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Review

The Role of BEACH Proteins in *Dictyostelium*

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The BEACH family of proteins is a novel group of proteins with diverse roles in eukaryotic cells. The identifying feature of these proteins is the BEACH domain named after the founding members of this family, the mouse beige and the human Chediak–Higashi syndrome proteins. Although all BEACH proteins share a similar structural organization, they appear to have very distinct cellular roles, ranging from lysosomal traffic to apoptosis and cytokinesis. Very little is currently known about the function of most of these proteins, few binding-partner proteins have been identified, and no molecular mechanism for any of these proteins has been discovered. Thus, it is important to establish good model systems for the study of these novel proteins. *Dictyostelium* contains six BEACH proteins that can be classified into four subclasses. Two of them, LvsA and LvsB, have clearly distinct roles in the cell. LvsA is localized on the contractile vacuole membrane and is essential for cytokinesis and osmoregulation. LvsB is most similar in sequence to the mammalian beige/Chediak–Higashi syndrome proteins and shares with them a common function in lysosomal trafficking. Structural and functional analysis of these proteins in *Dictyostelium* will help elucidate the function of this enigmatic novel family of proteins.

Key words: apoptosis, cell division, CHS, membrane traffic, neurobeachin

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What are BEACH proteins?

The BEACH proteins, defined as those containing the novel BEACH domain, are a diverse group of proteins found in all eukaryotes (1,2). BEACH proteins can be grouped into five different classes with multiple members in each class. Despite this large diversity, the function of most members of this family is not known. The cellular role of only three BEACH proteins is known in any detail: beige, LvsA and FAN. The first BEACH proteins to be identified, the mouse beige and its human ortholog Chediak–Higashi syndrome protein (CHS, also known as LYST), are very large proteins (>400 kDa) involved in lysosomal traffic (3,4). Cells defective in these proteins contain enlarged lysosomes and have secretory defects. Another large member of the BEACH family, the *Dictyostelium* LvsA protein (>400 kDa) is essential for cytokinesis and contractile vacuole function (5,6). This protein associates transiently with the membrane of the contractile vacuole during the expulsion phase (6). Not all BEACH members are large. The mammalian protein FAN is a medium-sized protein (~100 kDa) with a role in sphingomyelin signaling during apoptosis. FAN binds the cytoplasmic domain of CD40 and TNF-R55 receptors and activates a neutral sphingomyelinase in response to ligand binding (7–9).

Structural Organization of BEACH Proteins

All BEACH proteins have a similar structural organization (Figure 1). At the extreme C-terminus they have multiple WD motifs. The sequence identity among the WD motifs of BEACH proteins is low (~20%), but all are predicted to fold into a beta-propeller structure that creates a protein-interaction domain. The BEACH domain is adjacent to the WD domain and is highly conserved among the BEACH proteins (~50–60% identity). Given its size (~35 kDa), the BEACH domain may represent more than a simple protein-interaction domain and could actually harbor an enzymatic activity or have a unique structural role. However, no such activity has been discovered yet. Next to the BEACH domain is a novel domain that folds into a structure similar to that of PH domains (see below), although it does not share sequence similarity to PH domains from other proteins. Together, the WD, BEACH and PH domains comprise about 100 kDa, accounting for most of the size of FAN, but only 25% of the size of most other BEACH proteins. The rest of the BEACH proteins (the N-terminal 75% of their length) are mostly unique to each BEACH protein. Small areas of low similarity (~20% identity) are shared among some BEACH proteins, but the significance of these regions is unknown. In some cases, the BEACH proteins contain a PKA binding motif buried in the large N-terminal region.

Crystal Structure of the BEACH Domain

An exciting recent development is the crystallization of the BEACH domain from neurobeachin, a mammalian BEACH protein expressed in neurons (10). The crystal structure revealed that the BEACH domain folds into a unique structure

