

BEACH Family of Proteins: Phylogenetic and Functional Analysis of Six *Dictyostelium* BEACH Proteins

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Abstract The beige and Chediak-Higashi syndrome (BEACH)-domain containing proteins constitute a new family of proteins found in all eukaryotes. The function of these proteins, which include the Chediak-Higashi syndrome (CHS) protein, Neurobeachin, LvsA, and FAN, is still poorly understood. To understand the diversity of this novel protein family, we analyzed a large array of BEACH-family protein sequences from several organisms. Comparison of all these sequences suggests that they can be classified into five distinct groups that may represent five distinct functional classes. In *Dictyostelium* we identified six proteins in this family, named LvsA-F, that belong to four of those classes. To test the function of these proteins in *Dictyostelium* we created disruption mutants in each of the *lvs* genes. Phenotypic analyses of these mutants indicate that LvsA is required for cytokinesis and osmoregulation and LvsB functions in lysosomal traffic. The LvsC-F proteins are not required for these or other processes such as growth and development. These results strongly support the concept that BEACH proteins from different classes have distinct cellular functions. Having six distinct BEACH proteins, *Dictyostelium* should be an excellent model system to dissect the molecular function of this interesting family of proteins. *J. Cell. Biochem.* 86: 561–570, 2002. © 2002 Wiley-Liss, Inc.

Key words: beige; Lyst; CHS; lysosomal traffic

The Chediak-Higashi syndrome (CHS) is a rare recessive hereditary disease characterized by partial albinism and immune disorders among other symptoms [Introne et al., 1999; McVey Ward et al., 2000]. A similar disorder has been identified in mice (beige) and many other mammalian species. The common feature of these disorders is the presence of very large lysosomes in many cell types. In addition, protein traffic through the endolysosomal pathway is abnormal which leads to defects in the secretion of lysosomal enzymes. These characteristics have led to the proposal that the protein affected in CHS/beige cells is involved in the regulation of lysosomal trafficking. Thus, this protein has been called lysosomal-traffic regulator or Lyst.

The gene affected in CHS patients was identified recently by chromosome walking and YAC complementation of CHS cell lines [Barbosa et al., 1996; Nagle et al., 1996]. The identification of the CHS locus was confirmed by the cloning of the homologous mouse beige gene [Perou et al., 1996]. Unfortunately, little insight on the mechanism of disease was gained by the cloning of these genes. The CHS gene encodes a very large protein of 430 kDa with only two recognizable features near the C-terminus. Several WD-40 repeats are found at the C-terminus and are likely to fold into a beta-propeller structure that may bind to other interacting proteins. Adjacent to this region is a 30-kDa domain of unknown function that is highly conserved between CHS/beige and many proteins in the GenBank database. Thus, this domain has been termed the Beige and CHS (BEACH) domain.

Several proteins that have the BEACH and WD domain structure of CHS/beige have been identified in multiple organisms. This suggests the possibility that they represent a family of related proteins in all organisms, the BEACH family. Few of these proteins have a known function and most of them have only been

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identified as predicted proteins in genomic sequences. The best characterized BEACH protein is FAN, a mammalian protein that interacts with TNF-receptors via its WD domain and which activates a neutral sphingomyelinase in response to TNF stimulation [Adam-Klages et al., 1996]. LvsA is a *Dictyostelium* BEACH protein that is essential for cytokinesis and osmoregulation and is localized on the contractile vacuole [Kwak et al., 1999; Gerald et al., 2002]. Finally, additional BEACH proteins include the mammalian neurobeachin, LBA and the *Drosophila* AKAP550 protein; these proteins bind to the type II regulatory subunit of protein kinase A but their functions remain unknown [Han et al., 1997; Wang et al., 2000, 2001].

The existence of these related proteins in different species suggests that the BEACH family of proteins is a widespread group of proteins with a diverse array of functions. However, it is not currently known how many proteins belong to this family and whether they can be classified into distinct subfamilies. In addition, it is not known whether these proteins have similar molecular mechanisms and cellular functions. In this article, we analyze the diversity of the BEACH family by identifying all the related proteins from the genome databases of several sequenced organisms including *Dictyostelium*. To test the function of each member of the *Dictyostelium* BEACH-related family we cloned and disrupted each of them and analyzed the resulting phenotypes.

MATERIALS AND METHODS

Database and Sequence Analysis

We used the sequence from the BEACH domain of LvsA (amino acids 2,884–3,270) to search the *Dictyostelium* genome database (<http://dictybase.org>) for similar sequences using TBLASTN [Altschul et al., 1997]. This search yielded multiple raw sequences that could be compiled into six distinct contigs. One of them corresponded to the sequence of the *lvsA* gene and the others encoded five novel proteins containing a BEACH domain. We then searched the *Dictyostelium* database for more sequences that would extend the contigs for these five new proteins. These sequences were obtained from the *Dictyostelium* genome sequencing centers at Baylor College of Medicine in Houston, TX (<http://dictygenome.bcm.tmc.edu/>), at

the Department of Genome Analysis in Jena, Germany (<http://genome.imb-jena.de/dictyostelium/>), and the Sanger Center in Hinxton, UK (http://www.sanger.ac.uk/Projects/D_discoideum/). Following the *Dictyostelium* genetic nomenclature convention (<http://dictybase.org>) we named these five novel genes *lvsB*, *lvsC*, *lvsD*, *lvsE*, and *lvsF* and their corresponding proteins LvsB, LvsC, LvsD, LvsE, and LvsF. The final contigs for each gene were submitted to the GenBank database of third party annotated sequences and have the following accession numbers: *lvsB*-BK000072, *lvsC*-BK000073, *lvsD*-BK000074, *lvsE*-BK000075, *lvsF*-BK000076.

