Identification of *bdm-1*, a gene involved in G protein β-subunit function and α-subunit accumulation

Shin Kasahara, Ping Wang*, and Donald L. Nuss†

Center for Agricultural Biotechnology, University of Maryland Biotechnology Institute, University of Maryland, College Park, MD 20742-4450

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Targeted disruption of Gα and Gβ genes has established the requirement of an intact G protein signaling pathway for optimal execution of several important physiological processes, including pathogenesis, in the chestnut blight fungus *Cryphonectria parasitica*. We now report the identification of a G protein signal transduction component, *b*eta *d*isruption *m*imic factor-*1*, BDM-1. Disruption of the corresponding gene, *bdm-1*, resulted in a phenotype indistinguishable from that previously observed after disruption of the Gβ subunit gene, *cpgb-1*. The BDM-1 deduced amino acid sequence contained several significant clusters of identity with mammalian phosducin, including a domain corresponding to a highly conserved 11-amino acid stretch that has been implicated in binding to the G$\beta\gamma$ dimer and two regions of defined Gβ/phosducin contact points. Unlike the negative regulatory function proposed for mammalian phosducin, the genetic data presented in this report suggest that BDM-1 is required for or facilitates Gβ function. Moreover, disruption of either *bdm-1* or *cpgb-1* resulted in a significant, posttranscriptional reduction in the accumulation of CPG-1, a key Gα subunit required for a range of vital physiological processes.

Cryphonectria parasitica | fungal pathogenesis | phosducin

Heterotrimeric GTP-binding proteins are a highly conserved family of eukaryotic regulatory complexes composed of three subunits, designated α, β, and γ, that function to couple transmembrane receptors with intracellular effectors (1). Receptor activation by a variety of environmental stimuli results in the exchange of bound GDP for GTP, promoting disassociation of the three subunits as α-GTP and a $\beta\gamma$ heterodimer, both of which interact with and alter the activity of a diverse set of effector molecules, e.g., adenylyl cyclase, phospholipase C, and ion channels (2, 3). Hydrolysis of GTP to GDP by an intrinsic Gα-associated GTPase activity results in reassociation of the α-subunit with the $\beta\gamma$ heterodimer and signal termination.

Although signal specificity resides primarily at the receptor level (4), recent studies have identified a number of regulatory proteins that act directly on the G protein subunits to regulate various steps in the GTPase cycle. For example, Strittmatter *et al.* (5) reported acceleration of guanine nucleotide release mediated by the growth cone protein GAP-43. The regulators of G protein signaling family of proteins has been shown to activate the intrinsic GTPase activity of several Gα subunits (6–8). Signaling through the $\beta\gamma$ heterodimer has been shown to be modulated by binding proteins such as the β-adrenergic receptor kinases (9–11) and phosducin (12, 13).

The importance of G protein signaling in regulating diverse vital biological processes in filamentous fungi is now well established (14–21). Targeted disruption of Gα subunit genes for a number of pathogenic fungi has indicated a broad role for G protein signaling in fungal virulence and dependent developmental processes such as infection structure formation, mycelial growth, and reproduction (14, 15, 19, 20). Recent progress in cloning and characterization of filamentous fungal genes encoding other G protein subunits and regulatory proteins support this view, e.g., the Gβ subunit gene *cpgb-1* from *Cryphonectria parasitica* (18) and the regulator of G protein signaling protein flbA from *Aspergillus nidulans* (22).

We now report the identification of a *C. parasitica* gene encoding a component of G protein signaling, BDM-1, that is required for virulence and that has clusters of amino acid identity with mammalian phosducin. Although mammalian phosducins are postulated to modulate G protein signaling negatively by sequestering G$\beta\gamma$ heterodimers (4, 13, 23, 24), disruption of *bdm-1* produced a phenocopy of *C. parasitica* Gβ null mutants, suggesting a role in facilitating Gβ function. Additionally, disruption of either *bdm-1* or the Gβ gene *cpgb-1* caused a significant reduction in the accumulation of the key Gα subunit CPG-1 but not of CPG-1 mRNA. These results are discussed in terms of current views of the *in vivo* role of mammalian phosducin and the possibility that BDM-1 represents the prototype of a previously unidentified family of positive regulators of Gβ function. The increasing evidence for posttranscriptional regulation of G protein signaling in fungal processes ranging from reproduction to virulence is also considered.

