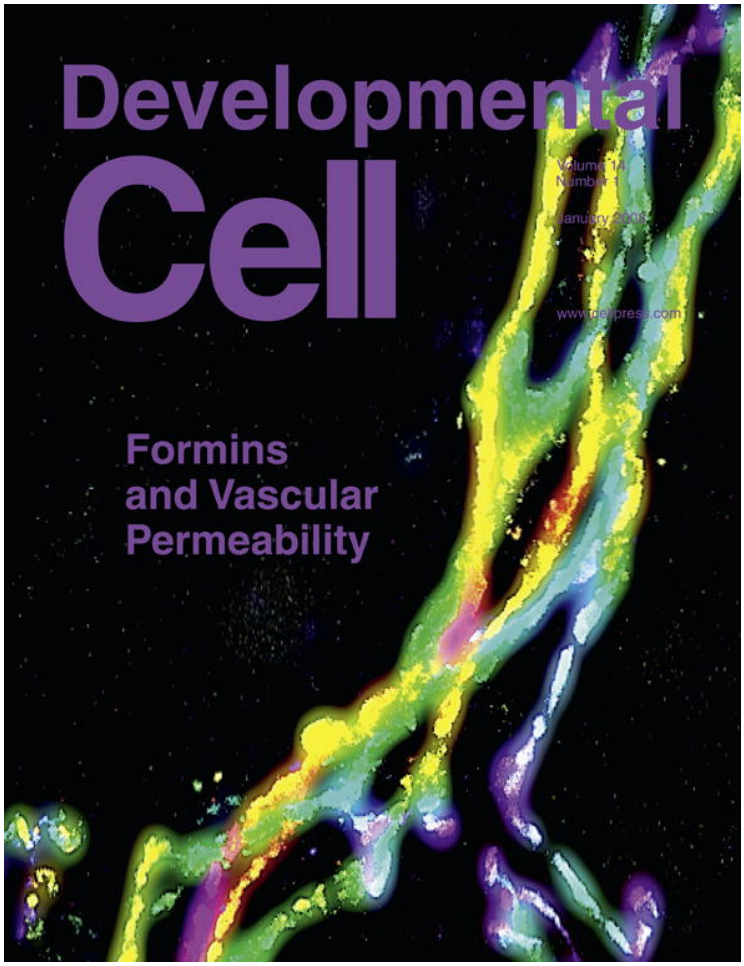

mutant animals. When endosome-to-Golgi retrieval of MPR is blocked by dominant-negative Rab9 expression, cells compensate by elevating the production of lysosomal hydrolases and by internalization of secreted hydrolases (Riederer et al., 1994). Similarly, these robust and redundant mechanisms probably insure that lysosomal function is unaffected by the mutation of retromer components. Trafficking of signaling molecules and their receptors appears to be more sensitive to perturbed retromer function. While no data yet suggests that cells normally modulate retromer-dependent trafficking to control cellular signaling pathways, this is an intriguing subject for future investigations.

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MIT Domainia

James H. Hurley[1,*] and Dong Yang[1]
[1]Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, U.S. Department of Health and Human Services, Bethesda, MD 20892, USA
*Correspondence: hurley@helix.nih.gov
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The AAA ATPase Vps4 disassembles the membrane-bound ESCRT-III lattice. Four recent publications show how Vps4 carries out this task in a partnership with another ESCRT-associated protein, Vta1. Vps4 and Vta1 both contain MIT domains, which bind to "MIT-interacting motifs" (MIMs) of ESCRT-III proteins. As new MIT domain proteins are rapidly being identified, these studies will likely have relevance well beyond Vps4.

The ESCRT (endosomal sorting complexes required for transport) complexes and associated proteins constitute a fundamental membrane scission machine (Hurley and Emr, 2006). Conserved throughout eukaryotes, the ESCRT machinery directs membrane budding away from the cytosol. ESCRTs are required for the budding of intralumenal vesicles from the limiting membrane of endosomes to form multivesicular bodies (MVBs), for the budding of many enveloped viruses from the plasma membrane of animal cells, and for the membrane abscission step in cytokinesis. The upstream components of the system contain specific phosphoinositide-binding domains, as well as specific ubiquitin-binding domains, which probably serve to concentrate ubiquitinated cargo. The interaction between lipid- and ubiquitin-binding domains and their partners is a major theme underpinning the ESCRT field and begs the question of whether there are other themes in molecular interactions that the field has missed.