The protein sequences encoded by these novel genes were compared with the GenBank database using BLASTP [Altschul et al., 1997]. This search identified the following BEACH-domain containing proteins (accession number in parenthesis): *Saccharomyces cerevisiae* BPH1 (CAA44309); *Schizosaccharomyces pombe* SPBC3H7.16 (CAA20312); *Leishmania major* L2581.05 (CAB58291); *Arabidopsis thaliana*, five predicted proteins AT2G45540 (AAC06163), F1003.12 (AAD25803), T18I24.16 (AAG50767), F16M22.8 (AAG50953), and T10P11.5 (AAC78268); *Caenorhabditis elegans*, three predicted proteins VT23B5.2 (T25148), T01H10.8 (1054706), and F10F2.1 (CAA84657); *Drosophila melanogaster* AKAP550 (AAF46011) and four predicted proteins CG11814 (AAF-47598), CG6734 (AAF53146), CG9011 (AAF-52302), and CG1332 (AAF47865); *Homo sapiens* CHS (AAB41309), FAN (CAA65405), BGL (a.k.a. CDC4L) (AAB09603), Neurobeachin (BAB-13370), and the predicted protein KIAA1607 (BAB13433). In addition, there are many other mammalian proteins closely related to the human sequences. We selected the following proteins as representatives: Mouse beige (T30851), Mouse LBA-Alpha (AAG14003), and Mouse FAN (AF013632).

To determine the phylogenetic relationship of these proteins we aligned the BEACH and WD-domain portion of all of these protein sequences using program Megalign (DNASStar, Inc., Madison, WI) with the default ClustalW parameters.

To determine the presence of WD repeats in the *Dictyostelium* Lvs proteins we used the predictive algorithm from the Biomolecular Engineering Research Center (<http://bmercwww.bu.edu/bioinformatics/wdrepeat.html>) in

addition to BlastP comparisons with proteins that have well-defined WD-motifs.

Design of an Improved Blasticidin-Resistance Plasmid for Gene Knockouts

We designed a plasmid suitable for our knockout constructs based on the blasticidin-resistance (*bsr*) marker from pUC-BSR Δ Bam [Sutoh, 1993]. We first removed the internal XhoI and SacI sites from this marker since they were not part of its coding region and we wanted to use them in our plasmid construct. We then excised the *bsr* marker from pUC-BSR Δ Bam with BamHI and HindIII and cloned it into the same sites of pSP72 (Promega Corporation, Madison, WI). This resulted in our plasmid pSP72-Bsr which is small (3.8 kb) and has multiple cloning sites on both sides of the *bsr* marker. The sequence for this vector has been deposited in the *Dictyostelium* database (<http://dictybase.org>).

Design of Knockout Constructs for the Novel *lvs* Genes

We used the polymerase chain reaction (PCR) to amplify two segments of each *lvs* gene from *Dictyostelium* genomic DNA. The oligonucleotides used for amplification contained restriction sites appropriate to clone into pSP72-Bsr. One of the gene segments (5' segment) was cloned into pSP72-Bsr at the 5' end of the Bsr gene and the other segment (3' segment) was cloned at the 3' end of the Bsr gene.

For *lvsB*, the 5' segment from nucleotide 7,398 to 9,285 (of the sequence deposited in BK000072) was amplified with oligonucleotides AO-308 (GGATCCGGAGCTCCAACAAC-TGATTCTGATTCAACTTCCG) and AO-309 (CTCGAGTCTAGAGCTGTTGTACTATTAATGGATG-ATGTTG), and the 3' segment from nucleotide 9,356 to 11,305 was amplified with oligonucleotides AO-310 (AAGCTTGGATCC-AGTAATATTGTTAAACCACCACAAAGAGG) and AO-311 (CTCGAGCCAAAGTATGATAGT-TCCAGATTCCATACC). For *lvsC*, the 5' segment is from 5,449 to 7,256 (in BK000073) with AO-314 (GGATCCTTGAATTCAATTC-ATGTTAAACTTTGGATGCC) and AO-315 (CTCGAGCTCGGAGGAGTTTTCAAATTTGA-GATATGAG), and the 3' segment is from 7,267 to 8,446 with AO-316 (CAAGCTTACT-TTTTAAAGATTACCAATAAGAATCC) and AO-317 (CTCGAGCTGTATAGATAAATCAT-TAAATCGGCCTGAG). For *lvsD*, the positions are 3,531–5,456 (in BK-000074) with AO-320