Materials and Methods

Fungal Strains and Growth Conditions. *C. parasitica* strains EP155 (ATCC 38755, virulent) and ΔGβ-a (strain EP155 in which the Gβ gene *cpgb-1* is disrupted; ref. 18) were maintained and propagated on potato dextrose agar (PDA; Difco) as described (25). A *C. parasitica* strain with a phenotype indistinguishable from ΔGβ-a, designated *bdmA*, was obtained during attempts to disrupt the gene that encodes the general amino acid control transcriptional activator CPC-1 (26).

Construction of a Cosmid Library. Genomic DNA was extracted and purified from 7-day-old mycelia of *C. parasitica* strain EP155 grown at room temperature (23°C) on cellophane-covered PDA as described (27). DNA was partially digested with restriction enzyme *Cfr*10 I. The cosmid vector pCOSNBn1 was constructed by replacing the *Sma*I–*Sal*I fragment of pWE15 (Stratagene) with the *Neurospora crassa* benomyl-resistance gene (28). The *Cfr*10 I-digested genomic DNA was partially filled in with dCTP by using the Klenow fragment of *Escherichia coli* DNA polymerase I. The *Not*I-digested vector pCOSNBn1 was also partially filled in with dGTP. After ligating the cosmid vector and *Cfr*10 I-digested insert, the DNA was treated with the Giga Pack III XL packaging extract (Stratagene) followed by transfection into *E. coli* XL-I Blue MR cells.

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Abbreviations: PDA, potato dextrose agar; kb, kilobase.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. AF140555).

*Present address: Department of Genetics, 317 CARL Building, Research Drive, Duke University Medical School, Durham, NC 27710.

†To whom reprint requests should be addressed. E-mail: nuss@umbi.umd.edu.

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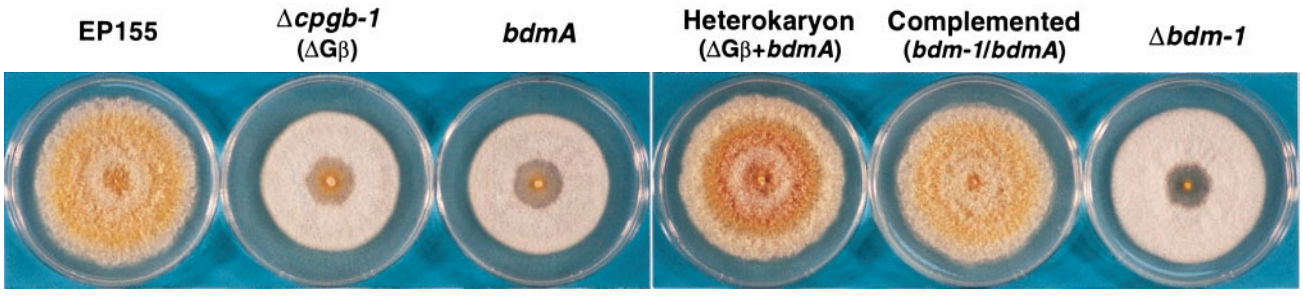

Fig. 1. Comparison of colony morphologies for wild-type *C. parasitica* strain EP155 and corresponding mutants, heterokaryon, and complemented strains. When grown on PDA, mutant *bdmA* had a phenotype indistinguishable from that previously reported (18) for Gβ gene *cpgb-1* disruptant mutants (Δ*cpgb-1*). Heterokaryons formed by anastomosis between *bdmA* and Δ*cpgb-1* had an overall phenotype very similar to that of wild-type strain EP155. *bdmA* transformants complemented with a 6.5-kilobase (kb) *Kpn*I fragment containing the gene *bdm-1* were indistinguishable from wild-type strain EP155. Disruption of *bdm-1* in strain EP155 (Δ*bdm-1*) resulted in a phenotype indistinguishable from *bdmA* and the Gβ null mutant Δ*cpgb-1*.

Complementation Cloning of the *bdmA* Locus. Mutant *bdmA* spheroplasts were prepared and transformed with cosmid library DNA as described by Churchill *et al.* (29). A single benomyl-resistant transformant with the wild-type (EP155)-like phenotype was purified to nuclear homogeneity by collecting uninuclear single conidial isolates capable of growing on PDA-benomyl plates. To recover the integrated complementing cosmid DNA from the transformant, genomic DNA was extracted, treated with RNase A, precipitated with polyethylene glycol/NaCl, packaged (3 μg/reaction) with a Giga Pack III XL packaging extract, and transfected into *E. coli* XL-I Blue MR cells.