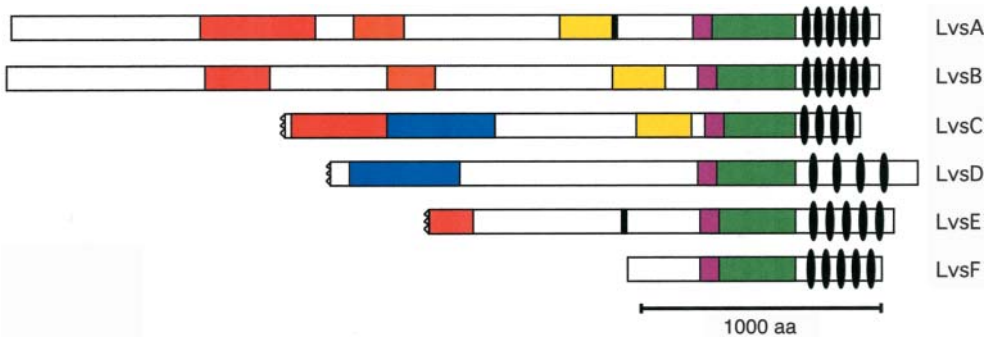

Figure 1: The domain organization of BEACH proteins. This diagram shows the domain organization of the six *Dictyostelium* BEACH proteins. The BEACH domain (green) is the most conserved portion of these proteins (50–60% identity). At the C-terminus of each protein there are multiple WD-40 repeats (ovals). Adjacent to the BEACH domain is a PH-like domain (purple). Other regions of homology (colored regions) are also shared among different Lvs proteins, but the similarity is low. The sequences of LvsC, LvsD and LvsE are truncated at the N-terminus. Accession numbers for these sequences are: LvsA, AAD52096; LvsB, AY159038; LvsC, AY159039; LvsD, AY159040; LvsE, AY159036; LvsF, AY159037.

never seen in any other protein. One of the most unusual features of the BEACH domain structure is the presence of multiple segments that cannot be classified as either beta-strands or random coils. These segments (ε-1 through ε-7) are found in the hydrophobic core of the domain and are stabilized by interactions with the adjacent alpha helices in the structure.

Disappointingly, the structure of the BEACH domain does not provide any insights into the potential role of this domain (10). The vast majority of the conserved amino acids among BEACH proteins are found in the interior of the molecule. There are no obvious pockets that may represent catalytic sites. An intriguing aspect of the BEACH crystal structure is that it displayed an intimate contact between two adjacent molecules. An exposed loop between segments ε-4 and ε-5 of one molecule was inserted into a cleft in the adjacent molecule. At this point it is not clear whether this represents a physiological interaction among beach proteins or simply an artifact of crystallization. Sequence comparisons (Figure 2) show that this portion of the BEACH domain is significantly conserved among BEACH proteins from different species (50% identity between *Dictyostelium* LvsA and Human CHS). It is possible that this exposed loop plays an important role in the function of BEACH proteins, perhaps by allowing BEACH proteins to interact with each other or other binding-partners.

The crystal structure of the BEACH domain provides a useful framework to understand the divergence in sequence among different BEACH proteins. Since most of the conserved residues among BEACH proteins are found in the interior of the domain, the surfaces of the different BEACH domains are quite different from each other. This implies that the BEACH domains from different proteins may bind to completely different sets of proteins or that their function does not involve binding to other proteins. Perhaps the function of the BEACH domain is simply to organize the other adjacent domains, the PH-like and the WD domains.

Other Domains Found in BEACH Proteins

A surprising discovery in the neurobeachin crystal structure is that the protein portion immediately upstream of the BEACH domain folds into a structure very similar to that of the PH domains (10). The PH-like domain of neurobeachin has the same set of beta strands that comprise the core of most PH domains. However, unlike other PH domains, the PH-like domain of neurobeachin probably does not bind to phosphorylated phospholipids. In neurobeachin, the portion of the PH-like domain that would bind phospholipids is blocked by an inserted alpha-helix and by portions of the BEACH domain. The PH-like domain has extensive interactions with the BEACH domain. These interactions are essential for the function of BEACH proteins, since the alteration of such interactions abrogates the function of FAN.

As mentioned above, all BEACH proteins contain multiple WD-motifs at their C-termini. Although this region of the BEACH proteins has not been crystallized, it is likely folded into the conserved beta-propeller structure seen in other WD-motif-containing proteins. The function of this domain is probably to serve as an interaction module with other proteins. In fact, the WD-domain of FAN interacts with the cytosolic domain of TNF-R55, and this interaction is essential for its role in activating the neutral sphingomyelinase at the plasma membrane (7). Unfortunately, other physiological interactions have not been discovered for the WD-domain of any other BEACH protein.