(CTCAAAGAATTCAATTCGTATTCAAAACG) and AO-321 (GGTACCGGTGATAATAAATA-ACGTCTACATTGTACACC), and 5,506–7,377 with AO-322 (AAGCTTGGATCCTTTCCA-AGAGGTTCAATTTGTGCTC) and AO-323 (CTCGAGGATTGAATGAATTGGTAATTTAT-TTGGATG). For *lvsE*, the positions are 1,903–2,749 (in BK000075) with AO-326 (GAGCTC-TACCATCACATATCTCTTACCTATGGG) and AO-327 (TCTAGAGTTGTTTGATGTGGTAAT-GGTGGTTC) and 3,897–5,782 with AO-328 (GGATCCAAGCTTTCATTGAATACTATCGC-AGGTAGAACC) and AO-329 (CTCGAGGC-AGCAGGACTATCGAAAGCATG). For *lvsF*, the positions are 671–2,311 (in BK000076) with AO-352 (CGAGCTCCCCAACCATTTGCAACC-AATCC) and AO-338 (CGGATCCTGAGCTT-CCATACCTTCACGTTCCG), and 2,780–3,715 with AO-354 (CAAGCTTAGGCGAAGAAAGA-AATAATAGTAATAATTGGGG) and AO-340 (GCTCGAGGCTTCAGTACCAGTTAATAAAA-TTGG).

Transformation of *Dictyostelium* and Selection of Knockout Clones

Before transformation each of the knockout constructs was digested at unique restriction sites at the 5' and 3' of the construct to separate it from the pSP72 plasmid. The linearized construct was then introduced into NC4A2 cells by electroporation as described [Vithalani et al., 1996]. Transformed cells were selected in 96-well dishes with HL5 medium [Sussman, 1987] supplemented with 60 U/ml penicillin and 60 μ g/ml streptomycin and containing 5 μ g/ml blasticidin [Larochelle et al., 1997]. Individual clones were screened by PCR for those that contained the designed knockout insertion as described previously [Gerald et al., 2002]. Briefly, primer pairs were designed that could distinguish between the wild type copy of each *lvs* gene and one in which the disruption construct inserted as planned. For each transformation we identified two or more clones that had an *lvs* gene knockout and one control sibling cell line where the construct inserted elsewhere. The phenotype of the two independent knockout cell lines was always compared to that of the sibling control and of NC4A2 cells transformed with pSP72-Bsr alone.

Southern Blot Analysis

Genomic DNA from each mutant cell line and wild-type control was prepared as described

[Manstein et al., 1989], digested with the indicated enzymes, and processed for transfer to a nylon membrane using standard methods. Probes derived from each *lvs* gene were labeled and hybridized to the membranes using the Gene Images™ AlkPhos Direct™ labeling and detection system (Amersham Pharmacia Biotech, Piscataway, NJ) following the manufacturer's instructions. The bound probes were detected with CDP-Star™ chemiluminescent detection reagent (Tropix, Inc., Bedford, MA) and exposed for 1 h on to an X-OMAT™ Scientific Imaging Film (Eastman Kodak, Rochester, NY).

Phenotypic Analysis

The development phenotype of the *lvs* mutant cells was determined under three conditions. First, cells were seeded on a bacterial lawn on an SM/5 agar plate as described [Vithalani et al., 1996]. The diameter of the colony was measured daily. Second, cells were plated on nonnutrient agar at 22°C, as described [Vithalani et al., 1998]. Third, cells were starved under a layer of buffer as described [De Lozanne and Spudich, 1987; Burns et al., 1995].

The ability of cells to grow in suspension culture was monitored as described [Larochelle et al., 1997]. The activity of the contractile vacuole in response to a hypoosmotic shock was monitored by video microscopy as described [Gerald et al., 2001, 2002].

RESULTS

Dictyostelium has a Family of Six BEACH-Domain Containing Proteins

To determine whether *Dictyostelium* contains several proteins with a BEACH domain we scanned the *Dictyostelium* genome database (<http://dicty.sdsc.edu>) for sequences similar to the BEACH domain of LvsA. This search identified five distinct sequences that encoded five new proteins closely related to LvsA. We named these novel genes *lvsB*, *lvsC*, *lvsD*, *lvsE*, and *lvsF*. Two sequences encompass the entire open reading frame of an *lvs* gene (*lvsB* and *lvsF*); three others lack a portion of the 5' end of the open reading frame (*lvsC*, *lvsD*, and *lvsE*).

Close inspection of the protein sequences encoded by these genes revealed that all of them have a similar domain structure (Fig. 1). At their C-termini, they have several WD motifs (between 4 and 6) recognized by their similarity to WD motifs from other proteins and

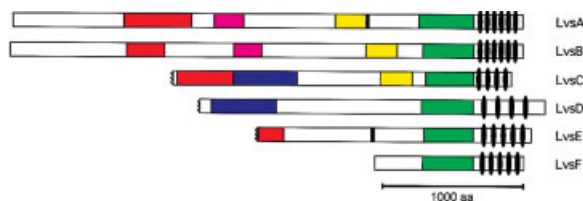


Fig. 1. The six Beach-domain containing proteins from *Dictyostelium*. This diagram shows the domain organization of the six Lvs proteins from *Dictyostelium*. The BEACH domain (GREEN) is the most conserved portion of these proteins (50–60% identity). At the C-terminus of each protein there are multiple WD-40 repeats (ovals). Other regions of homology (colored regions) are also shared among different Lvs proteins but the similarity is low (20–30% identity). The sequences of LvsC, LvsD, and LvsE are incomplete at the amino terminus.

by predictive algorithms (<http://bmerc-www.bu.edu/bioinformatics/wdrepeat.html>). Adjacent to their WD motifs, the Lvs proteins contain a highly conserved BEACH domain (50–60% identity) (Fig. 2). Upstream from the BEACH domain, the Lvs proteins share a few regions of homology, albeit of low similarity (20–25% identity).

