# The Divergent *Caenorhabditis elegans* β-Catenin Proteins BAR-1, WRM-1 and HMP-2 Make Distinct Protein Interactions but Retain Functional Redundancy *in Vivo*

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ABSTRACT

β-Catenins function both in cell adhesion as part of the cadherin/catenin complex and in Wnt signal transduction as transcription factors. Vertebrates express two related proteins, β-catenin and plakoglobin, while Drosophila has a single family member, Armadillo. *Caenorhabditis elegans* expresses three β-catenin-related proteins, BAR-1, HMP-2, and WRM-1, which are quite diverged in sequence from each other and other β-catenins. While BAR-1 and WRM-1 are known to act in Wnt-mediated processes, and HMP-2 acts in a complex with cadherin/α-catenin homologs, it is unclear whether all three proteins retain the other functions of β-catenin. Here we show that BAR-1, like vertebrate β-catenin, has redundant transcription activation domains in its amino- and carboxyl-terminal regions but that HMP-2 and WRM-1 also possess the ability to activate transcription. We show via yeast two-hybrid analysis that these three proteins display distinct patterns of protein interactions. Surprisingly, we find that both WRM-1 and HMP-2 can substitute for BAR-1 in *C. elegans* when expressed from the *bar-1* promoter. Therefore, although their mutant phenotypes and protein interaction patterns strongly suggest that the functions of β-catenin in other species have been segregated among three diverged proteins in *C. elegans,* these proteins still retain sufficient similarity to display functional redundancy *in vivo*.

β-CATENIN proteins function in two important processes during metazoan development. First, β-catenin is a component of the cadherin-catenin complex of proteins that function in cell adhesion at adherens junctions (reviewed in Kemler 1993; Yap *et al.* 1997). In this role, β-catenin is found at the plasma membrane where it binds to the cytoplasmic portion of cadherin, a transmembrane adhesion molecule, and to α-catenin, which itself can bind to α-actinin or vinculin and link to the actin cytoskeleton. The ability to make and break cell contacts via modulation of the cadherin-catenin complex is important to proper cell migration and morphogenesis during development (Gumbiner 1996), and modulation of this complex plays a role in the ability of oncogenic cells to become invasive (Ben-Ze'ev and Geiger 1998).

The second role for β-catenin proteins during development is as transcription factors acting in Wnt signaling pathways (reviewed in Miller and Moon 1996; Willert and Nusse 1998). In the absence of Wnt ligand, cytoplasmic β-catenin is present in a complex including the serine/threonine protein kinase glycogen synthase kinase 3β (GSK3β), the tumor suppressor gene product adenomatous polyposis coli (APC), the scaffold protein Axin, and other proteins. In this complex, β-catenin is phosphorylated at sites in its amino terminus by GSK3β, allowing β-catenin to be recognized by a ubiquitin ligase complex that targets it for destruction by the proteosome (Maniatis 1999). In the absence of ligand, transcription factors of the T cell factor/lymphoid enhancer factor (TCF/LEF) family are bound to specific sites in the promoters of Wnt-responsive genes, where they repress transcription (Eastman and Grosschedl 1999). In the presence of ligand, the activity of the destruction complex on β-catenin is inhibited and β-catenin is imported into the nucleus where it interacts with TCF/LEF proteins at Wnt-responsive promoters, leading to the expression of target genes in response to extracellular signal. Wnt signal transduction pathways are used extensively during metazoan development (Cadigan and Nusse 1997), and the improper activation of Wnt target genes due to aberrant stabilization of β-catenin can contribute to cancer in several cell types (Polakis 2000).

β-Catenin proteins have a common structure consisting of amino- and carboxyl-terminal domains flanking a well-conserved central domain (Willert and Nusse 1998). The carboxyl-terminal region contains a transcriptional activation domain in Drosophila and vertebrate β-catenin proteins (van de Wetering *et al.* 1997; Simcha *et al.* 1998; Hecht *et al.* 1999). The amino-terminal region acts in the regulation of protein stability as noted above and has been shown to also contain a transcription activation domain in some family mem-

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bers (Hsu *et al.* 1998; Simcha *et al.* 1998; Hecht *et al.* 1999). The central conserved domain is composed of 12 Armadillo repeat motifs folded into an extended helical structure, and many proteins interact with β-catenin via binding to this extended central domain (Willert and Nusse 1998).

