SELF-ASSEMBLY AND PHASE BEHAVIOR OF MEMBRANE SKELETONS

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ABSTRACT

Membrane skeletons are found at many if not all cell membranes, including nuclear membranes and the plasma membrane. Spectrin-family proteins and actin filaments are among the most common components of membrane cytoskeletons and are found in two of best studied cells: the red blood cell (RBC) and the outer hair cell of the inner ear (OHC). In current studies, we probe by simulation what associative interactions and constraints are sufficient to generate cell-realistic membrane networks of these proteins. At a higher level, we address principles of self-organization by simulating actin filaments as stiff rods in 2D interconnected by a soft, pseudo-3D network of spectrin. The phase behavior of this system under compression is qualitatively identical to that of a 2D fluid of hard rods, in which, short rods (typical of actin filaments in the red cell) show no order and long rods form a nematic phase. Networks with long actin filaments in the isotropic density regime can also be sheared into a nematic phase. Actin filaments that approximate the length of spectrin crosslinkers form a quenched nematic phase at zero pressure. Consistent with this model, the OHC cytoskeleton exhibits locked in nematic behavior due to its large actin filament length relative to spectrin. Tension studies of this quenched nematic state reveal a soft response in the direction perpendicular to the actin filaments – the direction of sound propagation through the OHC. Differing mechanical responses of cells to external stimuli highlight the importance of varied self-assembly mechanisms for cell cytoskeletons made of the same major components. The simulation approach introduces thermal noise into the usual mechanical analyses and thus highlights the strong role that entropy plays in these systems.

INTRODUCTION

Spectrin and actin monomers self-assemble into tetramers and filaments, respectively, to form membrane cytoskeletons in the RBC (Byers and Branton, 1985; Debreuil et al., 1987; Welch et al., 1994) and the OHC (Holley and Ashmore, 1988). Both cytoskeletal networks are assembled from the same two monomers, however, the connectivity and the relative sizes of the polymers differ in the two systems, as Figures 1A,B show. The displacement of the spectrin tetramers and actin filaments under externally applied forces determines the elasticity and responsiveness of the cell. These kinematic displacements have been studied via experiment and simulation on the red cell (Discher et al., 1994; Boal, 1994; Discher and Mohandas, 1996). Previous simulations exploring RBC

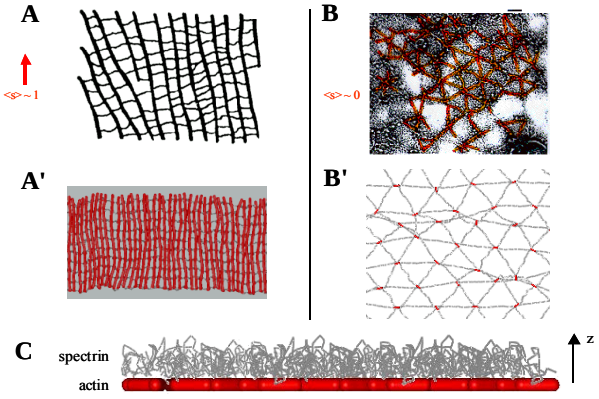

FIGURE 1 Electron Micrograph images of the cytoskeletons of the outer hair cell of the ear (OHC) and the red blood cell (RBC), respectively, and their real space bead-on-string models. (A) Vertical strips are stiff actin filaments interconnected by short, soft spectrin crosslinkers. Sound waves propagate through the network perpendicular to the actin filament orientation. (E.M. from Holley and Ashmore, 1990). (B) C6 symmetric spectrin-actin network with short actin filaments located at the nodes of this triangular network, connected by long spectrin crosslinkers. The orientation of the actin filaments is random (E.M. from Byers and Branton, 1985). (C) Side view of a RBC-like network emphasizing the confinement of the actin filaments to the xy plane and spectrin to z^+ space. Spectrin chains are shown without beads and actin filaments are shown as rods.

kinematics modeled the cytoskeleton as a C_6 triangular network of beads or springs, concentrating on the role of the spectrin tetramers in the elastic properties of the cell (Boal, 1994; Discher et al., 1997). The exclusion of actin filaments from the nodes of these network simulations seems valid based on micropipette deformation experiments carried out on the red cell, which showed no ordering or anisotropic behavior of the actin filaments during pure shear (Picart et al., 2000). Although the elastic properties of the RBC seem to be determined exclusively by its spectrin tetramers and not by its short actin filaments, it is obvious, by visual inspection, that the role of the actin filaments in the cytoskeleton of the OHC plays a nontrivial function in that particular cell's kinematics. With this is mind, we are obligated to explore the mechanical behavior of cytoskeletal-like structures by varying the spectrin tetramer to actin filament length ratio, which will provide a clearer picture of the role of nodal filaments in the kinematics of 2D membrane cytoskeletal networks.