Sequence comparison (BlastP) of each of these sequences against the GenBank database indicated that each of these *Dictyostelium* proteins is most closely related to a distinct group of BEACH-domain containing proteins. For example, the top BlastP scores for LvsB are obtained with the mouse beige protein and its orthologues from other species, whereas the best scores for LvsF are obtained with mammalian FAN proteins (data not shown).

We extended this search to identify all BEACH proteins present in other organisms with complete or nearly complete sequenced genomes. *S. cerevisiae* and *S. pombe* have only one BEACH protein each; *A. thaliana* has five; *D. melanogaster* has five; *C. elegans* has three; and *H. sapiens* has six. We then analyzed the relationship of these and several additional mammalian BEACH proteins using the ClustalW algorithm. For this analysis we compared only the BEACH and WD-domains of these proteins. The resultant phylogenetic tree illustrates the relationship among the BEACH-domain containing proteins (Fig. 3). This tree suggests that the diverse BEACH proteins may be grouped into five distinct classes according to sequence similarity. Most of these groups are represented by proteins previously characterized. Class I would be represented by the Lyst proteins; class II by LvsA; class III by FAN;

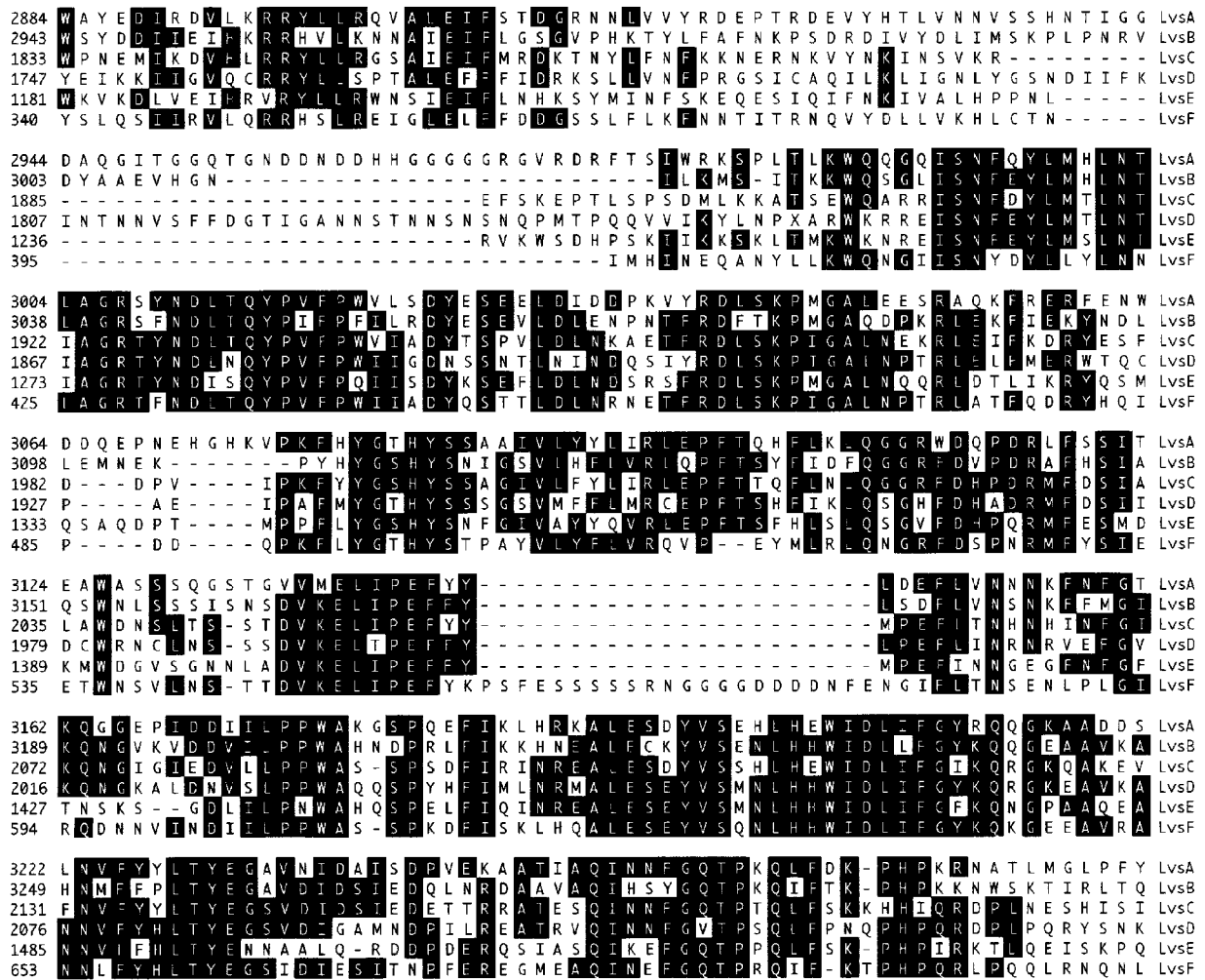


Fig. 2. Alignment of the BEACH domain of the *Dictyostelium* Lvs proteins. The BEACH domain, as defined by the Conserved Domain Database (<http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml>), starts at position 2,984 of LvsA (amino acid sequence TLKWQ) and ends at position 3,270 (amino acid sequence PHPKR). We find that a small region immediately upstream of the BEACH domain is also very conserved among many BEACH proteins.

class IV by AKAP550. Class V is the only group without a characterized representative and it encompasses the *Dictyostelium* LvsC, LvsD, and LvsE proteins and two predicted *Arabidopsis* proteins.

Importantly, although the phylogenetic tree was constructed solely with the BEACH and WD motifs of each protein, the proteins within each class share additional regions of sequence similarity (Fig. 4). This suggests that each BEACH protein class may in fact represent a distinct functional group. For example, the *Dictyostelium* LvsA protein shares several segments of sequence similarity with the *Drosophila* CG9011 protein but not with the human CHS protein nor with proteins of other BEACH classes. Similarly, the *Dictyostelium* LvsF pro-