*Drosophila melanogaster* has a single β-catenin gene, which encodes a protein, Armadillo, that functions in both Wnt signaling and adhesion, and these functions are independently mutable (Orsulic and Peifer 1996). Vertebrate genomes, however, express two highly related β-catenin proteins, β-catenin and plakoglobin (Butz *et al.* 1992; Peifer *et al.* 1992). β-Catenin functions in Wnt signaling and in cell adhesion, as described above. Plakoglobin binds to cadherin and α-catenin at adherens junctions but also functions in cell adhesion at desmosomal junctions, a function not shared by β-catenin (see Cowin and Burke 1996 for review). It is unclear whether plakoglobin normally functions in Wnt signaling; however, plakoglobin can bind to Wnt signaling components such as APC, axin, and TCF/LEF factors, and activation of the Wnt pathway can modulate plakoglobin levels in certain situations (Ben-Ze'ev and Geiger 1998; Kodama *et al.* 1999; Kolligs *et al.* 2000). When expressed in Drosophila *armadillo* mutants, both β-catenin and plakoglobin can provide the adhesive function of Armadillo, while only β-catenin can weakly provide the Wnt signaling function (White *et al.* 1998). In addition, mice homozygous for loss-of-function mutations in the β-catenin and plakoglobin genes die at different stages of development (Haegel *et al.* 1995; Bierkamp *et al.* 1996; Ruiz *et al.* 1996). Therefore, it appears that β-catenin and plakoglobin have both shared and diverged functions in vertebrates. Vertebrates contain another protein related to β-catenin called p120 catenin, which was originally identified as a tyrosine kinase substrate (Reynolds *et al.* 1992). p120 is one member of a growing class of related proteins; however, the function and regulation of these proteins is not well understood (Anastasiadis and Reynolds 2000).

Three proteins related to β-catenin have been identified through genetic screens in the nematode *Caenorhabditis elegans*: HMP-2, WRM-1, and BAR-1. Mutations in the *hmp-2* gene cause defects in cell migration and morphogenesis during embryogenesis, and the HMP-2 protein localizes at sites of epithelial cell contact in the embryo with an α-catenin homolog (HMP-1) and a cadherin homolog (HMR-1; Costa *et al.* 1998). *wrm-1* functions in early embryogenesis in the specification of the endodermal precursor cell E along with several genes encoding Wnt pathway factors, including *mom-1 (porcupine), mom-2 (Wnt), mom-5 (frizzled), apr-1 (APC)*, and *pop-1 (TCF/LEF)* (Lin *et al.* 1995; Rocheleau *et al.* 1997; Thorpe *et al.* 1997). The kinases LIT-1 and MOM-4 also function in this process with WRM-1 and are believed to downregulate the ability of POP-1 to bind DNA and repress transcription (Meneghini *et al.* 1999; Rocheleau *et al.* 1999; Shin *et al.* 1999). *bar-1* functions in three Wnt-mediated processes in *C. elegans* postembryonic life: cell fate specification by the vulval precursor cells, cell fate specification by the posterior ectodermal cells P11 and P12, and the migration of the progeny of the $Q_L$ neuroblast (Eisenmann *et al.* 1998; Maloof *et al.* 1999; Eisenmann and Kim 2000). Several other Wnt pathway genes also function in these processes, such as *egl-20 (Wnt), lin-17 (frizzled), mig-5 (dsh)*, and *apr-1* (APC; Harris *et al.* 1996; Jiang and Sternberg 1998; Maloof *et al.* 1999).

