434 Membrane domains: Polarity, trafficking and assembly in the eye - Minisymposium
Wednesday, May 10, 2017 11:00 AM–12:45 PM
Room 307 Minisymposium
Program #/Board # Range: 4271–4275
Organizing Section: Lens
Contributing Section(s): Anatomy and Pathology/Oncology, Biochemistry/Molecular Biology, Cornea, Glaucoma, Retinal Cell Biology

Program Number: 4271
Presentation Time: 11:00 AM–11:25 AM
An ankyrin-G-based mechanism for assembly and regulation of axon initial segments
Vann Bennett. Duke University Medical School, Durham, NC.
Presentation Description: Axon initial segments are specialized membrane domains of myelinated axons of jawed vertebrates and are the cellular sites of action potential generation. Axon initial segments thus operate as integrators of dendritic and somatic signals. Recent progress has resulted in elucidation of the molecular organization of axon initial segments and has identified giant ankyrin-G as a master regulator of their assembly. This lecture will summarize these studies and present new data related to regulation of ankyrin-G function by phosphorylation.
Commercial Relationships: Vann Bennett, None
Support: HHMI

Program Number: 4272
Presentation Time: 11:25 AM–11:45 AM
Tunneling nanotubes formed by trabecular meshwork cells: Their role in cellular communication and intraocular pressure regulation
Kate E. Keller. Casey Eye Insitute, Oregon Health & Science University, Portland, OR.
Presentation Description: The actin cytoskeleton of trabecular meshwork (TM) cells plays a key role in intraocular pressure (IOP) regulation. Actin microfilaments are assembled into higher ordered cellular structures such as stress fibers and filopodia. Relaxing stress fibers increases aqueous outflow via the conventional pathway, but the role of filopodia in IOP regulation has not been studied. Tunneling nanotubes (TNTs) are specialized filopodia that allow the direct transfer of molecular cargo between cells without secretion into the extracellular milieu. Using live-cell confocal microscopy, we describe TNT formation by cultured TM cells and the transfer of fluorescently-labeled vesicles and mitochondria between cells. Myosin-X (Myo10) is a critical regulator of TNT formation. The effects of Myo10 silencing on outflow rates in perfused human eyes and its distribution in normal and glaucomatous TM will be discussed. Our results provide important new understanding of how the actin cytoskeleton regulates IOP and describes a novel method by which TM cells can communicate in an aqueous environment.
Commercial Relationships: Kate E. Keller, None
Support: NIH Grant EY019643, Medical Research Foundation of Oregon

Program Number: 4273
Presentation Time: 11:45 AM–12:05 PM
Actin–rich membrane domains regulate lens fiber cell morphogenesis, transparency and mechanics
Velia M. Fowler. Cell and Molecular Biology, The Scripps Research Institute, La Jolla, CA.
Presentation Description: Lens shape, optics and mechanical integrity rely upon highly patterned morphogenetic differentiation and maturation of fiber cell layers. Age-related changes in lens structure and mechanics are linked to presbyopia, a reduction in the lens' ability to change shape during focusing (accommodation). While mouse lenses do not accommodate, mouse lenses are similar to primate and human lenses in their fiber cell morphogenesis and age-dependent stiffening, and thus can provide an excellent genetic model to elucidate connections between the lens cytoskeleton, fiber cell architecture, transparency and mechanics. Lens mechanical integrity during accommodation has long been hypothesized to rely on complex membrane interdigitations that form a 3D zipper between neighboring lens fiber cells, but little is known about the mechanisms and protein complexes required for formation and maintenance of these specialized cell architectures, and how fiber cell morphology correlates with mechanical properties.
To understand the role of the actin filament (F-actin) cytoskeleton in lens mechanical and cell shape properties, we have characterized the phenotypes of mouse lenses with disruptions in various F-actin binding proteins, including 1) Tmod1, an F-actin pointed-end capping protein, 2) γ-tropomyosin (γTM), a protein that binds and stabilizes F-actin, and 3) non-muscle myosin IIA (NMIIA), a contractile protein that generates force on filaments. Our analyses reveal that loss of Tmod1 leads to decreased lens stiffness at low mechanical loads that is linked to disassembly of the spectrin-actin network and abnormal lens fiber cell interdigitations. In contrast, disruption of γTM causes mild anterior cataracts and reduced lens stiffness at high loads along with abnormal resilience (recovery after release of load). A NMIIA motor domain mutation leads to anterior cataracts and reduces lens resilience without affecting lens stiffness or cell packing, while a NMIIA rod domain mutation leads to defective fiber cell hexagonal packing and loss of focusing, but no effects on lens stiffness or resilience. The disparate phenotypes of these mutant lenses imply that F-actin partners with diverse F-actin-binding proteins to control lens fiber cell morphogenesis and packing geometry, thereby non-coordinately regulating lens tissue level physiological functions of transparency, stiffness and resilience.
Commercial Relationships: Velia M. Fowler, None
Support: NIH Grant EY017724

