A. SPECIFIC AIMS

The objective of the proposed research is to determine the molecular requirements essential for the formation of cleavage furrows during cytokinesis. Errors in cytokinesis cause aneuploidy, which leads to genetic instability and abnormal cell behavior as seen in breast cancer and a variety of birth defects, for example. Not only are the molecular factors required for cleavage furrow establishment unknown, but also how these signals modify the cleavage furrow membrane remains a mystery. The goal of this research program is to gain insight into the mechanisms required to establish the cleavage furrow, identify factors necessary to establish and regulate furrow formation and to determine how Dynamin/DYN-1 coordinates this process. The study will focus on the early divisions of the nematode, *Caenorhabditis elegans*.

Hypothesis: The cleavage furrow is established by a clustering of lipid rafts from an unknown signal(s) from the spindle. This signal triggers Dynamin/DYN-1 to assemble and cluster in the equatorial membrane of the cell during the metaphase-anaphase transition. Dynamin/DYN-1 subsequently directs actin filament formation, which assemble into the acto-myosin ring and in turn, promotes cleavage furrow invagination. Dynamin/DYN-1 is, therefore, crucial for the formation of cleavage furrows during cytokinesis in animal cells.

Research Strategy: Dynamin has an essential yet undefined role in cytokinesis. **The long-term goal of this proposal is to determine the role of Dynamin/DYN-1 during cytokinesis by identifying and characterizing proteins that are essential for its regulation with the expectation that we also identify proteins required to target and maintain DYN-1 at cleavage furrows.**

A. Specific aims of this proposal are:

AIM 1: Determine the membrane-cytoskeletal consequences of DYN-1 depletion during cytokinesis. A. Define the effects of DYN-1 depletion on microtubules, actin, resident spindle midzone proteins and membrane dynamics during cytokinesis. **B.** Determine the localization and function of the midzone kinesin, ZEN-4, in the absence of DYN-1. **C.** Determine if previously characterized *C. elegans* cytokinesis proteins (~16) properly function in *dyn-1 ts (ky51)* and *dyn-1* (RNAi) embryos. **D.** Determine the role of DYN-1 in membrane and lipid raft dynamics during furrow formation.

AIM 2: Determine what proteins are essential for the recruitment of DYN-1 to cleavage furrows. A. Determine DYN-1::GFP dynamics during the cell cycle. **B.** Determine if DYN-1::GFP assembly is dependent actin, microtubules and secretion. **C.** Determine whether known cytokinesis genes (~16) regulate the localization of DYN-1::GFP to cleavage furrows.

AIM 3: Identify and characterize DYN-1-interacting proteins critical for cytokinesis. A. Determine if DIP-1/C49G7.1, a novel BRCA1 C-terminus (BRCT) domain-containing protein, plays a role in establishing the cleavage furrow and functions with DYN-1 during cytokinesis. **B.** Identify additional DYN-1 interacting proteins using TAP- and GST-tag methods and tandem mass spectrometry. Direct interactions between DYN-1 and the identified proteins will be determined. **C.** Characterize the role of newly identified DYN-1 interacting proteins in cytokinesis by genetics, RNAi and cell biological assays. Cell cycle localization of newly identified proteins that function during cytokinesis will be determined using GFP and live-imaging techniques.