A particular group of BEACH proteins has been shown to interact with the regulatory subunit of PKA and may therefore represent a novel class of A-kinase-adaptor proteins (AKAPs)

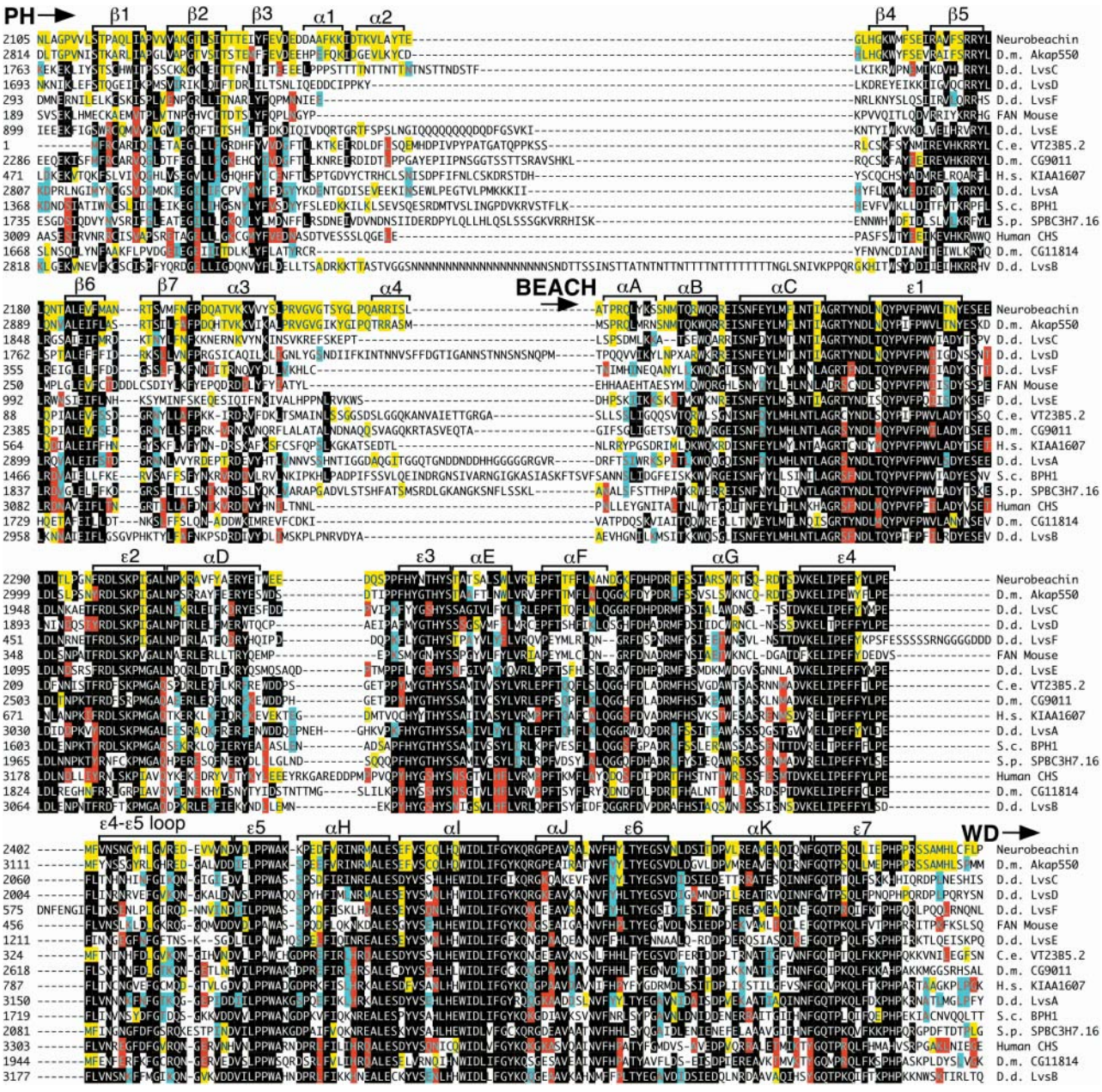

Figure 2: Alignment of the PH-like and BEACH domains of several BEACH proteins. The PH-like and BEACH domains of all *Dictyostelium* Lvs proteins were aligned to the corresponding portions of selected animal BEACH proteins. The top sequence is that of neurobeachin, which was recently crystallized (10). The different structural elements identified in the crystal structure are indicated on top of the neurobeachin sequence. The PH-like domain starts at β1 and ends at α4 and the BEACH domain starts at αA and ends at ε7. Positions where at least 5 sequences share the same sequence are indicated in white letters on black background. Those sequences identical to LvsA are highlighted by a blue background; those identical to CHS by a red background; and those identical to neurobeachin by a yellow background