Nucleic Acid Preparation and Analysis. Total nucleic acids were extracted from approximately 3 g of 7-day-old *C. parasitica* mycelia grown on cellophane-covered PDA plates as described above. Genomic DNA and single-stranded RNA fractions used for Southern and Northern blot analyses, respectively, were prepared according to the method described by Choi *et al.* (27). Standard protocols (30) were used for both DNA and RNA blotting analyses with slight modifications (18).

Nucleotide Sequence Accession Number. The GenBank accession number for the nucleotide sequence of *bdm-1* is AF140555.

Results

Phenotypic Characterization of *bdmA*. We previously reported that disruption of the *C. parasitica* Gβ subunit gene *cpgb-1* resulted in several phenotypic changes that included a flat colony morphology distinguished by densely bundled, sparsely branched, parallel arrays of hyphae, reduced orange pigment production, a 5–6 log reduction in asexual sporulation, and significantly reduced virulence (18). In a screen designed to disrupt the *C. parasitica* general amino acid regulatory gene *cpc-1*, we unexpectedly identified a mutant, subsequently designated *bdmA* (*b*eta *d*isruption *m*imic mutant *A*), that had a colony morphology indistinguishable from the *cpgb-1* disruptants (Fig. 1). Phenotypic similarities extended to reduced virulence (Table 1), reduced conidiation, and increased growth rates under a variety of growth conditions (data not shown). Microscopic examination of *bdmA* hyphae also revealed the distinctive bundled parallel arrays of hyphae with significantly reduced branch points as previously shown for the *cpgb-1* disruptants (see figure 6 in ref. 18).

Although *bdmA* phenocopied *cpgb-1* disruptants, heterokaryons formed by anastomosis of the two strains had a near wild-type morphology, indicating that the two mutations fall within distinct complementation groups (Fig. 1). Southern, Northern, and Western blot analyses confirmed that *bdmA* contained an intact *cpgb*-1 locus and wild-type levels of *cpgb-1* mRNA and CPGB-1 protein (Fig. 2). Hybridization analysis with a probe specific for the *cpc-1* disruption vector revealed a complex hybridization pattern (Fig. 3*B*) consistent with the presence of multiple integrated copies. This level of complexity necessitated mapping of the *bdmA* locus by complementation with a *C. parasitica* cosmid library.

Complementation Mapping of *bdm-1*. The cosmid rescued from chromosomal DNA of a complemented transformant with the wild-type phenotype (Fig. 1) contained an insert of approximately 35 kb. Transformation with portions of the original insert subcloned adjacent to the benomyl-resistance gene in a modified pBluescript II SK(+) vector (Stratagene) led to the identification of a 6.5-kb *Kpn*I fragment able to confer the wild-type EP155-like phenotype (Fig. 3*A*). Because the two subcloned *Xba*I–*Kpn*I fragments derived from this 6.5-kb *Kpn*I complementing fragment (Fig. 3*A*) failed to complement *bdmA*, the region flanking the *Xba*I site and a cDNA clone corresponding to that region were subjected to sequence analysis. Computer-assisted analysis of the sequence information revealed an ORF encoding a deduced protein of 32,885 Da. Two introns were identified within this gene, now designated *bdm-1*: one of 102 nt within the 5′ noncoding leader and one of 56 nt near the middle of the coding domain (Fig. 3*A*).

To confirm that a mutated *bdm-1* allele is responsible for the *bdmA* phenotype, the gene was subjected to independent targeted disruption. The 6.5-kb complementing *Kpn*I fragment was cloned into pBluescript II SK(+) and modified by replacing the 1.5-kb *Bam*HI–*Sma*I fragment containing the entire *bdm-1* coding region with a 2.3-kb hygromycin-resistance gene cassette (Fig. 3*A*). The modified 7.3-kb fragment released by *Kpn*I digestion was used to transform wild-type strain EP155. Transformants with the *bdmA* phenotype (Fig. 1) were recovered at a rate of 1 for every 20 hygromycin-resistant transformants. South-

Table 1. Mean canker areas induced on dormant chestnut stems by *C. parasitica* strains

Strains or mutants	Canker area, cm^2	
	2 Weeks	3 Weeks
EP155	11.95 ± 2.73	25.20 ± 5.01
Δ*cpgb-1* (ΔG_β)	2.94 ± 1.01	3.97 ± 1.46
bdmA	3.03 ± 0.59	3.25 ± 0.68
Heterokaryon (ΔG_β + *bdmA*)	8.99 ± 1.09	19.57 ± 2.40
Complemented (*bdm-1*/*bdmA*)	11.44 ± 1.74	24.00 ± 3.00
Δ*bdm-1*	2.05 ± 0.60	2.65 ± 0.73

Data are shown as means ± SD of 8 replicates and were determined at 14 and 21 days after inoculation.

