Pseudo-phosphatase Sbf1 contains an N-terminal GEF homology domain that modulates its growth regulatory properties

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Accepted 18 May 2001

SUMMARY

Sbf1 (SET binding factor 1) is a pseudo-phosphatase related to the myotubularin family of dual specificity phosphatases, some of which have been implicated in cellular growth and differentiation by virtue of their mutation in human genetic disorders. Sbf1 contains germline-encoded alterations of its myotubularin homology domain that render it non-functional as a phosphatase. We report here the complete structure of Sbf1 and further characterization of its growth regulatory properties. In addition to its similarity to myotubularin, the predicted full-length Sbf1 protein contains pleckstrin (PH) and GEF homology domains that are conserved in several proteins implicated in signaling and growth control. Forced expression of wild-type Sbf1 in NIH 3T3 cells inhibited their proliferation and altered their morphology. These effects required intact PH, GEF and myotubularin homology domains, implying that growth inhibition may be an intrinsic property of wild-type Sbf1. Conversely, deletion of its conserved N-terminal 44 amino acids alone was sufficient to convert Sbf1 from an inhibitor of cellular growth to a transforming protein in NIH 3T3 cells. Oncogenic forms of Sbf1 partially localized to the nucleus, in contrast to the exclusively cytoplasmic subcellular localization of endogenous Sbf1 in all cell lines and mammalian tissues tested. These data show that the N-terminal GEF homology domain serves to inhibit the transforming effects of Sbf1, possibly sequestering the protein to the cytoplasm, and suggest that this region may be a modulatory domain that relays growth control signals.

Key words: Pseudo-phosphatase, Sbf1, transformation, GEF

INTRODUCTION

The myotubularin family of dual specificity phosphatases consists of 12 members, several of which have been implicated in the regulation of cellular growth and differentiation. All contain a conserved motif similar to the active sites of protein tyrosine phosphatases (PTPs) and an adjacent domain (SID) proposed to function as a protein-protein interaction motif (Cui et al., 1998; Firestein et al., 2000). Myotubularin was originally identified as the product of a gene mutated in X-linked myotubular myopathy (XLMTM), a congenital disorder characterized by impaired terminal differentiation of myoblasts (Laporte et al., 1996). Several XLMTM mutations consist of single amino acid substitutions in the phosphatase catalytic pocket of myotubularin, implying that phosphatase activity is crucial for its function. Furthermore, inactivating mutations of a second family member, MTMr2, are associated with Charcot-Marie Tooth Syndrome (Bolino et al., 2000).

Several lines of evidence suggest that myotubularin-related proteins participate in lipid-mediated signaling. A subset contains motifs that have been implicated in binding phospholipids. For example, MTMr3 and MTMr4 contain FYVE domains, which are phosphatidylinositol 3-phosphate (PI(3)P)-binding motifs found in proteins linked to vesicular transport, cytoskeletal organization and signal transduction. Sbf1 (MTMr3) contains a pleckstrin homology (PH) domain that is responsive to phosphatidylinositol 3-kinase (PI3K) in yeast, implying that its PH domain is capable of binding phosphatidylinositol lipids (Isakoff et al., 1998). Furthermore, myotubularin displays a high affinity and specificity for hydrolysis of PI(3)P (Taylor et al., 2000), in addition to its previously reported ability to hydrolyze phosphoserine and threonine residues (Cui et al., 1998). A phosphatase-defective C375S myotubularin mutant induces an accumulation of PI(3)P in mammalian cells when hyper-expressed (Taylor et al., 2000), whereas expression of myotubularin reduces PI(3)P levels in S. pombe (Blondeau et al., 2000). Thus, mutations in myotubularin-related proteins may lead to impaired growth control and differentiation analogous to the association of PTEN phosphatase loss-of-function mutations with oncogenesis in various tissues (Ali et al., 1999).

Interestingly, a subset of myotubularin-related proteins (the so-called pseudo-phosphatases) contains conserved germline alterations of their PTP homology domains that are likely to abrogate phosphatase catalytic activity. Sbf1 (MTMr5) is the best characterized and has been linked to cellular growth and oncogenic transformation in vitro. Sbf1 transcripts are upregulated 27-fold in Ras transformed cell lines (Zuber et al., 2000). Truncated forms of Sbf1 are oncogenic in NIH 3T3 cells and primary B cell progenitors. The ability of Sbf1 to function as an oncoprotein is abrogated by restoring phosphatase activity to its catalytically inactive PTP motif. Experimentally,
phosphatases whose PTP motifs are catalytically inactive have been shown to bind phosphorylated substrates and prevent their dephosphorylation (Flint et al., 1997). Thus, myotubulin-related pseudo-phosphatases may act as naturally occurring substrate trapping mutants or regulate PI(3)P levels by opposing the actions of myotubulin phosphatases.

