Percolation in a network with long-range connections: Implications for cytoskeletal structure and function

Paulo S.P. Silveira\textsuperscript{a,b,*,1}, Adriano M. Alencar\textsuperscript{a,b}, Arnab Majumdar\textsuperscript{c,d}, Miriam Lemos\textsuperscript{a,b}, Jeffrey J. Fredberg\textsuperscript{a}, Béla Suki\textsuperscript{d}

\textsuperscript{a} Program in Molecular, Integrative and Physiological Sciences (MIPS), Department of Environmental Health, Harvard School of Public Health, Boston, MA 02115, USA
\textsuperscript{b} Department of Pathology, Medical School of the University of São Paulo, São Paulo, SP, Brazil
\textsuperscript{c} Center for Polymer Studies and Department of Physics, Boston University, Boston, MA, 02215, USA
\textsuperscript{d} Department of Biomedical Engineering, Boston University, Boston, MA, 02215, USA

A R T I C L E   I N F O

Article history:
Received 3 November 2008
Available online 16 December 2008

PACS:
87.15.A
87.18.Nq

Keywords:
Cell connectivity
F-actin
Mechanics
Model
Simulation

A B S T R A C T

Cell shape, signaling, and integrity depend on cytoskeletal organization. In this study we describe the cytoskeleton as a simple network of filamentary proteins (links) anchored by complex protein structures (nodes). The structure of this network is regulated by a distance-dependent probability of link formation as \( P = p/d^s \), where \( p \) regulates the network density and \( s \) controls how fast the probability for link formation decays with node distance (\( d \)). It was previously shown that the regulation of the link lengths is crucial for the mechanical behavior of the cells. Here we examined the ability of the two-dimensional network to percolate (i.e. to have end-to-end connectivity), and found that the percolation threshold depends strongly on \( s \). The system undergoes a transition around \( s = 2 \). The percolation threshold of networks with \( s < 2 \) decreases with increasing system size \( L \), while the percolation threshold for networks with \( s > 2 \) converges to a finite value. We speculate that \( s < 2 \) may represent a condition in which cells can accommodate deformation while still preserving their mechanical integrity. Additionally, we measured the length distribution of F-actin filaments from publicly available images of a variety of cell types. In agreement with model predictions, cells originating from more deformable tissues show longer F-actin cytoskeletal filaments.

© 2008 Elsevier B.V. All rights reserved.

1. Introduction

Many different types of cells contain filamentary structures that form the contractile apparatus and the cytoskeleton (CSK) network [1–3]. Structural integrity of cells require end-to-end connectivity of the CSK. Such solid-like structural integrity is required for fast transmission of mechanical signals over long intracellular distances, as has been demonstrated in recent experiments [4], and suggests that the CSK forms a percolating network. However, the CSK also has to be able to reorganize itself during dynamic cellular functions such as cell spreading, crawling, division and contraction, when the cell flows almost like a fluid. Under these conditions the connectivity of the CSK is likely reduced.

Airway smooth muscle (ASM) as well as other smooth muscle cells have intriguing mechanical properties which are related to the connectivity of their CSK. For example, the ASM can generate an almost constant force over a large range of muscle lengths. This phenomenon is known as length adaptation and holds true from about half to almost three times the

* Corresponding author at: Medical Informatics, Department of Pathology, Medical School, University of São Paulo, SP, Brazil.
E-mail address: silveira@usp.br (P.S.P. Silveira).

1 Av. Dr. Arnaldo, 455 - São Paulo, SP, CEP: 01246-903, Brazil.

0378-4371/$ - see front matter © 2008 Elsevier B.V. All rights reserved.
in situ length of the muscle [5,6]. To account for length adaptation, several mechanisms have been suggested in the literature: (a) anchorage of CSK filamentous structures can change [7], (b) contractile units can make quick transitions from parallel-to-series arrangements [8], and (c) elongated muscles can organize longer connections to span across the cell structures [9]. In particular, Solway and co-workers have suggested that length adaption may be tightly associated with actin filament length [10–14].