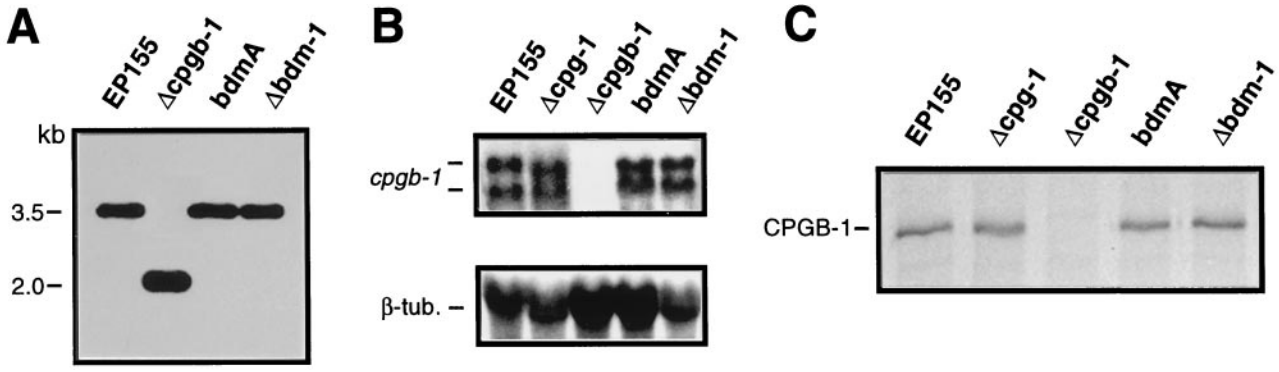

Fig. 2. Analysis of *C. parasitica* Gβ subunit CPGB-1 genomic DNA, mRNA, and protein in wild-type strain EP155 and corresponding mutants cultured under identical conditions. (*A*) Southern blots were performed on 10 μg of *Pst*I-digested genomic DNA from strain EP155, Gβ disruptant Δ*cpgb-1*, *bdmA*, and *bdm-1* disruptant Δ*bdm-1* by using a probe corresponding to the 5′ portion of the *cpgb-1* coding region as described (18). Sizes of expected hybridization bands are indicated at the left. (*B*) Northern hybridization analysis of *cpgb-1* transcript accumulation in wild-type strain EP155, *cpg-1* disruptant Δ*cpg-1*, *cpgb-1* disruptant Δ*cpgb-1*, *bdmA*, and *bdm-1* disruptant Δ*bdm-1* was performed as described in detail by Kasahara and Nuss (18). After hybridization, the blot was stripped and hybridized with a *C. parasitica* β-tubulin gene cDNA probe (27). (*C*) Western blot analysis of CPGB-1 levels in the same strains analyzed in *B*. Western blot analysis was performed by resolving 30 μg of extracted protein on a SDS/12% PAGE, followed by electrophoretic transfer to Immobilon-P poly(vinylidene difluoride) membrane (Millipore) and probing with affinity purified anti-CPGB-1 polyclonal antibodies (36). The CPGB-1-antibody complex was visualized with the aid of the amplified alkaline phosphatase goat-anti-rabbit immunoblot assay kit (Bio-Rad).

ern blot analysis of putative disruptants revealed a hybridization pattern consistent with the predicted disruption of the *bdm-1* locus (Fig. 3 *B* and *C*). The absence of *bdm-1* mRNA was confirmed by Northern analysis (Fig. 3*D*). The Δ*bdm-1* disruptants were also found to have an intact *cpgb-1* locus and to produce wild-type levels of *cpgb-1* mRNA and CPGB-1 protein (Fig. 2). These disruptants were also indistinguishable from *bdmA* and Δ*cpgb-1* in terms of growth characteristics (Fig. 1) and a significantly reduced level of virulence (Table 1). Additional confirmation was obtained by rescuing a *bdm-1* disruptant and *bdmA* by transformation with *bdm-1* cDNA under the control of the *C. parasitica* glyceraldehyde-3-phosphate dehydrogenase promoter in the standard *C. parasitica* transformation vector pCPXHY1 (31, 32) modified to contain a benomyl-resistance gene (data not shown). These combined results suggest that BDM-1 is required for, or facilitates, Gβ function and establish a role for this protein in the regulation of fungal virulence.