METHODS AND MATERIALS

Monte Carlo Simulations: As detailed in Picart et al. 2000, a coarse-grained network is created or now assembled by crosslinking flexible bead-on-string polymer chains to more rigid filaments. The polymer chains model spectrin and simply have finite length tethers interconnecting hard spheres. The actin-like filaments have the same interactions plus a potential that opposes bending. The harmonic potential is adjusted to give a persistence length for F-actin of ~10 μm. Networks of various sizes with variable length polymers and filaments are simulated in NPT ensembles for at least 1 million sweeps.

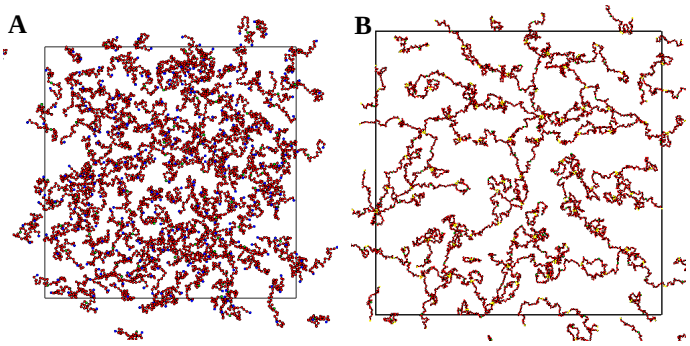

FIGURE 2 Assembly of spectrin-actin networks on a membrane (A) Initial configuration of NVT ensemble with no inter-spectrin binding. Density of spectrin tetramers is the same as that of electron micrograph data adjusted to zero pressure. Blue beads are end beads, which are allowed to irreversibly bind to one another, green beads are mid beads confined to 2D, and red beads are allowed to diffuse in psuedo-3D space. (B) Same network as in A but after blue beads have reacted (changed color to yellow) and stretched out to ease in the visualization of the symmetry at each of the nodes. Only one spectrin bead per chain was confined to the surface (2D) here; this is not enough to create a C6 symmetric network.

RESULTS

Self-assembly of spectrin-based membrane networks

The assembly of the spectrin-actin network in the red cell takes place during erythropoiesis when different elements of the cytoskeleton are expressed or directed towards the plasma membrane at different times. To better understand the relationships between these proteins (specifically spectrin, actin, ankyrin, band3, and 4.1) during assembly, we have modified the attachment of the spectrin tetramers to the plasma membrane *in silica* by confining specific beads comprising each tetramer to a 2D plane and then allowing the end beads of each spectrin chain to irreversibly bind to other end beads (Figure 2). We find that the symmetry and integrity of the resulting networks increases as more spectrin beads are confined to 2D. These results suggest that ankyrin and band3 proteins, which bind to spectrin domains and anchor them to the plasma membrane, are crucial for proper membrane cytoskeletal formation.

Phase behavior of spectrin-actin networks

The aspect ratio of the actin filaments determines the phase behavior of the network, as is the case with a 2D fluid of hard rods (Bates and Frenkel, 2000). Upon compression from an annealed, globally isotropic state, actin rods with an aspect ratio less than or equal to 6 (RBC model) undergo a first order transition to a frustrated glass that exhibits local ordering. Conversely, actin rods with an aspect ratio greater than or equal to 8 undergo a transition from an isotropic to a nematic phase. These results confirm the phase diagram of Bates and Frenkel, showing that the only effect of the added crosslinkers is to increase the needed pressure to compress the network (fluid). Both long and short filament networks can be ordered through pure shear of the box. The phase diagram will be presented.

Quenched C6 and C4 nematics and the OHC

Actin filaments that approximate the length of spectrin cross-linkers form a quenched nematic phase at zero external pressure. Consistent with this model, the OHC cytoskeleton has locked in nematic behavior due to its large actin to spectrin filament length ratio. Applied tension and zero-pressure box fluctuation studies of this quenched nematic state reveal a soft response in the direction perpendicular to the director, which is the direction of sound propagation through the OHC. For C6 networks, the ratio of the elastic moduli perpendicular / parallel to the filaments follows the form $R_S \approx \exp(R_L)$, where R_L is the ratio of the actin to spectrin length and R_S is the ratio of elastic moduli. Therefore, the more quenched these networks are, the softer they seem to be to external tensions in the direction of sound propagation. The connectivity rules of a C6 network places and upper bound on R_L but there is no such limit on C4 networks. Preliminary results on the same anisotropic softness of quenched nematic C4 networks (OHC) versus quenched nematic C6 networks show that the C4 networks have even greater responsiveness perpendicular to the actin direction, illustrating why the OHC cytoskeleton has C4 instead of C6 symmetry. The differing mechanical responses between the RBC and OHC to external stimuli illustrates the varying self-assembly mechanisms between cell cytoskeletons that are made of the same major components.

CONCLUSION

The RBC and OHC both have membrane cytoskeletons assembled from the same two main components, spectrin and actin. Relative lengths of spectrin and actin differ, however, resulting in distinct phase behavior and response to external stimuli.

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