We have recently presented a model of length adaptation based upon a network of rigid nodes connected by links. Conceptually, this model was able to unify the above-mentioned mechanisms. Specifically, length adaptation could be well accounted for by assuming that the probability to connect any two nodes decreases with the inverse of the distance between those nodes. Furthermore, when the connections were made longer this model was able to mimic features of the airway disease asthma [15,16]. These findings suggest that ASM mechanics may be closely related to long-range connectivity of the CSK networks.

In this study, we investigated the implications of CSK network organization that has long-range connectivity and is maintained in the vicinity of the percolation threshold [17–20]. Besides length adaptation, such organization would allow the cells to shift quickly between the solid-like and the fluid-like states. We hypothesized that the solid-like state would be achieved by increased polymerization, i.e., anchoring of a greater number of filaments such that the CSK network connectivity is beyond the percolation threshold, while the fluid-like state would correspond to depolymerization, or the temporary reduction of anchored filaments and resulting loss of CSK percolation.

To test this hypothesis, we study the percolation properties of networks with a wide range of connections. Networks with short-range connectivity, such as first-neighbors, are similar to many physical systems studied in the literature. Long-range networks similar to those recently found in the ASM CSK [4] are of great interest in physics [21] and have been studied in one dimension [22,23] and in two dimensions [24]. These studies show that with increasing system size, networks with short-range connections show finite percolation thresholds, while networks with long-range connections show decreasing percolation thresholds [22–24]. Here we further study networks with long-range connectivity and show that their properties are more sensitive to link density than those of short-ranged networks. Finally, in order to establish the connectivity properties of biological cells, we measured the distribution of F-actin filament lengths on images from cultured cells and compared them with the distribution of link lengths generated by models with long-range connectivity.

2. Network model formulation

We model the cytoskeleton as a network of links connecting nodes in a two-dimensional square lattice of \( L \times L \) nodes. These links represent the cytoskeletal filamentary structures such as actin filaments, while nodes represent dense bodies or focal adhesions, which are protein complexes organized in the cytoplasm and on the cell surface to which filaments can anchor. The probability \( P_{ij} \) of a link to connect nodes \( i \) and \( j \) is given by

\[
P_{ij} = p \left( \frac{1}{d_{ij}} \right)^{s}
\]

where \( d_{ij} \) is the distance between the nodes \( i \) and \( j \), and \( p \) is the probability of a nearest-neighbor link. The exponent \( s \) regulates the length distribution of the links. When \( s \to \infty \), only nearest neighbors with \( d_{ij} = 1 \) can be connected by a link. When \( s = 0 \), the probability of a link between any two nodes is independent of the distance \( d_{ij} \). Intermediate values of \( s \) can generate networks of all kinds, ranging from short-range to long-range connections (Fig. 1, left panels).

We constructed networks for a set of exponents \( s \) ranging from 0 to \( \infty \) for different system sizes \( L \). For each \( s \) and \( L \), random realizations of the network were generated repeatedly as a function of \( p \), while assigning links according to Eq. (1). In addition, the \( s \to \infty \) case was also simulated as a purely nearest-neighbor network, where each pair of neighboring nodes was assigned a link with probability \( p \) (Fig. 1c). We used periodic boundary conditions at the left and right boundaries of the lattice, while the top and bottom were considered to be ends, giving the network a cylindrical topology.

An assembled network was considered to be percolating when there was at least one path of adjoining links connecting the top boundary to the bottom. For each \( s \) and \( L \), the fraction \( f \) of percolating networks was computed as a function of \( p \) (Fig. 1, right panels). We found a transitional behavior around \( s = 2 \) (Fig. 1b). For \( s < 2 \), the lower knee of the \( f(p) \) curve shifted toward smaller values of \( p \) with increasing \( L \) (Fig. 1a), suggesting that larger networks were more likely to percolate for smaller values of \( p \). For \( s > 2 \), the lower knee of \( f(p) \) converged towards a larger value of \( p \) with increasing \( L \), as expected for nearest neighbor percolation (Fig. 1c).