tein has sequences uniquely in common with the human FAN protein.

Disruption of Each of the *Dictyostelium* *lvs* Genes

The presence of six BEACH proteins in *Dictyostelium* might indicate that they are used at different times during the *Dictyostelium* developmental program. To test this possibility we determined the expression pattern of each *lvs* gene during development. We used RTPCR with primers specific for each *lvs* gene in samples of RNA taken at different times of development. We found that all six *lvs* genes are expressed in the vegetative stage and at least through the first 18 h of development to the finger stage (data not shown).

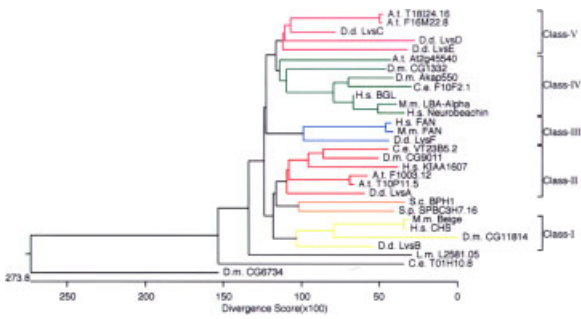


Fig. 3. Phylogenetic tree of the BEACH-domain containing protein family. The BEACH and WD domains of the indicated sequences were aligned by the ClustalW algorithm and the alignment used to construct this phylogenetic tree with DNStar. The proposed classes of BEACH proteins are indicated by the brackets on the right. The accession numbers for these sequences are listed in Materials and Methods. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

To assess the functional contribution of each of these novel BEACH proteins in *Dictyostelium* we decided to clone and knock out each of their corresponding genes. We engineered knockout constructs using portions of each gene that were amplified from genomic DNA by PCR. These constructs were designed to integrate into each gene by a double crossover recombination event, leading to the permanent disruption of their corresponding open reading frames. Each construct was introduced into *Dictyostelium* wild type cells and successful recombinants were identified by PCR (data not shown). Southern blot analysis was used to verify the correct disruption of each gene (Fig. 5). Several independent mutant cell lines were isolated for each gene disruption and were analyzed as described in the following sections. Since the knockout mutant cells for each gene displayed the same

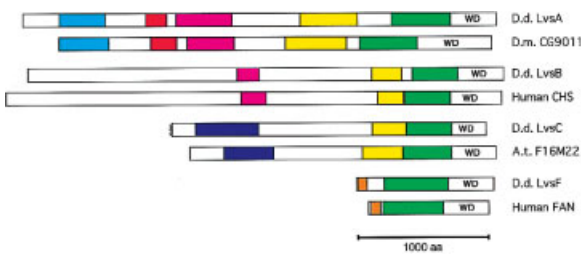


Fig. 4. *Dictyostelium* Lvs proteins share multiple domains with BEACH proteins of the same class. In addition to the BEACH domain (green) and the WD domain (WD), BEACH proteins within the same class have other domains in common. This diagram indicates in different colors the domains shared by BEACH proteins of the same class. These domains were identified as having a significant BlastP score in pairwise alignments.

