appears to be a fundamental and highly conserved component of centromeres in a variety of organisms, but its partners in mitosis and meiosis are divergent.

The study of Tanaka et al. (2009), together with other recent studies, highlight that despite the high degree of underlying structural and functional conservation, significant divergent and alternative kinetochore assembly pathways exist among yeasts and between yeast and higher eukaryotes. Importantly, these studies provide critical insights into how a centromere protein such as CENP-C is able to play an adaptive role in both mitosis and meiosis to ensure starkly different correct outcomes of chromosome orientation and polar segregation.

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Mechanisms of Cellular Protrusions Branch Out

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F-BAR domains bind curved membranes and induce membrane invagination. In a recent *Cell* paper, Guerrier et al. describe an "inverse" F-BAR family member that induces outward curvature and filopodia in migrating neurons. These findings suggest that F-BAR domains are functionally diverse and regulate different types of membrane morphology.

Cell migration and cell shape dynamics are central to the development of multicellular organisms with complex tissues and organs. Coupled to migration and cell morphology is the formation of membrane protrusions such as filopodia (Latin for "thread extensions"). Filopodia represent extreme cases of energetically unfavorable peripheral evaginations, and as such, cells must coordinate multiple mechanisms to efficiently deform and support the extension of these membrane structures. Filopodia are thought to serve as cellular "antennae" that explore the extra-cellular environment, sense cues, and signal back to the cell an appropriate response.

The genesis of filopodia depends primarily on the regulation of the actin cytoskeleton. Filopodia consist of bundles of unbranched actin filaments, and the generation of these filaments is promoted by proteins such as fascin, Mena/VASP, and formins (Mattila and Lappalainen, 2008). Now, recent studies, including that of Guerrier et al. (2009), support a newly emerging view that BAR domain superfamily proteins, which directly mold the membrane, also facilitate filopodial formation in conjunction with actin remodeling (Yang et al., 2009). The BAR superfamily of domains is composed of three main groups: the Bin/Amphiphysin/Rsv (BAR) domain, the Inverse BAR domain (I-BAR, also called IMD domain), and the Fes-Cip4 Homology BAR (F-BAR) domain (also called EFC domain) (reviewed in Itoh and De Camilli, 2006) (see Figure 1). All BAR superfamily members dimerize and display clusters of positively charged residues at their surface that interact with membrane lipids. The BAR domain recognizes membranes via a concave surface that invaginates membrane to facilitate endocytosis (Figure 1A).

I-BAR domains, such as that of IRSp53, induce membrane evagination, and overexpression of these domains potently induces cellular filopodia (reviewed in Mattila and Lappalainen, 2008) (Figure 1B). Because IRSp53 also contains an SH3 domain that interacts with regulators of the actin cytoskeleton, including Esp8, WAVE2, N-WASP, and mDia, it serves to couple I-BAR mediated membrane protrusive activity with regulated actin dynamics. IRSp53 may be held in an inactive state until it binds ligands via this SH3 domain, as it displays reduced filopodial activity in cells lacking its binding partners (Lim et al., 2008). Recent work, combining quantitative analysis of

time-lapse imaging and electron microscopy, suggests that the I-BAR domain may initiate membrane protrusions that are subsequently filled with actin filaments required for filopodial elongation (Yang et al., 2009).