This study was undertaken to further elucidate the role of Sbf1 in cell growth and onocogenic transformation. Characterization of a complete Sbf1 cDNA demonstrates that the predicted full-length Sbf1 protein contains an N-terminal GEF homology domain that is conserved with Rab3GEF and with several other proteins implicated in signaling and growth control. Consistent with a possible role in signaling, wild-type Sbf1 localizes to the cytoplasmic compartment in vitro and in vivo. Deletion of its N-terminal 44 amino acids converts Sbf1 from an inhibitor of cellular growth to an oncoprotein in NIH 3T3 cells. All transforming mutants of Sbf1 localize at least partially to the nucleus.

MATERIALS AND METHODS

DNA constructs

Sequences encompassing the 5’ end of the Sbf1 cDNA were cloned from an SW480-derived IgA10 cDNA library (Clontech). Library clones containing 5’ extension products were amplified by PCR using a 5’ primer specific to the IgA10 backbone (GAGCAAGGTCAGCC-TGGTAAAGTC) and a 3’ primer specific to Sbf1 base pairs 1072-1053 (GGTCCAGGACCATGCTACACACT). A 1 kb SacI/NaeI fragment containing the 5’ extension was used to reconstitute an Sbf1 cDNA encoding an open reading frame of 5934 base pairs. Sequence analysis and domain analyses were performed using the BLAST and CLUSTALW analysis programs. Sbf1 N-terminal mutants lacking the first 44 or 111 amino acids (ΔN44 and ΔN111) were constructed by PCR. Construct Sbf1ΔNCRS was generated by internal deletion of cDNA sequences encoding amino acids 200-280 using PCR techniques. Sbf1AC1642 consisted of an EcoRI/BamHI fragment of Sbf1 cloned into the EcoRI/BgIII site of MSCV. N-terminal Flag-tagged Sbf1 and MTM1 constructs were generated by cloning the respective full-length cDNAs in-frame with the Flag epitope in the mammalian expression vector pYD30. Reporter constructs for response element assays were obtained from commercial sources (Mercury System) (Clontech).

Chromosomal mapping of the Sbf1 gene

BAC clones containing the human Sbf1 gene were isolated using a human Sbf1 cDNA fragment as probe (Genome Systems). BAC clones were confirmed to encode Sbf1 by Southern blot hybridization or sequence analysis. Chromosomal localization of Sbf1 was determined by fluorescence in situ hybridization (FISH) using BAC clone F728 that was labeled with digoxigenin-dUTP by nick translation (Genome Systems). A total of 80 metaphase spreads were analyzed with 74 showing specific labeling.

Cell culture and transfections

NIH 3T3 cells were maintained in Dulbecco’s modified Eagle medium (DMEM) supplemented with 10% bovine calf serum. The Phoenix retroviral packaging cell line (kind gift of Garry Nolan) was maintained in DMEM supplemented with 10% fetal bovine serum. Retroviral infection of NIH 3T3 cells was performed as previously described (Pear et al., 1993). Transduced cells were selected in G418 (1 mg/ml) starting at 48 hours post-infection for a period of 2-4 weeks. Stable transductants were pooled and continuous cell lines were maintained in growth medium supplemented with G418 (200 μg/ml).

Soft agar and focus forming assays

Anchorage-independent growth was analyzed in 60 mm tissue culture plates containing 5 ml of base agar (0.7%) and 3 ml of top agar (0.33%) in DMEM containing 10% calf serum. NIH 3T3 cells from sub-confluent cultures were combined with the top agar and plated in triplicate at a concentration of 2×10^6 cells per plate. Cultures were re-fed with fresh growth medium every 2-3 days and colonies were scored at 21 days. For focus-forming assays, sub-confluent NIH3T3 cell lines were plated at 80% confluency on 60 mm diameter plates. Cultures were re-fed every 2-3 days and foci were scored at 21 days.

