

**MOTILE MEMBRANE SKELETONS:
WHAT NEUTROPHILS AND MUSCLES HAVE IN COMMON**

ELIZABETH J. LUNA, KERSI N. PESTONJAMASP, THOMAS NEBL,
YU CHEN, CHERYL GATTO, SANG W. OH and ROBERT K. POPE
Department of Cell Biology, University of Massachusetts Medical School,
Worcester, Massachusetts 01605, USA

Both nonmuscle and muscle cells contain actin-based plasma membrane skeletons that provide structural support during rapid cell movements. Plasma membranes from bovine neutrophils contain a detergent- and salt-resistant 26S protein complex. In negative stain electron microscopy, this complex forms a network of thick bundles that unwinds into a regular filamentous meshwork at low pH. Proteins in the complex are being identified by immunoblotting, binding to ¹²⁵I-labeled F-actin in blot overlays, and mass spectroscopy and/or microsequencing of tryptic fragments. Preliminary results indicate that the 26S complex contains fodrin, myosin II, the Rho-associated kinase ROCK2, phosphatidylinositol-4-kinase- α , F-actin, and a ~205-kDa F-actin binding protein we have named supervillin. Although the supervillin carboxy-terminus is ~29% identical and ~50% similar to subdomains 2 – 5 of gelsolin/villin and the villin headpiece, the major cytoskeleton binding sites reside in its amino-terminal 1009 amino acids. A focal adhesion association site lies within amino acids (aa) 1-171; at least two F-actin binding sites are in aa 1-340 and 570-830; and at least two functional nuclear targeting signals exist within aa 570-819 and 822-1009. Fodrin, myosin II, filamin, and vinculin— but not F-actin—bind to GST-tagged aa 1-171 *in vitro*, suggesting that supervillin may participate in both vinculin- and fodrin-containing membrane skeletons. Consistent with this hypothesis, stable overexpression of supervillin results in an increase in cell surface projections, loss of vinculin-containing focal adhesions, decreased cell-substrate attachment, and increased rates of cell spreading. Human and murine skeletal muscles contain an alternately spliced supervillin-like protein that has two conserved muscle-specific inserts, totaling ~45 kDa, between supervillin aa 278 - 356. While consequent alterations in binding to cytoskeletal proteins are not yet known, the muscle protein, called “archvillin”, immunolocalizes as “arches” at the sarcolemma, diffusely within myonuclei, and as punctae in register with myofibrillar Z-lines. In longitudinal sections, archvillin co-localizes with dystrophin at costameres, membrane-actin attachment sites that also contain fodrin, vinculin, myosin II, filamin, and F-actin. Thus, neutrophil and muscle membrane skeletons contain compositional similarities.