The percolation threshold \( p_c \) was estimated using an algorithm proposed by Stauffer and Aharoni [17]. Briefly, each pair of nodes is connected by a link with probability \( P_{ij} \) given by Eq. (1). For a given seed, the algorithm first generates a sequence of pseudo-random numbers \( r_{ij} \) uniformly distributed between 0 and 1, assigned to each possible link. Next, \( p \) is incremented in small steps and if \( P_{ij} > r_{ij} \), a link is formed between nodes \( i \) and \( j \) and the percolation condition is checked. The procedure is repeated for \( m \) different seeds and \( p_c \) is the average \( p \) for which percolation is first achieved. Networks corresponding to \( L = \{ 5, 10, 20, 40, 80, 128, 256 \} \) were simulated. We used \( m = 250 \) for networks with \( L \leq 40 \), \( m = 50 \) for \( 40 < L \leq 80 \), and \( m = 10 \) for \( L \geq 128 \), which was sufficient. We again found a transition behavior around \( s = 2 \) (Fig. 2). For \( s < 2, p_c \to 0 \) as \( L \to \infty \) indicating that large systems would achieve end-to-end connectivity even for any small non-zero value of \( p \). On the other hand, for \( s > 2 \), the \( p_c \) approached a finite value as \( L \to \infty \).

The existence of the transition around \( s = 2 \) has a simple geometric interpretation. Although Eq. (1) defines the connection between a pair of nodes by a probability that decreases with the distance between the nodes, in a 2-dimensional
network the number of nodes around any given node increases with the square of the distance. When \( s = 2 \), \( P_{ij} \) decreases with distance at about the same rate as the number of nodes that can be linked increases, and hence the probability of linking neighboring nodes becomes similar to that of linking nodes at greater distances. For \( s < 2 \), the number of nodes that can be linked increases faster than \( P_{ij} \) decreases, thus longer connection are likely to exist; whereas the reverse happens for \( s > 2 \), when a predominance of short-ranged links is expected. Formally, for a 2-dimensional network, we can define \( X(d, s) \) as the expected number of links connected from a given node to any other node located inside a circular region with radius \( d \), as

\[
X(d, s) = \int_{r=0}^{d} P_{ij} \pi r^2 dr
\]
which, from Eq. (1), becomes

\[ X(d, s) = \pi \int_{r=0}^{d} \frac{(pr^2)}{rs} \, dr. \]  

(3)

Therefore, for any fixed value of \( p \) and increasing \( d \), \( X(d, s) \) always increases for \( s < 2 \), decreases for \( s > 2 \), and remains constant for \( s = 2 \). As a consequence, \( p_c \to 0 \) with increasing network size if the parameter \( s < 2 \), as a consequence of the increased relative frequency of longer links (see Figs. 1 and 2).

3. Application to cytoskeletal networks

We assume, for simplicity, a direct relationship between cell activation and CSK filament density, as represented by the parameter \( p \) (Eq. (1)). For \( p > p_c \), more filaments are assembled, the network percolates and, thus, the cell assumes a solid-like state. On the other hand, \( p < p_c \) corresponds to the disassembly of links and loss of percolation resulting in a fluid-like state. Transitions between these two states can also be achieved by changing \( s \). This suggests that specific values of \( s \) may be associated with particular cell types, and thus by tuning \( s \) the model may be applicable to more general cases.

In order to characterize the connectivity properties of the network as the percolation transition occurs (i.e. in the neighborhood of \( p_c \)), we calculated the average number of links per node \( \langle k \rangle \) for networks with different values of \( s \) and examined how sensitive \( \langle k \rangle \) is to perturbations in link density \( p \). The results in Fig. 3 show that networks with long-range connectivity \( (s < 2) \) are extremely sensitive to changes in \( p \) since the slope of the \( \langle k \rangle - p \) relation is steep near \( p_c \). Therefore, for cells that maintain a relatively constant solid-like state, a higher value of \( s \) may be beneficial. Alternatively, cells that are exposed to stretching, and hence continuously accommodate changes in length, should be regulated with lower values of \( s \) that would allow a quick transition between the two sides of \( p_c \).
Fig. 3. Average number of links \( \langle k \rangle \) per node, in the numerical model, as a function of \( p \) for several values of \( s \) in a 40 \( \times \) 40 network. The dotted line corresponds to the percolation threshold (\( p_c \)). Symbols are the variation of \( \langle k \rangle \) when \( p \) varies \( \pm 10\% \) around \( p_c \). When \( s \) increases from \( s = 0 \) to \( s = 3 \) the slope of \( \langle k \rangle \) decreases significantly, thus suggesting that the smaller the value of \( s \), the easier to increase or decrease the connectivity of the network.