The upstream complexes are not thought to mediate membrane scission on their own. Rather, they recruit subunits of the ESCRT-III complex to the endosomal membrane, where they assemble into an insoluble array of poorly understood stoichiometry. The ESCRT-III array is disassembled by the ESCRT-associated ATPase Vps4, which, like many other AAA ATPases, forms a double hexameric ring. ATP hydrolysis is required for the disassembly, and Vps4 is essential for all identified ESCRT functions. Vps4 contains an N-terminal MIT (microtubule interacting and transport) domain and a C-terminal catalytic domain. ESCRT-III is the only component of the system known to form arrays on the membrane, whereas Vps4 is the only ATP-burning machine in the ESCRT pathway. Although there is almost no hard evidence that these proteins directly catalyze membrane scission, ESCRT-III and Vps4 are clearly at some level pivotal players in driving the membrane scission reaction. Thus an understanding of the interaction between Vps4 and ESCRT-III is a prerequisite for understanding the larger process of membrane scission.

MIT domains were first noted in a study of the sorting nexin SNX15 (Phillips et al., 2001) and subsequently identified in other trafficking proteins (Ciccarelli et al., 2003; Row et al., 2007; Tsang et al., 2006), many connected in some way to the ESCRT pathway. The MIT domain of Vps4 binds directly to a ~20 residue C-terminal

sequence found in ESCRT-III subunits (Scott et al., 2005). In a working model of Vps4 function, the MIT domains of the membrane-proximal hexamer bind to ESCRT-III subunits, which are then pumped one at a time through the central pore of Vps4 concomitant with ATP hydrolysis. Four new papers, two in this issue of *Developmental Cell* (Azmi et al., 2008; Xiao et al., 2008) and two in *Nature* (Obita et al., 2007; Stuchell-Brereton et al., 2007), add insights into this reaction mechanism. Vta1 is a Vps4-associated protein whose precise function has been unclear. Vta1 is now shown to be a very long rod that dimerizes, binds Vps4 through its C-terminal domain, and binds ESCRT-III subunits via its N-terminal domain (Xiao et al., 2008). Most strikingly, the N-terminal ESCRT-III binding domain of Vta1 is revealed to be a tandem pair of MIT domains. The Vta1 MIT2 domain binds just two of the six yeast ESCRT-III proteins, Did2 and Vps60 (Azmi et al., 2008). Vps4 is also selective for two ESCRT-III proteins, Did2 and Vps2 (Azmi et al., 2008; Obita et al., 2007; Stuchell-Brereton et al., 2007). Indeed, Did2, the only ESCRT-III protein capable of binding both Vps4 and Vta1, has been implicated as a key Vps4 coupling factor among the various ESCRT-III proteins (Lottridge et al., 2006; Nickerson et al., 2006). Azmi and colleagues show that Vps4 ATPase activity is potently activated by the appropriate combinations of Vta1 and the ESCRT-III ligands of Vps4 and Vta1. The picture that emerges is that the "real" functional complex that disassembles ESCRT-III in vivo is almost certainly a Vps4:Vta1 complex rather than Vps4 alone. This model may resolve a problem with Vps4 biochemistry, in that Vps4 is a monomer or dimer in the absence of ATP. It would have been inefficient for Vps4 to undergo a complete round of dodecamerization for every molecule of ATP consumed. The formation of a complex with Vta1 appears to resolve this issue by stabilizing the Vps4 dodecamer. Furthermore, Vta1 should in principle increase the processivity of the Vps4:Vta1 complex on the ESCRT-III lattice, and enhance its specificity for a Did2-containing lattice. As a late-acting ESCRT-III protein, incorporation of Did2 into the lattice might initiate its disassembly at the appropriate time.