Similar to the archetypical BAR domain, many F-BAR domains appear to regulate endocytosis (Figure 1A). Like IRSp53, they also coordinate actin polymerization with membrane dynamics. Structural and functional work on the F-BAR domain shows they facilitate membrane invagination by a self-assembly mechanism that utilizes dimerization, lateral, and end-to-end contacts between dimers (Frost et al., 2008). The sequential assembly of these contacts results in the generation of a helical coat that invaginates the lipid bilayer into larger and more rigid structures than the related BAR domain. Yet, the F-BAR domain has only been recognized recently, and being the newest member of the BAR domain superfamily only a limited number of F-BAR domains have been studied. Against this backdrop emerges the Guierrier et al. study of srGAP2 (Guerrier et al., 2009), an F-BAR containing Rho-GTPase activating protein previously implicated in cortical development downstream of neurogenin (Mattar et al., 2004). The F-BAR domain of srGAP2, when expressed in cells, appears to induce outward protrusions, which is exactly opposite of all other F-BAR domains studied to date (Figure 1B). The morphology of these protrusions closely resembles those induced by the I-BAR domain of IRSp53. The srGAP2 F-BAR domain alone evaginates membrane, since the purified protein also generates protrusions when added to the inside of lipid vesicles. Knockdown of srGAP2 alters cortical neuron morphology, resulting in decreased branching of neurites. These results alone are interesting because they demonstrate that srGAP2 has diverged from the canonical F-BAR domain to function as an "Inverse" F-BAR or IF-BAR domain. Because of the implication that srGAP2 functions downstream of neurogenin during cortical development, its role in migration was also examined. Overexpression of srGAP2 inhibited migration, while knockdown enhanced migration. The effects of srGAP2 overexpression could be mimicked by overexpression of just the IF-BAR domain alone, but also partially mimicked by expression of the RhoGAP and SH3 domains. These results suggest that the srGAP2 IF-BAR domain likely coordinates membrane protrusions with the regulation of actin dynamics via these associated domains. Interestingly, IRSp53 is also expressed in neurons, but knockout mice do not display any obvious abnormalities in neuronal morphology, including dendritic branching (Sawallisch et al., 2009). Thus, the srGAP2 IF-BAR domain may have specific functions for regulating neuronal protrusions not shared by the functionally related I-BAR containing protein IRSp53.

The results of this study not only highlight the role of srGAP2 in regulating cortical neuronal migration during development, but also detail how the cell has used individual domains within the BAR superfamily as scaffolds upon which to build new membrane deforming properties. Discovering the true diversity of these properties awaits further characterization of new members. Indeed, it seems very likely that other IF-BAR domains exist. Additional questions arise from this initial study of srGAP2. For example, what are the physiologic binding partners of its SH3 domain that help regulate membrane protrusion? Other closely related Rho-family GAPs, including WRP and srGAP1, interact with actin regulators such as WAVE1 and the migration guidance receptor Robo-1 (Soderling et al., 2007; Wong et al., 2001). It will be particularly exciting to analyze how the presumed IF-BAR domains of these members facilitate the functions of these pathways. This is especially true for srGAP1, since Robo receptor activation is also known to influence filopodial formation. Finally, what is the structural basis for the IF-BAR domain protrusive activity? Clearly the related F-BAR self assembles into a coat to facilitate membrane invagination, while evidence to date suggests the I-BAR domain may also coat membranes during protrusion (Yang et al., 2009). Whether the IF-BAR mechanism is similar will have to await further structural analysis. As these and other questions are addressed, we can expect to find new branches in the IF-BAR story—not unlike the unique protrusions they induce.

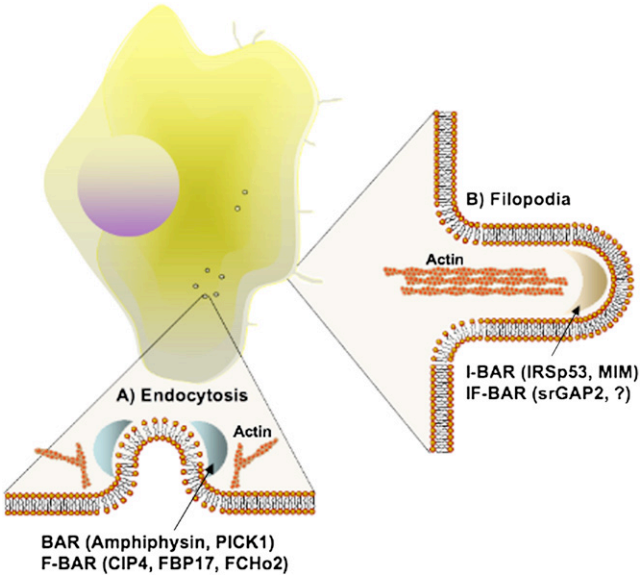

Figure 1. Membrane Morphology Regulated by the BAR Domain Superfamily

The BAR domain superfamily can recognize and induce either membrane invaginations or evaginations.

(A) Membrane invaginations such as those induced during endocytocysis are supported by both BAR and F-BAR domain containing proteins.

(B) The I-BAR and Inverse F-BAR, or IF-BAR, domains recognize and induce membrane projections such as filopodia.

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