Protein analysis and subcellular fractionation

Whole cell extracts for protein expression analysis were prepared from either NIH 3T3 or 293T cells by lysis at 95°C for 5 minutes in SDS lysis buffer containing 50 mM Tris-HCl (pH 6.8), 2% SDS and 10% glycerol. The heated lysate was passed three times through a 21 gauge syringe needle. Nuclear and cytoplasmic fractions were prepared from Raji cells as previously described (Jacobs et al., 1993). Lysates were subjected to SDS-PAGE and western blotting using standard methods.

Immunocytochemistry and fluorescence microscopy

The subcellular localization of Sbf1 and MTM1 was determined by indirect immunofluorescence microscopy. NIH 3T3 cells that had been transfected 48 hours previously were fixed in phosphate-buffered saline (PBS)/1% paraformaldehyde for 15 minutes. For detection of Sbf1, the cells were treated with PBS/1% sodium dodecylsulphate (SDS) at 50°C for 30 minutes for antigen retrieval purposes. Preparations were washed extensively in PBS, blocked in PBS containing 5% normal goat serum for 30 minutes followed by incubation with either the primary anti-FLAG mAb (M5; Sigma) at a dilution of 1:500 or anti-Sbf1 mAb (De Vivo et al., 1998) at a dilution of 1:100. Immune complexes containing the target protein were visualized with a fluorescein isothiocyanate-conjugated goat anti-mouse secondary antibody. Cells were counterstained with DAPI (4',6-diamidino-2-phenylindole; Boehringer Mannheim), mounted onto slides and visualized by indirect fluorescence microscopy. Immunohistochemical detection of Sbf1 was performed on formalin-fixed, paraffin-embedded tissues after antigen retrieval consisting of microwave treatment for 15 minutes in a 0.5 M Tris pH 10 solution. The primary antibody consisted of a mouse monoclonal (mAb 68) specific for Sbf1. Immune complexes were detected using biotinylated anti-mouse serum and avidin horseradish peroxidase complexes.

Growth analysis and cell cycle kinetics

Growth and cell cycle analyses were performed on NIH 3T3 cells that were stably transduced with the appropriate retroviral constructs. For growth assays, cells were seeded at a density of 1.5×10^5 cells per 100 mm diameter dish. Every 3 days the cells were trypsinized, total cell numbers determined by hemocytometer, and then replated at the initial density of 1.5×10^5 cells per 100 mm dish. The effect of Sbf1 on cell cycle progression was measured by pulse labeling the cells with 50 mM BrdU for 3 hours. BrdU incorporation was determined using an anti-BrdU antibody as recommended by the supplier (Sigma).

RESULTS

Sbf1 contains an N-terminal domain conserved with signaling proteins

A 5’ extension of the previously reported Sbf1 cDNA (Cui et al., 1998) was isolated by PCR amplification (see Materials and Methods) and used to reconstruct a full-length Sbf1 cDNA. Nucleotide sequence analyses showed that the resulting 6 kb cDNA contained a 5790 bp open reading frame (ORF) that
The full-length Sbf1 cDNA encodes a 1930 amino acid protein with a predicted molecular mass of 215 kDa (Fig. 1A). Sequences conforming to a Kozak consensus were identified at the first ATG ((A/G)c c ATG G; Kozak, 1989) and the ORF matched that predicted by translation of exonic sequences (as identified by GENSCAN). No alternative upstream ATG codons were identified. Western blot analysis of 293T cells transfected with an expression construct containing the full-length cDNA revealed the presence of an immunoreactive protein that co-migrated with endogenous Sbf1 and displayed an apparent molecular weight of approximately 220 kDa, consistent with the predicted size (Fig. 1C).

A motif search of the Sbf1 amino acid sequence revealed a heptad leucine repeat (HLR) (amino acids 240-261) and pleckstrin homology (PH) domain (Fig. 1A), in addition to the previously reported similarity with myotubularin (Cui et al., 1998). Furthermore, database searches revealed that sequences within the N-terminal region of Sbf1 (amino acids 207-292) spanning the HLR shared significant similarity with several putative signaling proteins and unknown ORFs. Proteins containing this novel leucine/proline-rich domain included calmodulin response activated gene (CRAG) (Xu et al., 1998), Rab6bp (Janoueix-Lerosey et al., 1995), Rab3GEF (Wada et al., 1997) and ST5 (Majidi et al., 1998). Hereafter, we refer to this conserved motif as the CRS domain (Fig. 1B).

