Cdc42 Interacting Protein 4 (CIP4), Involvement in Endocytosis and Membrane Protrusion

Genti Gjyzeli, Kelly Mitok, Corey O'Reilly
University of Wisconsin – Madison, Madison, WI 53706
Faculty Advisor: Michelle Harris, Ph.D. Research Mentors: Erik Dent, Ph.D, and Witchuda Saengsawang, Ph.D., UW-Madison

Abstract

Endocytosis is a critical process to all living cells. Human Cdc42 interacting protein 4 (CIP4) is known to function in collaboration with other molecules in endocytosis by helping to determine the curvature of the formed vesicle. To do this, certain positively charged residues on the concave surface of the FBAR domain of CIP4 interact with the negatively charged membrane phospholipids. CIP4 is important to the lab we are collaborating with because they have observed it in extending filopodia and lamellipodia of axonal growth cones. This is interesting because the conventionally accepted mechanism that CIP4 interacts with membranes along its concave surface is not consistent with our research that shows this protein is important for protrusion. CIP4-induced filopodial and lamellipodial protrusions would however, be consistent with it interacting with the membrane along its convex surface. This potentially novel function of CIP4 is important because it could add to our understanding of axon growth and neuron migration in prenatal nervous system development in humans. In our model of human CIP4 we are focusing on both the positively charged residues on the concave surface of the FBAR domain that have been shown to be important in endocytosis and the positively charged residues on the convex surface that may be important in protrusion. Further research could include carrying out point mutation studies of the positively charged residues on the convex surface of the FBAR domain to assess residues important in filopodia and lamellipodia protrusion.

Introduction

Cdc42 interacting protein 4 (CIP4) is a protein dimer with three main domains: FBAR, HR1, and SH3 (see Fig. 3). The FBAR domain has a long curved shape and has the most shallow degree of curvature of the BAR domains (Fig. 1). CIP4 is one of three proteins in the CIP4 subfamily of the FBAR family of proteins, a subset of the large BAR family that sense and induce membrane curvature.

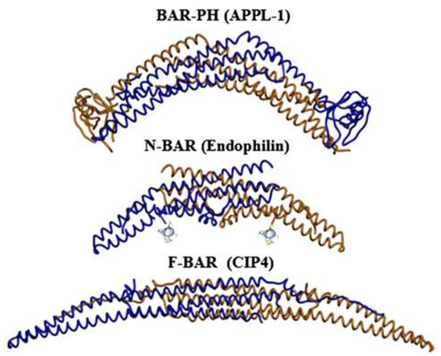

Figure 1: BAR domains of three different proteins of the BAR family (Frost *et al.*,2007).

CIP4 is interesting because it functions in both endocytosis and protrusion; essentially opposite events. In endocytosis it interacts with the plasma membrane to induce vesicle curvature. In the lab we are collaborating with, CIP4 has been observed at the tips of extending filopodial and lamellipodial protrusions in axonal growth cones of developing neurons from prenatal mouse brain (Fig. 2).

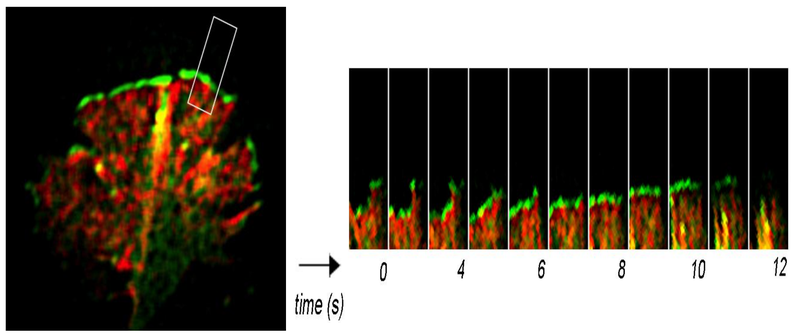

Figure 2: Lamellipodia protrusion on axon growth cone (left) and time lapse of boxed area (right). CIP4 is labeled green and is present at the tips of lamellipodia during extension (image provided by W. Saengsawang).

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Story

We are modeling CIP4 because it is important for the essential cellular process of endocytosis and for human nervous system development, which is the interest of the lab with whom we are collaborating.

- CIP4 has three domains (Fig. 3)
  - FBAR domain induces membrane curvature
  - HR1 and SH3 domains interact with and are important for actin polymerization, the primary polymer in cells that provides force for membrane movement
- These domains may reorient during endocytosis and protrusion to allow actin to properly facilitate either of these processes (Fig. 4)

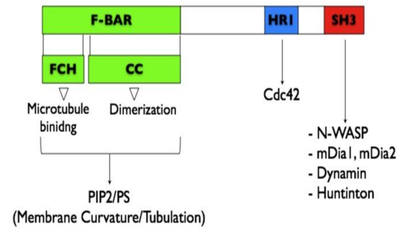

Figure 3: Linear representation of CIP4 monomer. Domains are highlighted, showing some proteins with which they interact (image provided by E. Dent).