Program Number: 4274
Presentation Time: 12:05 PM–12:25 PM
Differential adenoviral entry and trafficking in corneal cells
Jaya Rajaiya[1, 2]. [1]Ophthalmology, Mass Eye and Ear, Boston, MA; [2]Harvard Medical School, Boston, MA.
Presentation Description: Human adenoviruses within species D (HAdV-D) cause epidemic keratoconjunctivitis (EKC), manifest by severe, acute, ocular surface inflammation, and in up to one-third of infected patients a chronic, relapsing, stromal keratitis. This presentation will first review our earlier findings where we showed that human adenovirus type D37 (HAdV-D37) uses a lipid raft mediated caveolin-1 pathway to infect human corneal fibroblasts (HCF). In contrast, in corneal epithelial cells, our preliminary data is indicative of a combination of macropinocytosis and clathrin mediated viral entry. Dynamin 2, a molecule associated with fission and endocytosis of various endocytic vesicles including caveolae and clathrin, has been shown to be important in the entry of viruses. However, in HCF, dynamin 2 overexpression reduced entry of HAdV-D37 while dynamin 2 knock down increased viral entry. By ultrastructure, dynamin 2 knock down also induced movement of microtubule organizing centers (MTOCs) adjacent to nuclear membranes, facilitating viral nuclear entry and increased viral replication. Discussion of differences in viral trafficking between corneal epithelial cells and HCF will conclude the presentation. These studies challenge a previously reported role for dynamin

2 in viral entry, and suggest that manipulation of dynamin 2 expression could prove a useful strategy to improve delivery of transgenes in viral gene therapy.
Commercial Relationships: Jaya Rajaiya, None
Support: NIH grants EY013124, EY021558, and P30EY014104, Research to Prevent Blindness, the Massachusetts Lions Eye Research Fund, and the Falk Foundation.

Program Number: 4275
Presentation Time: 12:25 PM–12:45 PM
Membrane protein and lipid complexes that control diurnal photoreceptor outer segment tip clearance by the RPE
Silvia Finnemann. Biological Sciences, Fordham University, Bronx, NY.
Presentation Description: Photoreceptor outer segment renewal is a fundamental retinal process that is essential for vision. Defects in outer segment renewal cause severe forms of retinitis pigmentosa. Incomplete phagolysosomal degradation leads to accumulation of lipofuscin in the RPE in the human eye with age, and this likely contributes to RPE dysfunction associated with atrophic age-related macular degeneration. Rod outer segment renewal involves production of new outer segment disks and elongation of the outer segment in balance with removal of the distal, most aged fragment of the outer segment in a process known as disk shedding. Diurnal shedding of spent distal fragments of photoreceptor outer segments and their phagocytosis by the adjacent retinal pigment epithelium (RPE) require coordinated function of large complexes of signaling and cytoskeletal proteins. Our recent studies suggest that RPE cells orchestrate both the synchronized exposure of phosphatidylserine “eat me” signals by outer segments and the activation of the RPE engulfment machinery that follow light onset in light entrained experimental animals. In this presentation, I will discuss recent findings on the molecules and pathways that contribute to these processes with specific emphasis on soluble glycoproteins that are produced and secreted apically by RPE cells and that recruit, anchor, and/or ligate membrane proteins or lipids of outer segments and the RPE.
Commercial Relationships: Silvia Finnemann, None
Support: NIH grant EY026215