The N-terminal of Sbf1 negatively regulates its latent transforming potential

Previous studies have shown that forced expression of various forms of Sbf1 induced anchorage-independent growth of NIH 3T3 cells and immortalization of murine pre-B cells in vitro (Cui et al., 1998; De Vivo et al., 1998). To determine whether full-length Sbf1 displayed similar transforming properties, the growth of NIH 3T3 cells was evaluated in soft agar after their retroviral transduction with constructs encoding Sbf1 (full length) or a mutant Sbf1 that lacks the N-terminal 750 amino acids (\(\Delta N750\)) and has previously been shown to be oncogenic (Cui et al., 1998). Equivalent expression of each construct was confirmed by western blots of the retroviral packaging cell lines as well as the transduced NIH 3T3 cells (Fig. 2C). These studies showed that full-length Sbf1 was incapable of inducing anchorage-independent growth of NIH 3T3 cells, in contrast to \(\Delta N750\), which induced the growth of numerous colonies in soft agar when expressed under comparable conditions (Fig. 2A,B).

The potential role of N-terminal sequences in the negative regulation of Sbf1 transforming activity was further investigated using a series of mutants harboring N-terminal deletions (Fig. 3A). Possible roles for the CRS and PH domains were also investigated using mutants lacking either of these motifs (\(\Delta CRS\) and \(\Delta IC1642\), respectively; Fig. 3A). Equivalent expression of Sbf1 proteins was confirmed by western blots of stably transduced NIH 3T3 cells (Fig. 3B). Growth properties of the latter were evaluated in focus-forming assays at 21 days. Under these conditions, full-length Sbf1 was incapable of inducing anchorage-independent growth of NIH 3T3 cells (Fig. 4A,B). By contrast, cells expressing N-terminal deletion constructs lacking the first 44, 111 or 337 amino acids displayed robust focus-forming activity (Fig. 4A,B). Deletion constructs lacking the CRS domain alone (\(\Delta CRS\)) or the PH domain (\(\Delta IC1642\)) lacked...
focus-forming ability (Fig. 4B). These results indicate that the first 44 amino acids of Sbf1 are necessary for negatively regulating its latent oncogenic potential. Conversely, the conserved CRS and PH domains are not required for suppression or induction of Sbf1 oncogenic activity, consistent with previous observations that forced expression of the SID alone was sufficient for transformation (Cui et al., 1998; De Vivo et al., 1998).

Wild-type Sbf1 displays growth inhibitory properties in NIH 3T3 cells

Although wild-type Sbf1 was incapable of morphologically transforming NIH 3T3 cells, it did induce alterations in their growth properties. NIH 3T3 cells expressing exogenous Sbf1 grew at a significantly slower rate when compared with control cells or those expressing mutated forms of Sbf1 (Fig. 5A). Pulse-labeling of cells with BrdU, revealed an approximate threefold reduction in the number of Sbf1-expressing cells entering S phase (Fig. 5B) compared with control cells containing vector alone. In addition, NIH 3T3 fibroblasts stably transduced with Sbf1 exhibited an altered cellular morphology. They appeared less flattened and refractile, and exhibited a spindle-shaped appearance (Fig. 5C). The anti-proliferative and morphology altering effects of Sbf1 were abolished by N-terminal deletions (ΔN44 and ΔN337), deletions that removed the CRS or PH domains (ΔCRS and ΔC1642), or site-directed point mutations (Cui et al., 1998) that restored phosphatase activity to the PTP motif (Sbf1HCS; Fig. 5B). These observations indicated that forced expression of wild-type Sbf1 has an anti-proliferative effect. Its ability to alter the cytoskeletal structure and growth rate of NIH 3T3 cells is not mediated by a single domain, but is dependent upon the integrity of several motifs in the full-length protein.