(11,12). The mammalian neurobeachin and *Drosophila* AKAP-550 are closely related members in this group. The portion of these BEACH proteins that interacts with the RII subunit of PKA is embedded in the N-terminal portion in a region of low similarity among them. The significance of this interaction is not clear and has not been tested in any system.

Classification of BEACH Proteins

Since the BEACH domain is the most conserved portion of all BEACH proteins, it is also a useful segment to study the phylogenetic relationship among BEACH proteins (2). The availability of multiple sequenced genomes makes it also

possible to see the extent to which these intriguing proteins are represented in each organism. This analysis shows that both budding and fission yeasts contain a single BEACH protein, while *Dictyostelium* possesses six BEACH proteins. Among multicellular organisms humans have about six, *Arabidopsis* and *Drosophila* contain five, and nematodes contain only three members of the BEACH family. Therefore, it seems that the BEACH family of proteins is an ancient group of proteins that diversified quite early, before the separation of animals and plants.

The phylogenetic analysis of BEACH proteins indicates that they can be clustered into distinct classes (Figure 3). Some of these classes clearly have distinct functional roles in the cell, and it would be reasonable to predict that each class may have a different cellular role. Class I includes beige and its homologs CHS (LYST) and LvsB. Class II is represented by LvsA; Class III by FAN; and Class IV by neurobeachin and AKAP550. A fifth class includes a group of plant and *Dictyostelium* proteins closely related to Class IV, but their function has not been elucidated.

Interestingly, the proteins within each class share additional regions of sequence similarity with each other but not to proteins from other classes. This adds further support to the idea that each class represents a distinct functional group. This is an analogous situation to that found in many other protein families, like the myosins, kinesins, and small-GTPases, where each subgroup has a distinct cellular role.

LvsA Has an Essential Role in Cytokinesis and Contractile Vacuole Function

LvsA was the first BEACH protein identified in *Dictyostelium* during a cytokinesis screen searching for cytokinesis mutants (5). LvsA null mutants have a complete defect in cytokinesis when the cells are in suspension culture, but not when allowed to attach to a substrate. When attached to a substrate, *Dictyostelium* cells have alternative ways to divide during mitosis or even during interphase (13). Thus, it is possible to maintain cultures of LvsA mutants on a plate and then determine their cytokinesis defect while in suspension conditions. This analysis revealed that LvsA mutants fail in cytokinesis at a late stage of cytokinesis. The mutant cells are able to form an initial cleavage furrow at the correct equatorial location at the right time of the cell cycle. This indicates that the mechanisms for the formation of the contractile ring are intact in the LvsA mutant cells. However, the ingression of the cleavage furrow is never complete; the region of the furrow begins to swell until it becomes a large bulge in the middle of the cell. Eventually, the cell rounds up and becomes a binucleate cell.

Interestingly, this form of cytokinesis failure in LvsA mutant cells is very similar to that seen when *Dictyostelium* clathrin mutants fail in cytokinesis (14). This and other similarities in the phenotype of these two mutant strains strongly suggest that LvsA participates in a membrane traffic pathway that also involves clathrin. However, since clathrin mutants also have many other defects not found in LvsA mutants (15,16), it is clear that clathrin controls a broad set of membrane traffic pathways, one of which requires LvsA during cytokinesis.

