is sensitive to tension and may link mitotic forces to a cell cycle checkpoint. *J. Cell Biol.* **130**, 929-939.
- Ogston, A. G., Preston, B. N. and Wells, J. D.** (1973). On the transport of compact particles through solutions of chain polymers. *Proc. R. Soc. Lond.* **333**, 297-316.
- Okada, Y. and Hirokawa, N.** (1999). A processive single-headed motor: kinesin superfamily protein KIF1A. *Science* **263**, 1152-1157.
- Oren, A., Herschkovitz, A., Ben-Dror, I., Holdengreber, V., Ben-Shaul, Y., Seger, R. and Vardimon, L.** (1999). The cytoskeletal network controls c-Jun expression and glucocorticoid receptor transcriptional activity in an antagonistic and cell-type-specific manner. *Mol. Cell Biol.* **19**, 1742-1750.
- Ott, A., Magnasco, M., Simon, A. and Libchaber, A.** (1993). Measurement of the persistence length of polymerized actin using fluorescence microscopy. *Phys. Rev.* **E48**, R1642-R1645.
- Pawson, T.** (1995). Protein modules and signaling networks. *Nature* **373**, 573-580.
- Peyrelasse, J. and Boned, C.** (1990). Conductivity, dielectric relaxation, and viscosity of ternary microemulsions: the role of the experimental path and the point of view of percolation theory. *Phys. Rev.* **A41**, 938-953.
- Pollard, T. D., Selden, S. C. and Maupin, P.** (1984). Interaction of actin filaments with microtubules. *J. Cell Biol.* **99**, 33s-37s.
- Prahlad, V., Yoon, M., Moir, R. D., Vale, R. D. and Goldman, R. D.** (1998). Rapid movements of vimentin on microtubule tracks: kinesin-dependent assembly of intermediate filament networks. *J. Cell Biol.* **143**, 159-170.
- Presley, J. F., Smith, C., Hirschberg, K., Miller, C., Cole, N. B., Zaal, K. J. and Lippincott-Schwartz, J.** (1998). Golgi membrane dynamics. *Mol. Cell Biol.* **9**, 1617-1626.
- Press, W. H., Teukolsky, S. A., Vetterling, W. T. and Flannery, B. P.** (1992). *Numerical Recipes in C*. Cambridge University Press. 866pp.
- Rodionov, V., Hope, A., Svitkina, T. and Borisy, G.** (1998). Functional coordination of microtubule-based and actin-based motility in melanophores. *Curr. Biol.* **8**, 165-168.

- Rogers, S. and Gelfand, V. I.** (1998). Myosin cooperates with microtubule motors during organelle transport in melanophores. *Curr. Biol.* **8**, 161-164.
- Rosales, C., O'Brian, V., Kornberg, L. and Juliano, R.** (1995). Signal transduction by cell adhesion molecules. *Biochem. Biophys. Acta* **1242**, 77-98.
- Sackmann, E.** (1994). Intra- and extracellular macromolecular networks: physics and biological function. *Macromol. Chem. Phys.* **195**, 7-28.
- Sahimi, M.** (1994). *Applications of Percolation Theory*. Taylor & Francis, London. 255pp.
- Saxton, M. J.** (1994). Anomalous diffusion due to obstacles: a Monte Carlo study. *Biophys. J.* **66**, 394-401.
- Schmalz, D., Kalkbrenner, F., Hucho, F. and Buchner, K.** (1996). Transport of protein kinase C alpha into the nucleus requires intact cytoskeleton while the transport of a protein containing a canonical nuclear localization signal does not. *J. Cell Sci.* **109**, 2401-2406.
- Seksek, O., Biwersi, J. and Verkman, A. S.** (1997). Translational diffusion of macromolecule-sized solutes in cytoplasm and nucleus. *J. Cell Biol.* **138**, 131-142.
- Shyy, J. Y., Lin, M. C., Han, J., Lu, Y., Petrim, M. and Chien, S.** (1995). The cis-acting phorbol ester '12-O-tetradecanoylphorbol 13-acetate'-responsive element is involved in shear stress-induced monocyte chemotactic protein 1 gene expression. *Proc. Nat. Acad. Sci. USA* **92**, 8069-8073.
- Small, J. V., Herzog, M., Häner, M. and Aebi, U.** (1994). Visualization of actin filaments in keratocyte lamellopodia: negative staining compared with freeze-drying. *J. Struct. Biol.* **113**, 135-141.
- Svitkina, T. M., Verkhovsky, A. B. and Borisy, G. G.** (1996). Plectin sidearms mediate interaction of intermediate filaments with microtubules and other components of the cytoskeleton. *J. Cell Biol.* **135**, 991-1007.
- Tang, J. X. and Janmey, P. A.** (1996). The polyelectrolyte nature of F-actin and the mechanism of actin bundle formation. *J. Biol. Chem.* **271**, 8556-8563.
- Tang, J. X., Ito, T., Tao, T., Traub, P. and Janmey, P. A.** (1997). Opposite effects of electrostatics and steric exclusion on bundle formation by F-actin and other filamentous polyelectrolytes. *Biochemistry* **36**, 12600-12607.
- Tempel, M., Isenberg, G. and Sackmann, E.** (1996). Temperature-induced sol-gel transition and microgel formation in  $\alpha$ -actinin cross-linked actin networks: a rheological study. *Phys. Rev. E* **54**, 1802-1810.
- Wang, D. L., Wung, B. S., Shyy, Y. J., Lin, C. F., Chao, Y. J., Usami, S. and Chien, S.** (1995). Mechanical strain induces monocyte chemotactic protein-1 gene expression in endothelial cells. Effects of mechanical strain on monocyte adhesion to endothelial cells. *Circ. Res.* **77**, 294-302.
- Vetterling, W. T.** (1992). *Numerical Recipes Example Book (C)*. Cambridge University Press. 325pp.
- Zalewski, P. D., Forbes, I. J., Giannakis, C. and Betts, W. H.** (1991). Regulation of protein kinase C by  $Zn^{2+}$ -dependent interaction with actin. *Biochem. Int.* **24**, 1103-1110.