Sima Lev

Sreekumar Ramachandran
Vladimir Litvak
Nili Dahan
Tal Lavie
Roy Amarilio
Rachel Argov
Assaf Lask

Membrane Trafficking and Cytokinesis

Department of Neurobiology

Tel. 972 8 934 **2126** **Fax.** 972 8 934 **4131**
E-mail: sima.lev@weizmann.ac.il
Web page: www.weizmann.ac.il/ neurobiology/labs/lev/lev.html

Cytokinesis is the critical final stage of eukaryotic cell division. It ensures the production of two daughter cells endowed with a complete set of chromosomes and cytoplasmic organelles, and is tightly coordinated with mitotic progression. As the final step of mitosis, it must occur following chromosome segregation to ensure the integrity of genetic transfer to each future daughter cell. Thus, cytokinesis is essential for the completion of cell division, and defects in this process can lead to chromosomal instability- a driving force of tumorigenesis and cancer development.

In animal cells, cytokinesis requires the constriction of an equatorial actomyosin ring, which assembles between the spindle poles and provides the necessary force to constrict the cytoplasm. Contraction of the actomyosin ring pulls the plasma membrane inward and creates an ingressing cleavage furrow. Ingression of the furrow is followed by disassembly of the contractile ring, resealing of the plasma membrane and cell separation (Fig. 1).

Growing lines of evidence suggest that membrane trafficking events mediate crucial aspects of animal cytokinesis. Insertion of membranes at the cleavage furrow provides the additional surface area necessary for ingression of the cleavage furrow, and membrane fusion events are required for resealing of the plasma membranes at the final stage of cytokinesis. In addition, membrane trafficking may be necessary for insertion of specific proteins or lipids that are required for localization of the cytokinetic machinery or that alter membrane curvature.

While cytokinesis has been studied for decades, its underlying molecular machinery is not completely understood. The major goal of our studies is to understand the mechanisms and regulation of cytokinesis, and to define the role of membrane trafficking events in this fundamental process.

During the past few years, our studies have been focused on a novel family of proteins designated Nir/rdgB, which are implicated in regulation of lipid trafficking and membrane biogenesis. Current studies from our laboratory indicate that Nir2, a representative of this protein family, plays an essential role in cytokinesis, is tightly regulated during mitosis, and affects specific membrane trafficking events.

Nir2 belongs to a highly conserved family of proteins that have been identified in a variety of eukaryotic organisms. It shares a high sequence homology with the *Drosophila* retinal degeneration B (rdgB) protein, a protein that is required for photoreceptor cell viability and light response, and it consists an amino-terminal phosphatidylinositol (PI)-transfer domain. Nir2 mainly localizes in the Golgi apparatus in interphase cells, but is recruited to the cleavage furrow and midbody during cytokinesis, where it colocalizes with the small GTPase RhoA (Fig. 1C). Biochemical studies indicate that Nir2 interacts with RhoA via its Rho-inhibitory domain (Rid), which resides within the N-terminal region adjacent to the PI-transfer domain. Overexpression of Rid markedly affects cell contractility, and causes aberrant cytokinesis progression, whereas expression of a truncated Nir2 mutant, which lacks the N-terminal region consisting the PI-transfer domain and Rid, induces multinucleate-cell formation due to cleavage furrow regression. These results suggest that Nir2 is essential for cytokinesis and its N-terminal region is critical for this process.

More recently, we found that Nir2 is tightly regulated during mitosis by the mitotic kinase cyclin-depenent kinase 1 (Cdk1). We showed that at the onset of mitosis, Cdk1 phosphorylates Nir2 at multiple sites and facilitates its dissociation from the Golgi apparatus. We also found that phosphorylation of Nir2 by Cdk1 provides a docking mechanism for

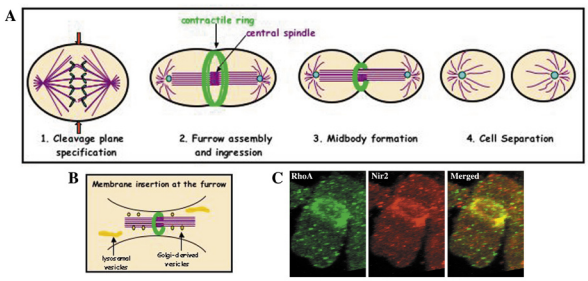

Fig. 1 *Animal Cytokinesis. (A). The different stages of animal cytokinesis. 1. Localization of the cleavage plane is specified by the position of the spindles. 2. The contractile ring of actin and myosin filaments is assembled under the cortex of the cell at the cleavage plane. Sliding of the actin and myosin filaments pull the plasma membrane inwards, forming a cleavage furrow. 3. The cleavage furrow narrows to form the midbody. 4. The contractile ring and the spindle midzone disassemble, the plasma membrane fuses, and the two daughter cells separate. (B). Membrane insertion occurs at the furrow during scission. The inserted membrane may be in the form of Golgi-derived vesicles transported along the polarized midzone microtubules or in the form of larger lysosomal vesicles. (C). Three dimensional image of anaphase cell that double immunostained with anti-Nir2 and anti-RhoA antibosies. Colocalization appears in yellow. The two proteins are localized to at the cleavage furrow, and visualized in a ring-like structure.*

an additional key mitotic kinase, the Polo-like kinase (Plk1). Expression of a Nir2 mutant lacking the Cdk1 phosphorylation sites, which fails to interact with Plk1, affects the ingression of the cleavage furrow and thereby the completion of cytokinesis. These results suggest Nir2-Plk1 interaction is required for cleavage furrow ingression, and that this interaction may link Plk1 at the central spindle to RhoA-mediated effects at the cell cortex. Collectively, our results suggest that Nir2 acts as a scaffold protein that plays an important role in cleavage furrow ingression. However, it is possible that Nir2 is also involved in regulation of membrane addition or lipid composition at the cleavage furrow. It is now evident that perturbation in lipid trafficking to the plasma membrane directly affects cytokinesis, and that membrane lipid composition can affect ingression of the cleavage furrow. Thus, it could be that Nir2 coordinates membrane-remodeling events with the cytoskeletal dynamics necessary for cleavage furrow ingression. We are currently investigating these possibilities applying different experimental approaches, including real-time imaging, immunoflourescence analysis, confocal and electron microscopy, RNA-interference (RNAi), and a variety of molecular and biochemical approaches.

Overall, our results on the Nir/RdgB protein, are novel and provide the first functional studies of these family members in mammalian cells. Future studies will further examine the roles of membrane lipids and membrane trafficking in cytokinesis, as well as more general mechanisms of cytokinesis, including those that are regulated by key mitotic kinases or small GTPases, such as Plks and Aurora B, or RhoA and Arf1, respectively.

Selected Publications

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