Endocytosis vs. **Protrusion**

- Important for cells to retrieve substances from the environment
- Four clusters of positive residues on concave surface of FBAR domain interact with negative membrane (Fig. 4 and 5)
- Many CIP4 dimers wrap around an endocytosing vesicle, forming a tubule(Fig. 6)

- Important for neurons to properly migrate during prenatal development
- Hypothesized that certain positive residues on convex surface of the FBAR domain interact with negative membrane (Fig. 4)
- CIP4 overexpression in neurons = large lamellipodia phenotype (Fig. 7)

Figure 4: Hypothetical model of different interacting surfaces of the FBAR domain and different orientations of the HR1 and SH3 domains of CIP4 in endocytosis (left) and protrusion (right) (image provided by Corey O'Reilly).

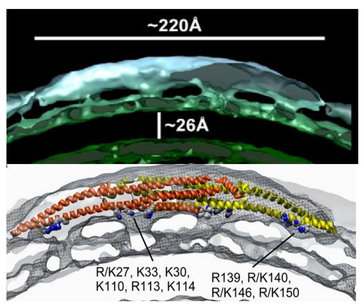

Figure 5: Cross-section through CIP4 dimer on membrane shows four points of interaction. Superposition of FBAR shows residues involved. (Frost *et al.*, 2008).

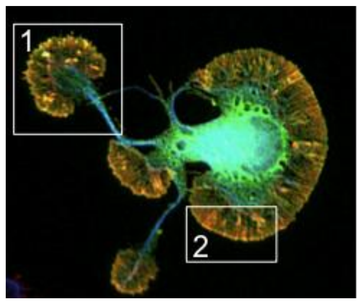

Figure 7: Overexpression of CIP4 produces large lamellipodia in neurons (image provided by S. Saengsawang).

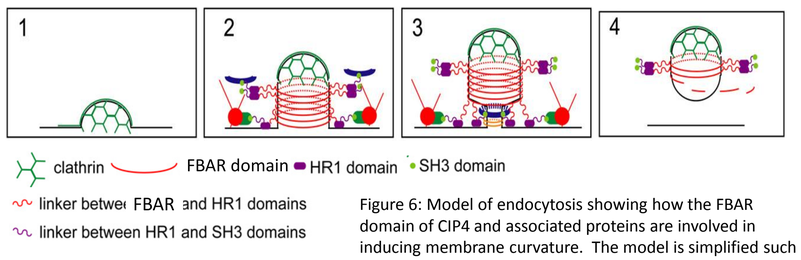

Figure 6: Model of endocytosis showing how the FBAR domain of CIP4 and associated proteins are involved in inducing membrane curvature. The model is simplified such that only a few of the HR1 and SH3 domains are shown. (modified from Shimada *et al.*, 2008).

Cancer

- CIP4 is overexpressed in some highly invasive cancers
- This is consistent with our hypothesis because cancer cells, like migrating neurons, need to become highly protrusive to invade surrounding tissues.

Determination of Amino Acids Important for Endocytosis

Hypothesis: membrane deformation by FBAR requires electrostatic interactions

↓

Result: endocytosis requires anionic headgroups to be present on membrane at >10%mol, confirming hypothesis

↓

New hypothesis: contact between protein's cationic residues and phospholipid headgroups constrain membrane to match curvature of the FBAR domain

↓

Result: Reconstruction (Fig. 7) shows contacts, superposition of atomic coordinates shows that they are clusters of cationic residues on concave surface

↓

Mutation of these positive residues on concave surface of FBAR compromises membrane binding and endocytosis

Ideas for Further Research

- Mutate positive residues on convex surface of FBAR domain to determine if they are important for large lamellar phenotype in CIP4 overexpressing neurons (Fig.8)

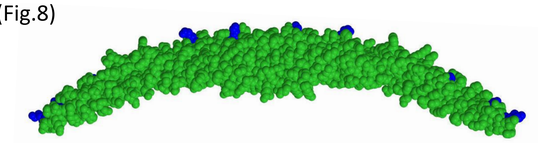

Figure 8: FBAR domain of CIP4 in spacefill. The positive residues highlighted blue on the convex surface may be important for the filopodia and lamellipodia phenotype. They can be mutated in the lab in the future to assess this hypothesis (Shimada *et al., 2008).*

- Phosphorylation of conserved amino acids located between domains may result in the different conformations exposing the two sides of the FBAR (Fig. 9)
- Mutate possible phosphorylation sites and analyze changes in lamellipodial and endocytic phenotype

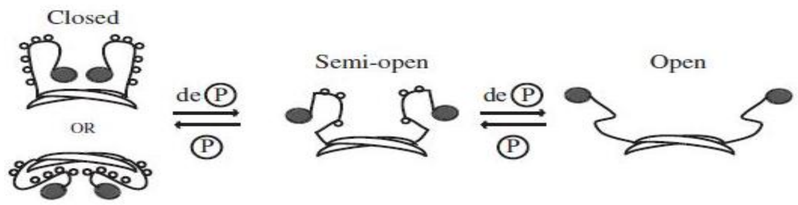

Figure 9: Hypothetical model of CIP4 phosphoregulation. Phosphorylation or dephosphorylation may result in a conformation that blocks CIP4s ability to bind the membrane surface with its concave or convex surface. It has not yet been determined which conformation phosphorylation induces (Roberts-Galbraith *et al.*, 2010).

Summary

CIP4 function is important in endocytosis and for the proper development of the human nervous system. CIP4 senses and induces membrane curvature by using positive residues on its concave surface of its FBAR domain during endocytosis. The positive charges on the convex surface might be used to induce filopodia and lamellipodia in neurons. Future mutagenesis studies could include point mutations of the positive residues on the convex surface to find out which ones are important for protrusion in neurons.

References

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