Although all three *C. elegans* β-catenin homologs have the conserved structure of β-catenin proteins, they are more diverged in primary sequence than other members of the family, making the relationship between the *C. elegans* proteins and β-catenin, plakoglobin, and p120 unclear. In addition, it is not known whether each of the three *C. elegans* proteins can perform both adhesion and Wnt signaling functions. We previously proposed that the functions of β-catenin may have been segregated among these three proteins, with BAR-1 and WRM-1 functioning in Wnt signaling only, and HMP-2 functioning in a cadherin-catenin complex only (Eisenmann *et al.* 1998). Recent work examining the protein-protein interactions of these three proteins has supported this hypothesis (Korswagen *et al.* 2000). Here we confirm and extend those findings by showing that (1) *C. elegans* contains no other proteins obviously related to β-catenin but does contain a p120-related protein; (2) BAR-1 has redundant transcription activation domains in its amino- and carboxyl-terminal domains; (3) HMP-2 and WRM-1 can also activate transcription; (4) HMP-2, WRM-1, and BAR-1 make distinct protein-protein interactions; and, surprisingly, (5) both WRM-1 and HMP-2 can substitute for *bar-1* in postembryonic development when expressed at high levels. On the basis of these results we suggest that *C. elegans* possesses three diverged β-catenin homologs, each of which normally performs only one of the functions of β-catenin in other organisms, but which still retain functional redundancy *in vivo* despite their high sequence divergence.

## MATERIALS AND METHODS

**Database searching and phylogenetic analysis:** To search for additional β-catenin homologs in *C. elegans* the advanced BLAST program (Altschul *et al.* 1990) was used via the National Center for Biotechnology Information web site (http://www.ncbi.nlm.nih.gov/BLAST). We performed tblastn searches against translated *C. elegans* genomic DNA sequence using the following proteins (with accession numbers): human β-catenin (P35222); hydra β-catenin (JC4835); Drosophila Armadillo (P18824); mouse p120 (P30999); and human ARVCF (NP001661), NPRAP (BAA36163), p0071 (NP003619), plakophilin 1 (NP000290), plakophilin 2 (NP004563), and plakophilin 3 (NP009114). The predicted p120 homolog Y105C5B.21 (CAB60320) was found fourth on the list of β-catenin-related

proteins and first on the list of *C. elegans* predicted proteins similar to p120/ARVCF/p0071/NPRAP/plakophilin. Further characterization of this putative *C. elegans* p120 homolog will be presented elsewhere. Other Armadillo repeat-containing proteins, such as importin and smgGDS homologs, were also found in these searches, but with much lower BLAST scores (data not shown). For Table 1, percentage identity was calculated by aligning each pair of proteins using the program CLUSTAL W (v1.4) for Macintosh (Higgins *et al.* 1996) and dividing the number of identical residues by the consensus length determined by the program (this results in numbers slightly different from those reported previously).

**Genetics and molecular biology:** Standard *C. elegans* genetic procedures were followed in this work (Brenner 1974; Epstein and Shakes 1995). Strains were maintained at 20°. Plasmid DNA was injected at 100 μg/ml along with punc-30(+) (Jin *et al.* 1994) at 100 μg/ml into either *unc-30(e191) IV; bar-1(ga80) X* or *unc-30(e191) IV*. Multiple non-Unc $F_2$ lines carrying the injected DNAs as an extrachromosomal array were identified. As all lines generally behaved the same, phenotypic data for several lines (2–4) were combined and averaged. Molecular biological techniques were performed using standard protocols (Ausubel *et al.* 1987). When constructs were made via polymerase chain reaction (PCR), PCR products were first ligated into either pCRII (Invitrogen, Carlsbad, CA) or pGEM-T-Easy (Promega, Madison, WI) and then removed using restriction enzymes corresponding to sites introduced via the PCR. All constructs were sequenced using an Applied Biosystems (Foster City, CA) ABI 310 capillary electrophoresis DNA sequencing apparatus per manufacturer's protocols or at an outside facility.