Given the rapid growth in the number of identified MIT domains (Row et al., 2007; Tsang et al., 2006; Xiao et al., 2008) (Figure 1A), the determination of the structures of three related MIT domains in complex with ESCRT-III C-terminal fragments could not have been more timely. The term "MIT-interacting motif" (MIM) has been proposed for these C-terminal ESCRT-III fragments. There are two isoforms of human VPS4, A and B. The solution NMR structures were determined for VPS4A and VPS4B bound to the MIMs of DID2A and VPS2B, respectively (human DID2A is also known as CHMP1A, while human VPS2B is also known as CHMP2B) (Stuchell-Brereton et al., 2007). The MIT domain of yeast Vps4 was cocrystallized with the Vps2 MIM (Obita et al., 2007). MIT domains are structurally similar to the dihelical tetratricopeptide repeat (TPR) domain. The three helices of the MIT domain correspond to 1.5 dihelical TPR repeats. In all three complex structures, the MIM appears to supply this missing half of the second dihelical repeat, except that the N-to-C direction of the helix is reversed. The affinities of all the complexes are very similar as well (roughly 30 μM). This modest affinity is typical of binary interactions that normally occur in the context of multivalent assemblies in trafficking. The affinity of the complex derives from hydrophobic interactions with three Leu residues located on the same face of the MIM helix at three consecutive turns, together with salt bridges involving three charged MIM side chains. These side chains collectively define a motif that determines specificity for Vps4 (Obita et al., 2007).

However, we are still far from having a predictive MIT-MIM code for proteins other than Vps4. A comparison of the Vps4 MIT domain to the Vta1 MIT2 domain highlights how far there is still to go. Vta1 MIT2 and Vps4 MIT both bind Did2, yet none of the three critical charged residues in the Vps4 MIT domain (Figure 1B) are present in the Vta1 MIT2. By the same token, it is hard to draw up general rules about MIT recognition by MIMs. The Vta1 MIT2 binds Did2 and Vps60, but not Vps2. This seems inconsistent with the observation that the MIMs of Did2 and Vps2 are much more similar to each other than to the corresponding region in Vps60. In time, the

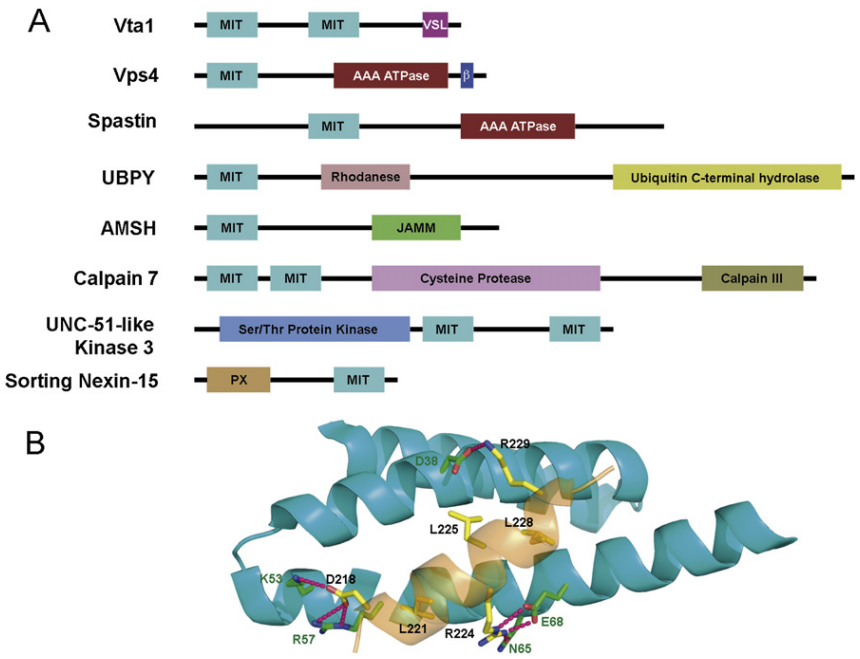

Figure 1. MIT Domain Protein Architectures and Structure

(A) Architectural diversity of MIT-domain-containing proteins. VSL, Vta1/SBP1/LIP5, the region of Vta1 responsible for binding to Vps4; β, the C-terminal β sheet region of Vps4 responsible for binding to Vta1; JAMM, JAB1/MPN/Mov34, a metal-dependent deubiquitinating enzyme catalytic domain; PX, Phox homology domain.