Sbf1 is a cytoplasmic protein but N-terminal deletion induces partial nuclear localization

Sbf1 was originally isolated in a yeast two-hybrid screen as a result of its interaction with SET domain proteins, which are nuclear factors implicated in gene regulation. Therefore, we evaluated whether wild-type Sbf1 may also be a nuclear protein. Subcellular fractions of nuclei and cytosol were prepared from Raji cell extracts. Western blot analysis showed that Sbf1 was predominantly present in the cytoplasmic fraction (Fig. 6A). Furthermore, immunohistochemistry using

Fig. 2. Wild-type Sbf1 lacks oncogenic effects in NIH 3T3 cells. (A) Soft agar assays were performed using NIH 3T3 cell lines expressing the indicated constructs. Data are the average of three experiments performed in triplicate. (B) Representative colony growth in soft agar is shown for the cell lines tested. (C) Western blot analysis using an anti-Sbf1 monoclonal antibody demonstrates comparable expression of wild-type Sbf1 and Sbf1ΔN750 in stably transfected NIH 3T3 cells.

Fig. 3. Structure and expression of mutant Sbf1 proteins. (A) A schematic depiction of wild-type Sbf1 is shown at the top. Bars depict amino acid compositions of various Sbf1 deletion mutants. (B) Western blot analysis with an anti-Sbf1 monoclonal antibody demonstrates equivalent expression of Sbf1 deletion constructs in stably transfected NIH 3T3 cell lines.

Fig. 4. Oncogenic activation of Sbf1 by N-terminal deletions. (A) Representative focus-forming assay shows that the ΔN111 mutant, but not wild-type, Sbf1 induces foci in monolayers of stably transfected NIH 3T3 cells. (B) Bar graph indicates focus-forming activity of mutant Sbf1 proteins. Data are the average of three experiments performed in triplicate.
an Sbf1 monoclonal antibody on various mouse tissues showed that in the brain (Fig. 6B) and testis (R.F. and M.L.C., unpublished), two tissues that express high levels of Sbf1, the protein was exclusively localized in the cytoplasm. Thus, endogenous Sbf1 appears to be a cytoplasmic protein.

The subcellular localization of Sbf1 as well as that of MTM1 was also evaluated by immunocytochemistry. Immunolocalization of exogenously expressed FLAG-tagged Sbf1 and MTM1 revealed that both were exclusively cytoplasmic under conditions of hyper-expression (Fig. 6C).

Localization of wild-type Sbf1 in the cytoplasm would appear to be inconsistent with previous observations that oncogenic forms of Sbf1 modulate the activities of nuclear SET domain proteins (Cui et al., 1998; Firestein et al., 2000). One possible explanation for this apparent discrepancy may be that the localization of oncogenic Sbf1 proteins differs from wild-type Sbf1 and their altered subcellular localization might correlate with oncogenic activity. This was tested by determining the subcellular localization of Sbf1 mutant proteins in stably transfected NIH 3T3 cells. Transforming mutants (ΔN44, ΔN111, ΔN337 and ΔN750) localized to both the nuclear and cytoplasmic compartments. By contrast, non-transforming mutants ΔCRS and ΔC1642 localized exclusively to the cytoplasm (Fig. 7). The correlation between transformation and nuclear localization is consistent with the possibility that Sbf1 acts in the nucleus to transform cells.

**DISCUSSION**

In this study, we have determined the complete structure of the Sbf1 pseudo-phosphatase and shown that its N-terminal regions contain structurally conserved domains that modulate its growth regulatory properties. Wild-type Sbf1 displays features of a growth inhibitory protein, and deletion of its conserved N terminus activates its latent transforming potential. Localization of wild-type Sbf1 exclusively to the...
cytoplasmic compartment suggests that it normally functions in cytoplasmic rather than nuclear signaling pathways involved in growth control. Conversely, altered subcellular localization may be a contributing factor to oncogenic activation of Sbf1.

We have previously shown that truncated forms of Sbf1 interact with and modulate the function of SET domain proteins such as Mll (Hrx) and SUV39H1 in vitro (Cui et al., 1998; Firestein et al., 2000). Our current study, however, suggests that interaction with these proteins is unlikely to constitute a normal physiological role for wild-type Sbf1, as the endogenous protein does not localize to the nucleus. The recent finding that SET domain proteins can localize to non-nuclear structures such as gap junctions underscores the possibility that wild-type Sbf1 may interact and mediate its signals through cytoplasmic targets (Nakamura et al., 2000).