Database homology search analysis (BLASTP) revealed mammalian phosducins as having the highest level of amino acid sequence identity with the newly identified gene. This result was intriguing, given the reports that this family of proteins also modulates G protein signal transduction, primarily by binding to Gβγ subunits (13, 23, 24, 33). As shown in Fig. 4, optimal alignment of the two proteins excluded the negatively charged 64-residue N-terminal portion of the *C. parasitica* protein. Although the overall level of sequence identity is low (23%), further inspection revealed several clusters of reasonably high similarity. For example, residues 83–95 had 69% identity; residues 188–205 showed 50% identity and 78% conservation; and residues 247–261 had 40% identity and 73% conservation (Fig. 4). Interestingly, two of these clusters corresponded to regions of phosducin that contact the Gβ subunit as determined by x-ray-crystallography (24). The N-terminal BDM-1 cluster, amino acids 83–95, contained identical amino acid residues at eight positions within an 11-amino acid stretch, TGPKGVINDWR, that is highly conserved in mammalian phosducin and phosducin-like proteins (34, 35). This 11-amino acid stretch lies within a domain containing eight Gβ contact points, six of which are retained in the BDM-1 sequence. The C-terminal cluster, amino acids 247–261, corresponds to the C-terminal phosducin/Gβγ interface and shares identical residues at two of the five contacts and conserved residues at two additional contact points.

Disruption of *bdm-1* or *cpgb-1* Results in Posttranscriptional Reduction in Accumulation Of Gα Subunit CPG-1. Gao and Nuss (36) recently reported that mutation of the putative myristoylation site (Gly-2) of the *C. parasitica* Gα subunit CPG-1 resulted in complex phenotypic changes and a 5-fold increase in CPG-1 accumulation. Moreover, Northern analysis of *cpg-1* mRNA revealed that this increase was regulated at the posttranscriptional level, i.e., *cpg-1* mRNA was found to be present at wild-type levels. This observation stimulated an examination of whether the disruption of the Gβ gene and subsequent loss of CPGB-1 protein accumulation would have an effect on the accumulation of the presumptive Gα partner, CPG-1. As indicated in Fig. 5*B*, CPG-1 levels are consistently reduced to ≈10% of the wild-type level in the *cpgb-1* disruptant. Interestingly, a nearly identical result was obtained for the *bdm-1* disruptant. As shown in Fig. 5*A*, the level of *cpg-1* mRNA is not reduced but slightly increased in the Gβ null mutant and found at wild-type levels in the *bdm-1* disruptant and in *bdmA*. Note additionally in Fig. 2, that CPGB-1 accumulation is not altered in *bdm-1* disruptants. Thus, the phenotypic similarities resulting from *cpgb-1* and *bdm-1* disruption extend to a posttranscriptional reduction in Gα CPG-1 accumulation.

Discussion

The observation that *bdm-1* disruption phenocopies *cpgb-1* null mutants strongly suggests that this newly identified gene encodes a positive regulator of Gβ function. Consistent with a role for BDM-1 in Gβ function, the deduced BDM-1 amino acid sequence contained several clusters of identity with mammalian phosducin that correspond to regions of physical interactions between the latter protein and Gβ subunit. The level of identity within the N-terminal cluster, BDM-1 amino acids 83–95, is provocative in that in addition to having identical residues at six of eight Gβγ contact points, the BDM-1 sequence also shows identity at eight positions of a contiguous 11-residue peptide, TGPKGVINDWR, that is absolutely conserved in all mammalian phosducins (34, 35).

Mammalian phosducins are generally thought to act as negative regulators of G protein signaling. First isolated as a complex with the transducin Gβγ heterodimer Tβγ from the retina (23), where it is present in high concentration, phosducin is postulated to reduce signal amplification by sequestering Tβγ subunits, thereby preventing reassociation with the inactive