Fig. 4. (a) Publicly available images of cells on the Internet are analyzed. The example shown is a chondrocyte obtained from http://www.bms.ed.ac.uk/services/impact/pages/Gallery.htm (accessed October, 2008). Images were processed with an image analyzer (Image-Pro Plus 6.0). All identifiable filaments were measured. (b) Distribution of filament lengths obtained from cell images. Filament length was normalized by main cell axis; inset: average filament length for the four measured cell types in descending order. (c) Distribution of link lengths from simulation for several values of \( s \). Link length was normalized by the diagonal length of the lattice; inset: average length as a function of \( \langle k \rangle \).

Since each value of \( s \) corresponds to a link length distribution, we collected publicly available images of cells and measured the length distribution of F-actin filaments, an essential component of the CSK, using an image analyzer (Fig. 4a). The distribution of F-actin filament lengths from these images and the distribution of link lengths from the model are compared in Fig. 4b and c. The reasonable agreement between data and model prediction suggests that living cells regulate their CSK network structure around and below \( s < 2 \). Furthermore, cells that are subjected to larger magnitude stretches, such as a skin fibroblast, have longer internal connections than cells that are adapted to a stiff environment, such as a chondrocyte that produces and maintains the cartilaginous matrix.

4. Discussion

The present study suggests that the connectivity properties of the CSK in living cells depend on fine regulation of the CSK connection lengths. The stiffness and the contractile abilities of cells are a function of the CSK network structure. Thus, the modulation of the mechanical and connectivity properties may be given by the parameter \( s \) in Eq. (1) in such a way that these cells favor long-ranged networks (i.e., \( s < 2 \)). It was previously shown that a dynamic network with connection probabilities given by Eq. (1) and with \( s = 1 \), can mimic the dynamics of the length adaptation phenomenon observed in ASM, and is a major factor that regulates the mechanics of the ASM [5,15]. In addition, the use of \( s < 1 \) generated a network that overadapted to length and qualitatively mimicked some features of asthma [16].
Results from the present model indicate that for $s < 2$, network percolation can be easily achieved (Fig. 2). Additionally, Fig. 4c (see inset) suggests that by using $s < 2$ more long-ranged links are anchored in fewer nodes. This perhaps reflects an optimization feature: the extension of links, which are relatively simple protein polymers, is less expensive than the construction of dense bodies or dense plaques, which are complex protein aggregates. In addition, one can also argue that if the cell were a bag filled with fluid or the CSK had only short-ranged denser network ($s > 2$), then an elastic perturbation at the cell surface would quickly die out with distance. Thus, long-range networks formed with $s < 2$ would allow direct mechanical connection to different regions of the cell. This is in agreement with the immediate signal transmission from the cell surface to the nucleus or to remote cytoplasmatic structures that has been experimentally observed [4].

In conclusion, the presence of long-range connections alters the connectivity properties as well as the percolation threshold of networks. As a consequence, the mechanical and contractile properties of cells with long-range CSK connections should also be different. With regard to cell structure and function, we identified some general structural properties of the cytoskeletal network that are, to our knowledge, not known in the literature. We argued that these long-range connections have important physiological and biological consequences. Furthermore, long-range connections are not considered in the dynamics of mechanical systems, especially in the context of the cytoskeleton [25–27]. We suggest that the implications of such behavior warrant further studies in both man-made and living systems that may show a mixture of short and long-range connections and are involved in mechanical work.

Acknowledgements

This work was supported by National Institutes of Health grants HL 059215, HL 065960, and HL 084224, Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP 2007/08165-8), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, process number BEX0659/02-9), Department of Pathology at the School of Medicine of the University of São Paulo, and Laboratórios de Investigação Médica (LIM01 and LIM05, HC-FMUSP). The authors also thank Prof. Dr. Venâncio Avancini Ferreira Alves (head of the Department of Pathology at the School of Medicine of the University of São Paulo) for his support and Prof. Dr. Raymundo Soares Azevedo for providing additional computational resources used for simulations.

References