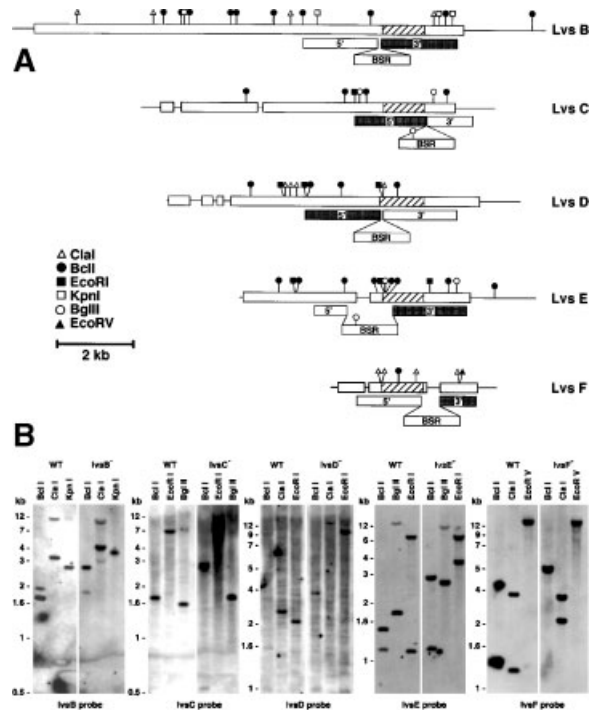


Fig. 5. Disruption of the *Dictyostelium* *lvs* genes. (A) Diagram of each *Lvs* locus and portions used for gene disruption. These diagrams show the genomic organization of each of the *Lvs* genes. The open bar on each gene indicates the open reading frame. The single line represents either flanking regions or introns that interrupt the open reading frame. The hatched portion of the open reading frame indicates the location encoding the BEACH domain. The boxes labeled 5' and 3' correspond to the portions of each gene that were cloned by PCR and used for the disruption of each *Lvs* gene as described in Materials and Methods. The Blasticidin resistance gene (BSR) was cloned in between these cloned regions. The crosshatched bar also indicates the probe used for the Southern Blot analysis shown in (B). The locations of restriction sites used for the Southern analysis are shown for each gene. (B) Southern blot analysis of knockout cell lines. These Southern blots demonstrate the disruption of genes with the appropriate knockout constructs. After transformation and selection of clones by PCR, we prepared genomic DNA of wild type and each mutant cell line. The DNA was digested with the indicated enzymes and processed for Southern blot analysis with probes specific for each of the *lvs* genes. Notice how the restriction pattern of each gene probe has been altered in the mutant cell lines compared to the wild type. All these changes are consistent with the double crossover insertion of the knockout constructs into each *lvs* gene.

phenotype, only one example will be described in detail.

Only LvsA is Required for Cytokinesis and Osmoregulation

Since the LvsA protein is known to play an essential role in cytokinesis and osmoregulation [Kwak et al., 1999; Gerald et al., 2002] we

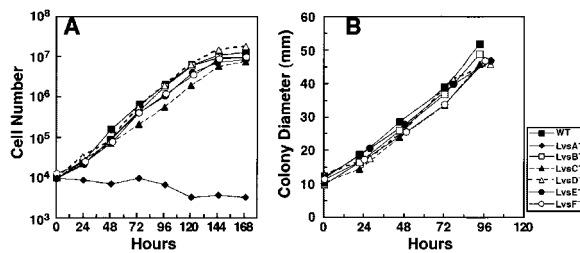


Fig. 6. Growth properties of the Lvs mutants. **(A)** Suspension cultures. Each cell line was placed in suspension cultures to test their ability to carry out cytokinesis in these conditions. Due to their cytokinesis defect, LvsA mutants cannot grow in suspension [Kwak et al., 1999]. In contrast, all other mutants grew at the same rate as wild type cells. **(B)** Bacterial lawns. To test for their ability to phagocytose and move across a substrate we inoculated each cell line on a pregrown bacterial lawn on an agar plate. The colony of cells clears the bacteria at the inoculum site and begins advancing across the plate to reach for more bacteria. Colonies from all tested mutant cell lines grew as fast as those from wild-type cells.

wanted to determine whether any of the other Lvs proteins might also contribute to those same functions. Clearly, the ability to obtain knockout strains in each of the *lvs* genes demonstrated that they are not essential for growth, at least under the conditions used for their selection. However, LvsA mutants are known to fail in cytokinesis when placed in suspension culture [Kwak et al., 1999]. Thus, we tested the ability of the new mutants strains to accomplish cytokinesis and divide in suspension conditions. Except for LvsA mutants, all other Lvs-mutant cell lines grew in suspension at the same rate as wild-type cells (Fig. 6A) indicating that the other Lvs proteins do not function in cytokinesis.

To test their ability to osmoregulate, we placed each cell line in hypoosmotic conditions. As shown before, LvsA mutants swelled dramati-

cally after a few minutes in water (Fig. 7). In contrast, all of the other Lvs-mutants resembled wild-type cells in their ability to maintain their shape even after prolonged (>1 h) immersion in water (Fig. 7). Close inspection of each cell line demonstrated that, except for LvsA mutants, all other Lvs-mutants contained contractile vacuoles that expelled water like those of wild-type cells (data not shown).

Dictyostelium Lvs Proteins are Dispensable for Growth and Differentiation

To determine if the Lvs mutants had any general defects in growth or development we plated each of them on bacterial lawns and monitored their behavior. Wild-type cells are able to ingest the bacteria and translocate across the agar plate as they consume the lawn of bacteria. This requires that the cells are able to phagocytose and move effectively. Figure 6B shows that the diameter of a colony of wild-type cells increases rapidly over the course of several days. Colonies from all the Lvs mutant strains were also able to grow at the same rate as wild-type cells.