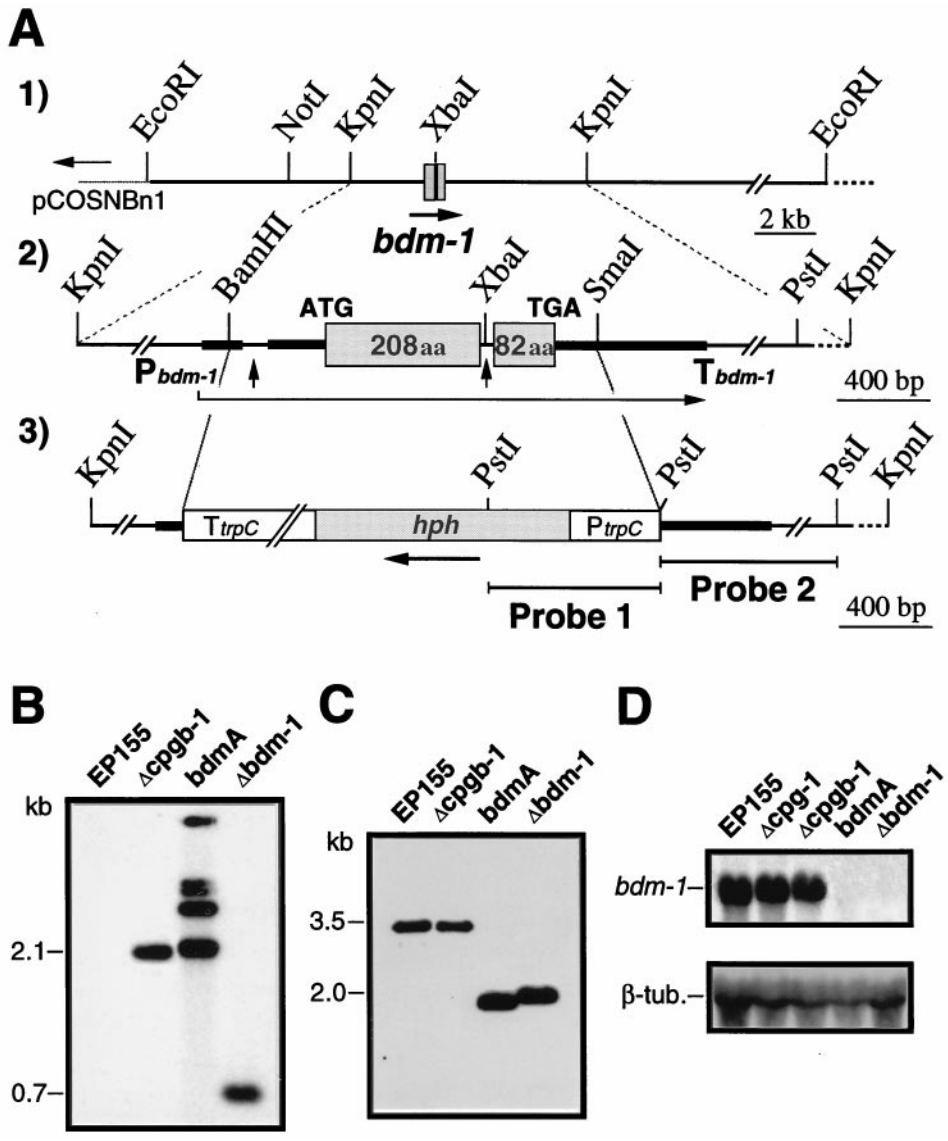

Fig. 3. Complementation mapping and disruption of *bdm-1*. (*A1*) Restriction map of cosmid clone rescued from the chromosome of a *bdmA* complemented transformant. (*A2*) Detailed organization of *bdm-1* in the context of the 6.5-kb *Kpn*I *bdmA* complementing fragment. The *bdm-1* promoter and terminator are indicated by P*bdm-1* and T*bdm-1*, respectively. The protein-coding regions are represented by boxes. Thick solid lines denote 5′ and 3′ noncoding region. Two introns are marked by vertical arrows. (*A3*) DNA fragment used to disrupt *bdm-1*. The *Bam*HI–*Sma*I fragment within the *bdm-1* structural gene was replaced with a hygromycin-resistance gene (*hph*) cassette that contained the *A. nidulans* TrpC promoter and terminator, P*trpC* and T*trpC*, respectively (49). A 7.3-kb fragment released by digestion with *Kpn*I was used directly for transformation of *C. parasitica* strain EP155 spheroplasts. (*B*) Southern blot analysis of *Pst*I-digested genomic DNA (10 μg) isolated from wild-type *C. parasitica* strain EP155, Gβ disruptant Δ*cpgb-1*, *bdmA*, and *bdm-1* disruptant Δ*bdm-1* by using probe 1 (specific for the *hph* gene) shown in *A* (3) above, under conditions described in ref. 18. Sizes of expected hybridization bands are indicated at the left. (*C*) Southern blot analysis of the same strains listed in *B* by using probe 2 shown in *A* (3), which is specific for *bdm-1*. (*D*) Northern blot analysis of RNA (15 μg) isolated from the same strains shown in *C* and the *cpg-1* disruptant Δ*cpg-1* by using the entire coding region of *bdm-1* cDNA as probe as described in ref. 18.