(B) Structure of the MIT domain of Vps4 (light blue) bound to the MIM of Vps2 (orange with residues labeled black). Key charged interactions between the Vps4 MIT domain and the Vps2 MIM are shown with dashed magenta lines.

principles of such a recognition code will be more fully mapped out. As these principles emerge, we can expect MIT domains to take their place alongside ubiquitin- and phosphoinositide-binding domains as a major organizing theme in the ESCRT system and related pathways.

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Subversion of Myosin Function by *E. coli*

Seema Mattoo,[1] Neal M. Alto,[3] and Jack E. Dixon[1,2,*]
[1]The Howard Hughes Medical Institute, Leichtag Biomedical Research Building
[2]Department of Pharmacology, School of Medicine
University of California at San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0721, USA
[3]University of Texas Southwestern Medical Center, 6000 Harry Hines Boulevard, NA6.308, Dallas, TX 75390, USA
*Correspondence: jedixon@ucsd.edu
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Enteropathogenic *E. coli* establish close contact with host cells by nucleating localized actin rearrangements and directly evading phagocytosis. Iizumi et al. now show in a recent issue of *Cell Host and Microbe* that the type III secretion effector EspB, initially thought to be involved in the translocation of other bacterial effectors, mediates antiphagocytosis and microvilli lesions by inhibiting myosin function.

Enteropathogenic *Escherichia coli* (EPEC) colonize the gut mucosa and elicit diarrheal disease (Caron et al., 2006). The intestinal and colonic epithelia colonized by EPEC form a highly specialized cell type consisting of an apical brush border surface that faces the gut lumen and a basolateral membrane that makes contact with the interstitial tissue environment. EPEC infection is characterized by attaching and effacing (A/E) lesions, caused by bacterially mediated effacement of the intestinal brush border microvilli and subsequent intimate attachment of the bacterium to the host epithelium.

EPEC belong to a large class of Gram-negative bacterial pathogens that utilize a specialized type III secretion system (T3SS) to directly inject "effector" proteins into the host cell; these effectors can severely alter the host's cell-signaling mechanisms (Caron et al., 2006). For EPEC, this arsenal of T3SS effectors is critical for virulence. EPEC effectors initiate signaling events that promote actin rearrangement and formation of a characteristic "pedestal"-shaped membrane protrusion from the host cell. The bacteria make close contact with these pedestals, which move along the surface of the host cell in an actin-dependent manner. This movement is accompanied by the concurrent clearing of the microvillus brush border as EPEC establish their colonization niches. Several proteins involved in actin dynamics (Figure 1) are present in EPEC-induced pedestals (Freeman et al., 2000). Efficient establishment of infection by EPEC also requires crosstalk between host cytoskeletal networks, and a role for disruption of microtubules by EPEC effectors has been shown (Caron et al., 2006). Interestingly, EPEC establish pedestal formation and microvilli effacement while simultaneously avoiding phagocytosis by macrophages. This antiphagocytic activity directly correlates with T3SS-dependent tyrosine dephosphorylation of several host proteins, for which a responsible effector has not yet been identified (Goosney et al., 1999). While the dynamics of EPEC-induced actin rearrangement have been extensively studied, the mechanism by which these pathogens evade phagocytosis remain unknown.

EspB is another EPEC effector implicated in actin-mediated cytoskeletal rearrangement. EspB is a bifunctional protein that is targeted to the plasma membrane and cytosol of host cells, where it modulates the cell cytoskeleton by decreasing actin stress fibers (Taylor et al., 1999). It functions as a translocator protein