Once the cells have depleted the bacteria that surround them, they initiate a characteristic developmental program that culminates in the formation of fruiting bodies. All mutant strains were able to progress through each step of this developmental program and formed mature fruiting bodies (Fig. 8). These fruiting bodies contained spores with the characteristic cylindrical shape of wild type spores (data not shown). To look more closely at the initial stages of development we plated the cells on Petri dishes under a starvation buffer. Under these conditions all mutant strains behaved as wild type

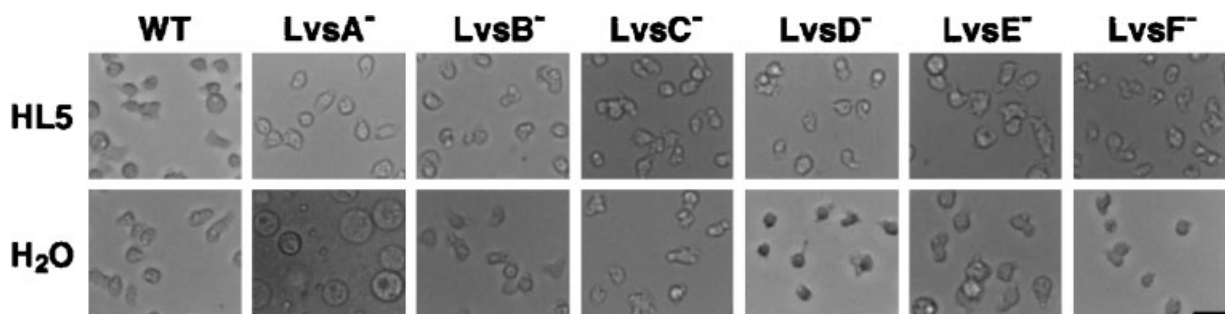


Fig. 7. Only LvsA is required for osmoregulation. *Dictyostelium* has the ability to survive in hypoosmotic environments due to the activity of the contractile vacuole. When wild type cells are changed from their growing medium (HL5) to distilled water (H₂O) they are able to retain their cell shape and to move across the substrate. In contrast, LvsA mutant cells, which are defective in contractile vacuole function, swell rapidly, loose their attachment to the substrate and lyse after prolonged exposure to water [Gerald et al., 2002]. All other Lvs mutants have a normal osmoregulatory response. Scale bar = 20 μ m.

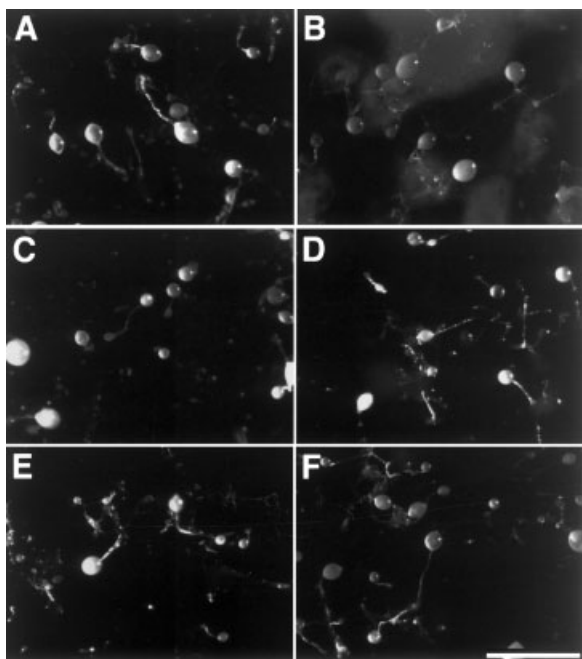


Fig. 8. *Dictyostelium* Lvs mutants can complete development. Each cell line was placed in starvation conditions to induce the *Dictyostelium* developmental program. All cell lines were able to complete development with the formation of mature fruiting bodies. (A) Wild type; (B) LvsB; (C) LvsC; (D) LvsD; (E) LvsE; (F) LvsF. Scale bar = 1 mm.

cells and were able to form waves of aggregating cells that coalesced into large streams (data not shown). Thus, all aspects of development do not seem to require any of the Lvs proteins.

Only LvsB is Required for Lysosomal Trafficking

Given the closed similarity of LvsB with the mammalian lysosomal trafficking proteins beige and CHS, we explored the requirement of LvsB in lysosomal trafficking in *Dictyostelium*. Remarkably, LvsB mutants have enlarged lysosomes reminiscent of the large lysosomes found in beige and CHS mutant cell lines [Cornillon et al., 2002; Harris et al., 2002]. This function in lysosomal trafficking is specific to LvsB since none of the other Lvs mutants displayed any defects in lysosomal processing [Cornillon et al., 2002; Harris et al., 2002].

DISCUSSION

The BEACH-domain containing proteins (BEACH family) are an interesting but poorly understood set of proteins. As an initial step toward dissecting their function we have shown here the phylogenetic diversity of this family of

proteins and the mutational analysis of the *Dictyostelium* members of the BEACH family.