GDP-bound form of the transducin Gα subunit Tα (13, 37). The latter step is essential for reactivation. Phosducin and phosducin-like proteins were subsequently reported to be expressed in numerous tissues (38, 39) and to modulate G protein signaling by competing with a variety of other G$\beta\gamma$ binding proteins, e.g., the β-adrenergic receptor kinase (40) and the olfactory receptor-specific kinase GRK3 (33). Binding of phosducin to G$\beta\gamma$ subunits is, in turn, regulated by cAMP-dependent phosphorylation at Ser-73 (12, 37, 41, 42), with the phosphorylated form having a much reduced affinity for the G$\beta\gamma$ dimer. Phosducin has also been reported to interact with Gα subunits, resulting in reduced GTPase activity or rate of GDP release (12, 13).

The positive regulatory role proposed for BDM-1 raises intriguing questions regarding currently envisioned *in vivo* roles of mammalian phosducins and the potential for a family of BDM-1 homologs in higher eukaryotes. Evidence supporting the model for mammalian phosducin as a negative regulator of G$\beta\gamma$ signaling is based almost exclusively on data from *in vitro* biochemical or transient expression studies. There have been no gene disruption experiments like those performed for BDM-1. In this regard, Lem *et al.* (43) recently reported a surprising observation in rhodopsin knockout mice. Transgenic mice containing a single opsin gene had an accelerated recovery time to light response relative to wild-type mice. Measurement of eight different phototransduction proteins confirmed wild-type levels, except for a 50% increase in the level of phosducin. Because photoresponse recovery depends on reunion of the Tα subunit with the T$\beta\gamma$ subunit, a step that is proposed to be negatively

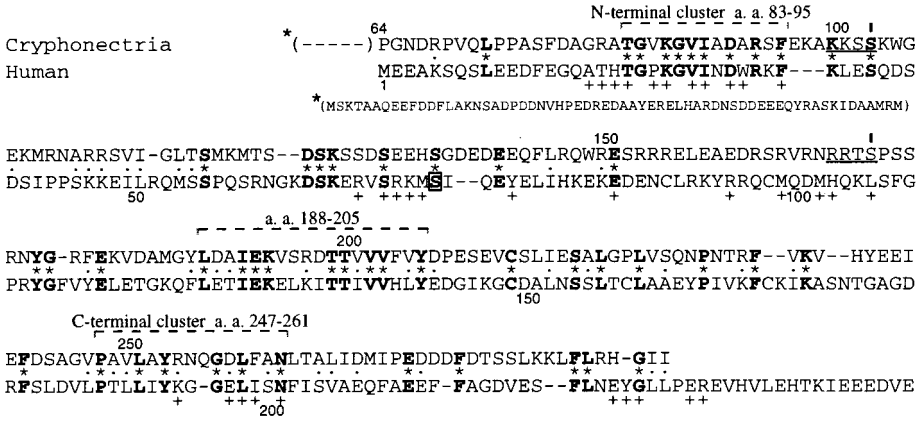

Fig. 4. Alignment of the deduced *C. parasitica* BDM-1 amino acid sequence (GenBank accession no. AF140555) with that of human retinal phosducin (SWISS PROT accession no. P20941). This alignment was completed by using GENETYX-MAC, version 8.0 (Software Development, Tokyo). Identical amino acids are indicated in bold and with an asterisk, whereas chemically similar amino acids are marked with a dot. Clusters of sequence homology discussed in the text (BDM-1 residues 83–95, 188–205, and 247–261) are marked by dashed lines. The 11-amino acid peptide, TGPKGVINDWR, absolutely conserved in all mammalian phosducins is located within the N-terminal homology cluster spanning BDM-1 residues 83–95. Ser-73 of human phosducin, a substrate for protein kinase A, is boxed. The BDM-1 sequence contains two protein kinase A consensus motifs (R/K-X-X-S/T) that are underlined, with the potential phosphorylation sites indicated with wedges. Phosducin residues that contact Gβ are indicated below the phosducin sequence by a + (24).

