late-stage ovarian cancer patients is attributed to chemoresistance against drugs like cisplatin and paclitaxel. Though alterations in physical properties of the tumor microenvironment in a variety of different epithelial cancers, including ovarian cancer, is well appreciated, how such alterations influence normal versus drug-resistant cells remains obscure. In this study, we have compared the behaviour of normal and cisplatin-resistant ovarian cancer cells in response to changes in extracellular matrix (ECM) density. In comparison to control cells, cisplatin-resistant cells were less spread, possessed less number of cell matrix adhesions, and lower degradation potential. Further, cisplatin-resistant cells were found to possess higher baseline contractility compared to normal ovarian cancer cells, as assessed by trypsin de-adhesion assay. However, the enhanced contractility of cisplatin-resistant cells remained insensitive to changes in ECM density. Western blots revealed lower expression levels of the focal adhesion protein vinculin and higher expression levels of the actin crosslinking protein α-actinin in cisplatin-resistant cells compared to control cells. Together, our results point to a potential mesenchymal to amoeboidal transition in ovarian cancer cells that attain drug resistance.

Key Words: chemoresistance, tumor microenvironment, extracellular matrix, contractility, focal adhesions, actin bundling proteins.

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A Single-Chain Model to Predict Buckling in Active Gels

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We present a single-chain theory to describe the dynamics of active actin gels, driven by motor proteins. Molecular motors create active cross-links between the semiflexible filaments. We model the semiflexible filaments as bead-spring chains; the active interactions between filaments are accounted for using a mean-field approach in which filaments have prescribed probabilities to undergo a transition from one motor attachment state into the other depending on the state of the probe filament. The level of description of the model includes the change in the end-to-end distance of the filaments, the attachment state of the filaments, and the motor-generated forces, as stochastic state variables which evolve according to a proposed differential Chapman-Kolmogorov equation. The motor-generated forces are drawn from a stationary distribution of motor stall forces that can be measured experimentally. The general formulation of the model allows accounting for physics that is not possible, or not practical, to include in available models that have been postulated on coarser levels of description. To obtain analytical results that provide insight into the microscopic mechanisms underlying the dynamics of active gels we first treat the special case of filaments as one-dimensional dumbbells, approximate the elasticity of the semiflexible filaments with a Hookean spring law, and assume that the transition rates are independent of the tension in the filaments. We show that even in this simplified form, the model predicts the buckling of individual filaments that is thought to be the underlying mechanism in the self-contraction of non-sarcomeric actin-mysoin bundles [Lenz et. al., PRL 108, 238107 (2012)]. The active dumbbell model also explains the violation of the fluctuation-dissipation theorem observed in microrheology experiments on active gels [Mizuno et. al., Science 315, 370-373 (2007)].

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Metavinculin Induced Changes at the Actin Interprotomer Contacts and the Mechanism of Resulting Severing

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Vinculin is a ubiquitously expressed adhesion protein of cell-matrix and cell-cell junctions. A larger splice-isoform of vinculin, metavinculin, is specifically expressed in cardiac and smooth muscles where it co-localizes with vinculin. Metavinculin has a 68 amino acid insert at its C-terminal actin binding tail (metavinculin tail: MVT). Mutations in the MVT insert are associated with disrupted intercalated disc organization and dilated cardiomyopathies, suggesting a distinct and important role for this isoform in cardiac cells. In vitro, metavinculin was shown to sever and organize actin filaments into fine meshworks while vinculin bundles them. Therefore, we focused on characterizing the structural changes MVT induces in F-actin and the severing activity of MVT. By using mutagenesis, cross-linking and site-directed labeling, we determined that MVT causes changes in the actin interprotomer contacts and dynamics. Specifically, the spectra of acrylodan attached to cysteine residues engineered in dynamic loops of actin -D-loop, W-loop, and C-terminus- are changed in the presence of MVT indicating a rearrangement of actin-actin contacts. Enhanced excimers fluorescence due to pyrenes attached to cysteines 265 and 374 shows that MVT binding affects mainly lateral actin contacts. By using a seeded actin elongation assay as a reporter of severing by MVT, we confirmed that MVT has a biphasic mode of actin severing. At partial decoration with MVT -as defined by co-pelleting of MVT-actin complexes- we observed increasing amounts of filament severing. At higher decoration, above 60%, MVT had a stabilizing effect on filaments. Our work enhances our understanding of MVT induced severing and changes in actin filaments structure, which appears to be important for the physiological role of this tissue-specific vinculin isoform.

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Mechanical Properties of Branched Actin Networks Assembled from Yeast Extracts

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The actin cytoskeleton is essential for cell mechanics and motility. Its dynamic nature and the host of actin binding protein (ABP) that modifies its architecture and growth make its description challenging to achieve. Here we propose to study actin branched networks through their mechanical properties. We previously developed a powerful experimental method to measure the mechanics of in vitro dense branched actin networks assembled from a minimal number of regulatory proteins (Pujol et al. PNAS 2012). This method is based on magnetic beads and allows for the rapid acquisition of orders of magnitude more experiments than previous techniques. We now focus on a recent in vitro system that reconstitutes actin branched gels in a near-physiological environment, from yeast cell extracts. These reconstituted actin networks are analogous to endocytic actin patches assembled in vivo, with more than 80 regulatory proteins present (Michelot et al., Curr. Biol, 2010). Compared to gels assembled from purified proteins, these gels were notably stiffer and more prone to plastic deformations, exhibiting failure and long-time relaxation. Because of the large number of experiments that can be carried out with the magnetic technique and the ease of yeast mutant generation, we are able to test the effects of the absence of numerous ABPs on the actin network mechanics. The lack of two cross-linkers Sac6 (fimbrin) and Scp1 (calponin) softens the gels and modifies their long-term evolution. The absence of Aip1, a protein involved in actin filament severing and network disassembly, changes the plastic reorganization properties of the actin networks. In the future, the combination of the mechanical magnetic probing technique and the yeast actin reconstituted system will provide an avenue to precise the role of many other actin protein partners implicated in the mechanics of the cytoskeleton.

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Response of Actin Networks at Intermediate Distances

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We report the observation of a large-distance intermediate response in an experimental system of entangled F-actin gels. The tools of 1-point and 2-point microrheology were used to characterize the local and distance-dependent responses of the actin networks, respectively. The 2-point response at intermediate distances, arising from the effect of mass displacement rather than momentum diffusion, is enhanced by the much softer local microenvironment of the tracers compared to the bulk properties of the gel. Consequently, the intermediate behavior sets in at particle separations much larger than the mesh size, ξ, of the actin gel. Results from several networks with different mesh sizes will be presented, emphasizing this inherent property of complex fluids and its relation to ξ.

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Actin Filament Severing by Vertebrate Cofilin is Driven by Linked Cation Release

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The dynamic remodeling of actin cytoskeleton drives cell movement. The essential actin regulatory protein cofilin accelerates actin remodeling by