A comprehensive search through the databases for organisms with completely sequenced genomes revealed that most organisms contain multiple members of the BEACH family. The only exceptions are the two yeasts *S. cerevisiae* and *S. pombe*, which have only one BEACH protein each. Phylogenetic analysis of the BEACH and WD domains of all the retrieved sequences suggested that these proteins could be grouped into five distinct classes. These five sequence groups may represent five different cellular functions modulated by BEACH proteins.

The first group, Class I, is represented by the human CHS protein and its orthologues in other mammals. This group includes the *Dictyostelium* LvsB protein and the *Drosophila* CG11814 gene product. We have shown here that the loss of LvsB does not affect the growth and developmental properties of *Dictyostelium* cells. However, recent studies showed that LvsB mutant cells contain enlarged lysosomes and have defects in the secretion of lysosomal enzymes [Cornillon et al., 2002; Harris et al., 2002]. This phenotype is very similar to that caused by mutations in the CHS locus, which allow normal development to occur but cause severe lysosomal traffic defects typified by enlarged lysosomes. Thus, we postulate that Class I BEACH proteins are involved in lysosomal function and predict that the *Drosophila* CG11814 gene product will have a similar function. Surprisingly, we did not find a nematode sequence that clearly belongs to this class. It is possible that nematodes do not use a BEACH protein to modulate lysosomal traffic. However, we believe it is more feasible that a nematode BEACH protein (such as T01H10.8) has diverged from the Class I proteins but still retains its function in lysosomal traffic.

The second group, Class II, encompasses proteins closely related to Class I proteins but it seem to participate in a different function. This group is represented by the *Dictyostelium* LvsA protein, which we have shown to play an essential role in cytokinesis and osmoregulation [Kwak et al., 1999; Gerald et al., 2002]. This group includes one predicted sequence from human, fly and nematode, and two from *Arabidopsis*. The presence of a single distinct member of this group in each organism suggests that it functions in a basic process common to all cells. We postulate that, like LvsA, the

other Class II proteins are also involved in cytokinesis.

The single BEACH proteins found in the two yeasts are almost equally related to the Class I and Class II BEACH proteins. Mutation of the *S. cerevisiae* bph1 was reported as viable and sensitive to low pH [Winzler et al., 1999]. Further characterization of these proteins will be necessary to determine whether they have a function in lysosomal trafficking, in cell division, or both.

The third group, Class III, is represented by the mammalian FAN proteins. These are a unique group among the BEACH protein family because they are only about a quarter of the size of all other members of the family (~100 vs. >400 kDa). While FAN seems to play a role in apoptosis [Segui et al., 1999] and TNF signaling [Adam-Klages et al., 1996], it is not an essential protein in mice. FAN knockout mice seem to have only a defect in epidermal barrier repair [Kreder et al., 1999]. Intriguingly, *Dictyostelium* is the only other organism with a BEACH protein closely related to mammalian FAN. We showed here that the *Dictyostelium* LvsF protein is not essential for growth and development but it will be interesting to determine if LvsF, like FAN, functions in apoptosis in *Dictyostelium* [Arnoult et al., 2001].

The fourth group, Class IV, includes a specific subset of BEACH proteins characterized by their ability to bind the type II regulatory subunit of protein kinase A. The only BEACH proteins where this property has been demonstrated is the *Drosophila* AKAP550 and mammalian neurobeachin. However, the mammalian LBA (or BGL) and nematode F10F2.1 proteins are so closely related to neurobeachin and AKAP550 that they are predicted to have similar properties. On the other hand, the *Drosophila* CG1332 and *Arabidopsis* At2g45540 proteins are more distantly related members of this group and are less likely to bind to protein kinase A.

Finally, Class V BEACH proteins include only members from *Arabidopsis* and *Dictyostelium*. This group cannot currently be ascribed to any specific cellular function. We found that the *Dictyostelium* LvsC, LvsD, and LvsE proteins are not essential in any of the assays tested here. However, the high degree of similarity between these three proteins raises the possibility that they share similar functions and thus could compensate for the loss of each of them.

Engineering a triple mutant will be necessary to test this hypothesis.

The mutational analysis described here clearly demonstrates that the function of LvsA and LvsB is unique among the *Dictyostelium* set of BEACH proteins. Although all proteins are expressed in the vegetative stage of *Dictyostelium*, none can compensate for the deletion of LvsA or LvsB. In addition, none of the single *lvs* mutants share the phenotypic characteristics of LvsA or LvsB mutants. We postulate that this same distinction in cellular roles will be found for the different BEACH proteins in other organisms.

The molecular function of BEACH proteins still remains obscure. Clearly, the WD domain region of these proteins is a module for the interaction with other proteins. This interaction has been demonstrated in the case of the mammalian FAN protein which binds to the cytoplasmic domain of the p55 TNF-receptor [Adam-Klages et al., 1996]. Unfortunately, no binding partner has been identified for the WD domain of any other BEACH protein. The function of the BEACH domain also remains elusive. While highly conserved among the different BEACH proteins, the BEACH domain could provide different functions for each BEACH protein. Clearly, a detailed structure–function analysis of different members of this novel family will help elucidate some of the molecular details of their function.

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