regulated by phosducin, the increased ratio of phosducin to T$\beta\gamma$ should have caused a delay rather than an acceleration of the photoresponse recovery. Such unanticipated *in vivo* results suggest that caution should be exercised when considering interpretations of phosducin function based predominantly on *in vitro* and transient transfection data. The phenotypic consequences associated with the disruption of mammalian phosducin will be of particular interest. The apparent opposite functional roles proposed for mammalian phosducin and fungal BDM-1 also raise the possibility that the latter protein represents the prototype of a previously unidentified family of positive regulators of Gβ function that is perhaps ancestrally related to mammalian phosducin. Like mammalian phosducin, such regulatory proteins may also represent potential targets for the development of G protein modulating agents of therapeutic value (44).

Similarities between the *bdm-1* disruptant and the Gβ null mutant extended to the reduced accumulation of the Gα subunit CPG-1. The literature contains several examples of coordinated regulation of G protein subunit accumulation at varying levels (45–47). However, analogies to the 90% posttranscriptional reduction in CPG-1 accumulation observed on disruption of either *bdm-1* or *cpgb-1* (Fig. 5) are not apparent in the literature. Altered accumulation of CPG-1 levels has been reported previously under several apparently unrelated conditions. A decrease in CPG-1 levels was observed after hypovirus CHV1-EP713 infection and on cosuppression as a result of stable transformation with a *cpg-1* sense copy (48). Mutation of the putative myristoylation site (Gly-2) resulted in a substantial increase in CPG-1 accumulation (36). As was observed for *bdm-1* and *cpgb-1* disruptants, mutation of the putative myristoylation site or alterations in CPG-1 accumulation after hypovirus infection were regulated at the posttranscriptional level (ref. 36 and data not shown, respectively). This apparent propensity of CPG-1 for posttranscriptional regulation is of considerable importance, because vital processes ranging from mycelial growth to asexual sporulation to virulence are surprisingly sensitive to even minor modulations of G protein signaling (15, 18, 36). A clear understanding of the mechanisms by which BDM-1 affects Gα stability and facilitates Gβ function will provide important advances in the areas of *C. parasitica* biology and fungal pathogenesis and is likely to have broad implications for the general field of G protein signal transduction.

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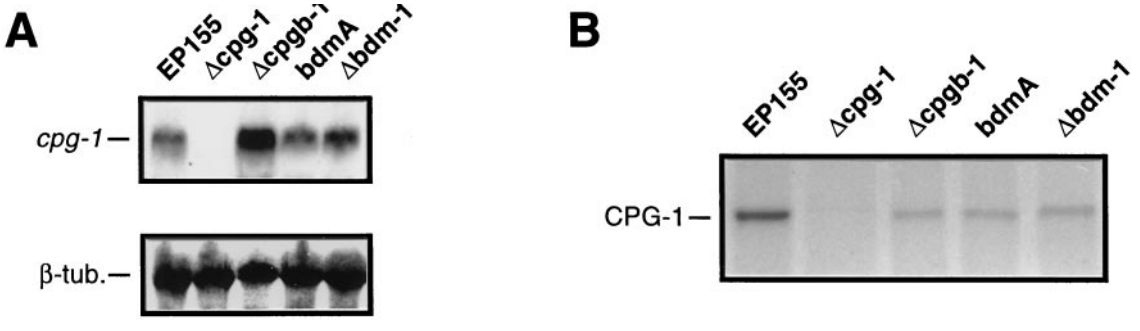

Fig. 5. Analysis of *C. parasitica* Gα subunit CPG-1 mRNA and protein accumulation in wild-type strain EP155 and corresponding mutants cultured under identical growth conditions. (*A*) Northern hybridization analysis of *cpg-1* transcript accumulation in wild-type strain EP155, *cpg-1* disruptant Δ*cpg-1*, *cpgb-1* disruptant Δ*cpgb-1*, *bdm*A, and *bdm-1* disruptant Δ*bdm-1*. The blot was probed with the entire *cpg-1* cDNA clone. (*B*) Western blot analysis of CPG-1 levels in the same strains analyzed in *A* with affinity purified anti-CPG-1 antibody as described (36). Signal intensity of visualized CPG-1 on poly(vinylidene difluoride) membranes was quantified with National Institutes of Health IMAGE 1.61 in combination with a UMAX POWERLOOK 2000 scanner. Purified recombinant CPG-1 (6-His fusion) was used as a standard.

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