Specific Aims

Genomic and proteomic screens have revealed that there are core sets of molecular mechanisms which regulate cell asymmetry and division[1-6]. Regulatory and signaling mechanisms that are highly conserved between animals, plants and other eukaryotes also frequently serve essential roles in early development and disease[7]. Historically, exocytic mechanisms have received primary emphasis amongst trafficking routes responsible for cell polarization[8, 9]. Accumulating evidence now reveals that endocytosis plays an equally important role in the proper localization of key polarity proteins, such as the PARs. Intriguingly, some polarity proteins, such as the anterior PAR protein PAR-6, can also regulate the endocytic machinery[5, 10, 11]. Although studies in yeast, polarized mammalian cells and *Drosophila* have been pivotal for our understanding of many of the cellular mechanisms and interactions that occur (reviewed in[11]), some of the most important aspects of the role of membrane trafficking during early embryonic development are missing from almost all organismal models. In recent years, we discovered that dynamin/DYN-1, an essential endocytic factor, plays a necessary role in both cell polarity and cytokinesis events. Preliminary data from our lab also suggest that anterior PAR proteins are involved in cytokinesis.

Here, we will test the hypothesis that DYN-1 and PAR proteins cooperatively regulate the temporal and spatial organization of membrane trafficking events during the maintenance of cell asymmetry and cell division. Our hypothesis has been formulated on the basis of published[10, 12], and preliminary data from our laboratory, and others[13-18], suggesting that membrane trafficking proteins play essential and conserved roles in both cytokinesis and polarity. The collective evidence reviewed in this proposal strongly supports the conclusion that there is a need to identify the mechanisms by which cells establish and maintain proper plasma membrane function throughout development.

Our ***long-term goal*** is to understand the role of membrane trafficking and polarity machinery in regulating cell asymmetry and cell division in development. We will then incorporate what we uncover from these studies and identify factors required to target, maintain and regulate membrane trafficking and polarity machinery at particular membrane sites throughout the cell cycle. The ***objective*** of this proposal is collectively expected to identify how dynamin cooperates with PAR protein machinery throughout development. Focusing on DYN-1 and PAR proteins, we will pursue the following three specific aims:

1. **Identify factors that target DYN-1 and PAR proteins to specific membrane domains.** We postulate that factors regulate the localization of DYN-1 and PAR proteins to cortical membranes throughout the cell cycle ensuring that cell asymmetry will be maintained. We will determine the contributions of the exocyst complex and other conserved cell cycle regulated factors in regulating polarized membrane trafficking during embryonic development.

2. What is the function of PAR proteins and DYN-1 during cytokinesis? Preliminary data suggests that PAR-6/PAR-3 double mutants lead to multi-nucleate phenotypes, suggesting a novel role for these proteins in cytokinesis. Our goals are to determine how DYN-1 and PAR proteins cooperate during furrow establishment, actomyosin ring assembly and cell abscission.

3. **What are the mechanisms that mediate the spatial and temporal localization of polarity and endocytic machinery during development?** DYN-1 appears to play a necessary role in the maintenance of cell polarity but how polarity cues and endocytosis are spatially and temporally restricted is unclear. Our goals are to understand how PKC-3 mediates DYN-1 asymmetry and function, determine if DYN-1 and endocytosis regulates posterior PAR protein localization and identify the extent that membrane micro-domain clustering plays in cell polarity cue localization and maintenance during development.

Regarding *expected outcomes*, the combination of work in this proposal is collectively expected to identify how DYN-1 and PAR protein networks cooperate during embryonic development to achieve cell asymmetry. This work will have an important positive impact, because the identified components are highly likely to provide new targets for preventative and therapeutic interventions, in addition to, fundamentally advancing the fields of cell polarity, cytokinesis and membrane trafficking. This work will also be used in various scientific art outreach events and numerous ***minority outreach opportunities*** that my lab offers.