The first 44 amino acids of Sbf1, which suppress its latent transforming activity, display similarity with a Rab6-binding protein and a GEF of the Rab3 family of small GTP-binding proteins. This similarity raises the possibility that Sbf1 may function in vesicular transport or secretory pathways controlled by the Rab family of GTPases (Martinez and Goud, 1998). Although Rab3GEF has not been reported to display oncogenic activity, other GEFs undergo oncogenic activation when truncated in their N-terminal regions. For example, the proto-oncoprotein Vav and several other Dbl family proteins such as Dbl, Ost and Tiam1 are oncogenically activated by N-terminal mutations (Cerione and Zheng, 1996). No sequence similarity is shared between these proteins to suggest that a specific domain is involved. However, it has been shown for Vav that deletion of its N terminus leads to an increase in its membrane soluble fraction (Abe et al., 1999), suggesting that altered subcellular localization may play a role in transformation. Altered subcellular localization may also activate the oncogenic potential of ST5, a CRS-containing protein whose transforming activity is induced by C-terminal deletions that reposition a putative membrane targeting motif (Majidi et al., 2000). Our data show that N-terminal deletions of Sbf1 lead to its partial nuclear localization, raising the possibility that the N terminus regulates Sbf1 by sequestering it to a specific subcellular address. It remains to be determined whether nuclear localization is required for oncogenic activity of Sbf1 or simply an indicator that its normal subcellular distribution is altered by N-terminal deletion.

The ability of myotubularin to dephosphorylate PI(3)P (Taylor et al., 2000) and the presence of PI(3)P-binding FYVE domains in several members of this family (Laporte et al., 1998) imply that myotubularin proteins regulate growth control via lipid second messengers. Lipid phosphatase activity appears to be crucial for myotubularin function, as mutations in the PTP motif that abrogate its ability to dephosphorylate PI(3)P result in XLMTM (Taylor et al., 2000). Furthermore, a phosphatase defective C375S myotubularin mutant causes an accumulation of PI(3)P in mammalian cells when hyper-expressed (Taylor et al., 2000). Interestingly, mutations in the PTP motif of Sbf1 also alter its growth regulatory properties. Restoration of catalytic activity to the PTP motif of either wild-type or truncated Sbf1 abrogates growth inhibition and oncogenic transformation, respectively. One possibility is that the catalytically inactive PTP motif in Sbf1 may function as a phosphatidylinositol-binding motif analogous to FYVE domains. The latter have been shown to function as docking motifs that bind PI(3)P and function in vesicular transport, cytoskeletal organization and signal transduction (Driscoll and Vuidepot, 1999). The docking ability of the Sbf1 PTP domain

### Table 1. Growth properties and subcellular localization of Sbf1 proteins

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may be abrogated by mutations that restore catalytic activity, thereby disrupting its role in signaling processes. The combination of RabGEF homology, PH and phosphatidylinositol-binding domains strongly imply a role for Sbf1 in lipid-mediated signaling events. Although we interrogated several signaling pathways, our results did not reveal any significant differences in wild-type or oncogenic Sbf1 (data not shown). It remains to be determined which signaling pathways lie downstream of Sbf1 and how activating N-terminal mutations may alter its function and contribute to transformation.

The ability of wild-type Sbf1 to inhibit growth and alter the morphology of NIH 3T3 fibroblasts is dependent on the integrity of several domains. Deletion of the CR3 domain, the PH domain, or the first 44 amino acids, as well as restoration of catalytic activity in its PTP motif, all abrogate the ability of Sbf1 to inhibit growth. These data imply that growth inhibition is an intrinsic property of wild-type Sbf1 and not a dominant-negative effect resulting from its overexpression. It is of interest that PTEN, a lipid phosphatase in the PIP pathway, is a tumor suppressor protein whose loss of function is associated with several human cancers. Although mutant Sbf1 is a potent transforming protein in different cell types, an oncogenic role in human cancer has not been reported. The chromosomal localization of Sbf1 to 22q13.33 should aid in discovery of malignancies that may carry mutations in the Sbf1 gene.

This work was supported by a grant from the National Institutes of Health (CA55029). R.F. was supported by a training grant from the National Institute of General Medical Sciences (5T32GM07365). We thank Joe Lipsick for providing an anti-Myb antibody, Bich-Tien Georgiou, D. M., Christodoulou, K., Hausmanowa-Petrusewicz, I., Sci. USA 94, 8392-8396. Myotubularin, a protein tyrosine phosphatase mutated in myotubular myopathy, dephosphorylates the lipid second messenger, phosphatidylinositol 3-phosphate. Proc. Natl. Acad. Sci. USA 97, 8285-8289.


REFERENCES