How these two proteins are required in cytokinesis is not yet clear, but several scenarios are possible. Since beige and LvsB seem to control the fusion/fission rate of endosomes (see below), it is reasonable to postulate an analogous function for LvsA. In several other systems, it is known that specific sets of vesicles fuse in the region of the cleavage furrow during cytokinesis. Perhaps a clathrin-dependent pathway generates this set of vesicles and LvsA controls their fusion to the membrane

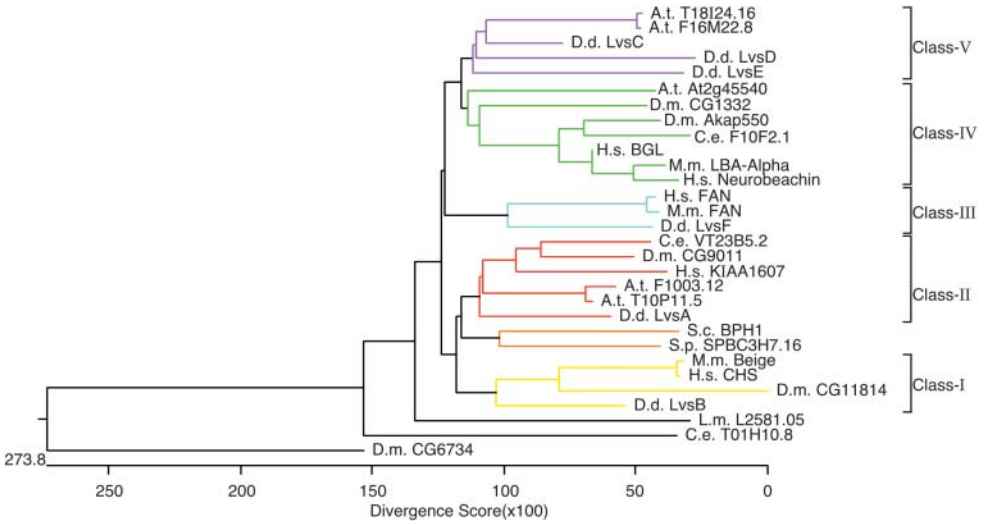

Figure 3: Phylogenetic tree of family of BEACH proteins. The BEACH and WD domains of the indicated sequences were aligned by the ClustalW algorithm and the alignment was used to construct this phylogenetic tree. The different classes of BEACH proteins are indicated by the brackets on the right.

of the furrow. These vesicles may deliver special proteins or lipids necessary for the completion of cytokinesis.

In addition to its role in cytokinesis, LvsA is also required for the function of the contractile vacuole during osmoregulation (6). This organelle is comprised of a set of membrane sacs and tubules that are derived from the endolysosomal system (17,18). *Dictyostelium* cells can withstand a hypoosmotic environment by disposing of excess water through the contractile vacuole. The function of this organelle is not required for cytokinesis, since contractile vacuole mutants do not have cytokinesis defects (19). Thus, the role of LvsA in osmoregulation is probably a separate function from its role in cytokinesis. In fact, recent genetic experiments show that the cytokinesis defect of LvsA mutants can be suppressed while retaining their osmoregulation defect (J. Yajnik and A. De Lozanne, unpublished observations). Nonetheless, understanding the function of LvsA in osmoregulation may shed light onto its role in cytokinesis. Interestingly, LvsA associates with the membrane of the contractile vacuole, but only when the vacuole has reached its maximum diameter. LvsA stays on the vacuole until it has contracted completely, and then comes off into the cytoplasm (6). This suggests that LvsA may have a role during the expulsion phase of the contractile vacuole. Perhaps LvsA regulates the fusion of the contractile vacuole with the plasma membrane for water expulsion. In fact, we have observed that some LvsA mutants display very large vacuoles that do not contract normally as if they fail during expulsion (6).

An alternative scenario for the function of LvsA is suggested by the activity of FAN (7). This mammalian protein activates a neutral sphingomyelinase that resides on the plasma membrane. Since FAN interacts with TNF-R55 receptors via its WD domain, it is presumed that the BEACH/PH-like domains are involved in the activation of the sphingomyelinase. Perhaps a common function of BEACH proteins is to induce lipid remodeling by activating a lipidase or other lipid-modifying enzyme. Thus, perhaps the function of LvsA is to change the lipid composition of the contractile vacuole for expulsion and of the cleavage furrow for cytokinesis. Curiously, changes in lipid composition have been implicated for both events. A change in lipid composition has been proposed to induce the change in membrane curvature that drives water expulsion in the contractile vacuole of *Paramecium* (20). In mammalian cells, the sudden exposure of phosphatidylethanolamine (PE) on the outer leaflet of the membrane at the cleavage furrow is essential for disassembly of the contractile ring and for successful cytokinesis (21). It will be interesting to test whether these events are mediated by LvsA or related BEACH proteins.

LvsB Has an Important Role in Lysosomal Function

LvsB is the *Dictyostelium* protein most closely related to the mammalian beige and CHS proteins (Figures 2 and 3). Interestingly, disruption of the LvsB gene produces a phenotype very similar to that found in beige and CHS mutant cell lines (22,23). LvsB/beige/CHS mutants contain enlarged lysosomes and display secretory defects. In *Dictyostelium,* these defects do not result in any harmful effects to the cell, and the mutants are able to grow vigorously and develop normally (2). While the loss of beige/CHS is relatively mild in mammalian cells in culture, it results in severe immune and neurological disorders (1). The cause of enlarged lysosomes is not completely understood. Overexpression of beige/CHS protein in mammalian cells leads to the formation of smaller than normal lysosomes. This result suggested the possibility that this protein induces the fission of the lysosome into smaller compartments. This possibility also explains the enlargement of lysosomes in the absence of beige/CHS. An alternative view would be that beige/CHS proteins are negative regulators of homotypic fusion of endosomes and lysosomes. Increased expression of beige/CHS protein could inhibit the fusion of prelysosomal vesicles, resulting in small lysosomes; the absence of beige/CHS could increase fusion of vesicles, resulting in giant lysosomes.

To distinguish between these two possibilities, an *in vivo* fusion assay was used to study the formation of large endosomes in *Dictyostelium* LvsB mutant cells (22). Using either fluid-phase markers of endocytosis or bacteria for phagocytosis, an enhanced rate of endosome fusion was clearly demonstrated for the LvsB mutant cells. This result provides greater support for the idea that these proteins are negative regulators of homotypic endosome fusion. However, there is still the possibility that beige/LvsB proteins are positive regulators of fission. While the lysosomal compartment is enlarged in the mutant cells, the number of lysosomes per cell is greatly reduced. This could potentially increase the observed rate of fusion of new endosomes because the number of potential targets is greatly reduced. A definitive answer may be obtained by using an *in vitro* endosome fusion assay (24). Further studies of the *Dictyostelium* LvsB mutant should provide important insights into the function of this important family of proteins.

Other Dictyostelium BEACH Proteins

The function of the other four BEACH proteins in *Dictyostelium* is not known at this time. The knockout mutations of each of their genes did not reveal any significant phenotype in cell growth, osmoregulation, secretion, endocytosis or development (2,22). Further work will be necessary to elucidate their function.

In the case of LvsC, LvsD and LvsE, it seems possible that they may have redundant functions as they are closely related to each other and form part of the same BEACH protein class V. It may be necessary to disrupt all three genes to elicit a mutant phenotype. Since this group of proteins is closely related to class IV, it is possible that these proteins may also be AKAPs. Biochemical analysis of these proteins should reveal if they can bind the RII subunit of PKA. Localization of

these proteins may also be interesting, since some class IV proteins (neurobeachin) have been shown to localize in a near-Golgi membrane compartment (12).

Dictyostelium LvsF protein is closely related to mammalian FAN. Interestingly, this smallest member of the BEACH protein family is only found in mammals and *Dictyostelium*. No ortholog of FAN seems to exist in either flies or nematodes. In mammals, FAN plays an important role in TNF-signaling and apoptosis (7,9). Programmed cell death plays an important role during *Dictyostelium* development. Some cells in a fruiting body die and become encased in a rigid cellulose wall to form the rigid stalk that supports the spore head (25). Recently, a molecular pathway for apoptosis has been described in *Dictyostelium* (26). Perhaps LvsF also plays a role in apoptosis in this organism.

The Future

Dictyostelium is the only unicellular model system that contains the full complement of BEACH proteins found in metazoans. With the extensive array of tools available to study *Dictyostelium* cells, it is clearly a great system to dissect the function of this novel group of proteins. The comparative study of the function of each Lvs protein should provide greater insight into the molecular function of these proteins. What is the significance of the poorly conserved N-terminus of all BEACH proteins? Do they bind to different partners or provide specific functions? Is there a common mechanism of action to all these proteins? These and other questions can be addressed in *Dictyostelium and* will provide an excellent framework to understand the role of BEACH proteins in other organisms.

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