**BAR-1 structure/function analysis in yeast:** We created fusions of all or portions of the *bar-1* cDNA to the coding region for the Gal4 DNA-binding domain (1–147) in the vector pAS1 (gift of Steve Elledge). Plasmids (BAR-1 residues in parentheses) contain the indicated *bar-1* regions inserted into the pAS1 polylinker. pDE227 (1–811) contains the full-length *bar-1* open reading frame (ORF) flanked by *Nde*I sites from the cDNA plasmid pDE219 (Eisenmann *et al.* 1998). pDE245 (85–762) contains an internal *Nco*I/*Bam*HI fragment from pDE227. pDE246 (1–85) was made by digesting pDE227 with *Nco*I and religating. pDEL250 (85–540) contains an internal *Nco*I/*Pvu*II fragment from pDE219. pDE278 (685–811) was made by PCR with oligonucleotides ODE57 and ODE40. pDE283 (685–762) was made by digesting pDE278 with *Bam*HI and religating. pDE284 (539–667) was made by PCR with ODE174 and ODE149.

Plasmids were transformed into the yeast strain PJ69-4A (*MAT***a** trp1-901 leu2-3,112 ura3-52 his3-200 gal4Δ *gal80*Δ *GAL2-ADE2 LYS2::GAL1-HIS3 met-2::GAL7-lacZ*; gift of Philip James; James *et al.* 1996), using standard methods (Ausubel *et al.* 1987). Multiple independent $Trp^+$ colonies were picked and replica plated to test for growth on SC-Ade, SC-His + 3 mm 3-amino-1,2,4-triazole (3AT), SC-His + 25 mm 3AT, SC-His + 50 mm 3AT, and for color on 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-Gal) plates. All plates were lacking in tryptophan to select for the plasmid. The protein produced from pDE250 is stable in yeast, as it can be detected on a Western blot and can interact with other proteins in a yeast two-hybrid assay (data not shown).

β-Galactosidase activity was assayed using the Gal-Screen chemiluminescent reporter system from Tropix (Bedford, MA; Nevels *et al.* 1999). Chemiluminescence was measured by a Dynex MLX luminometer using Dynex Revelation software and Dynex microfluor white round bottom 96-well plates. Readings were taken every 0.01 sec and averaged over 1 sec. A standard curve was generated by serial dilutions of β-galactosidase enzyme (Sigma, St. Louis) reconstituted in 0.1 M $NaH_2PO_4$/50% glycerol at 1.0 mg/ml (740 units/mg). One unit hydrolyzes 1.0 μmol of *o*-nitrophenyl β-D-galactoside to *o*-nitrophenol and D-galactose per min at 37° (pH 7.3). Three individual yeast colonies containing each construct were inoculated into minimal media and grown to saturation. An aliquot of each culture was inoculated into two tubes of minimal media and grown to an $OD_{600}$ of 0.6–1.0. Cells were counted and 75,000 cells added per well. The six readings for each construct were averaged and normalized to the activity of full-length BAR-1 (pDE227).

**HMP-2 and WRM-1 yeast constructs:** Fusions of WRM-1 and HMP-2 to the Gal4 DNA-binding domain were made by PCR using *wrm-1* (gift of C. Rocheleau and C. Mello) and *hmp-2* cDNAs (gift of M. Costa and J. Priess) as templates. PCR products were cloned into the pAS1 polylinker using appropriate enzymes. The following primers were used: WRM-1FL, ODE158 and ODE160; WRM-1R112, ODE172 and ODE173; HMP-2FL, ODE169 and ODE170; and HMP-2R19, ODE157 and ODE107. For HMP-2R19 an internal *Nco*I/*Bam*HI fragment was used. Plasmids were transformed into yeast and analyzed as described above.

**Yeast two-hybrid analysis:** For yeast two-hybrid analysis (Fields and Song 1989), the strain PJ69-4A was sequentially transformed with two plasmids, one expressing a β-catenin::Gal4 activation domain fusion protein and the second expressing a potential β-catenin-interacting protein::Gal4 DNA-binding domain (Gal4DBD) fusion protein. Multiple $Trp^+$ $Ura^+$ colonies were picked to a master SC-Trp-Ura plate, which was replica plated to SC − Ade, SC − His + 3 mm 3AT, SC − His + 25 mm 3AT, SC − His + 50 mm 3AT, and X-Gal plates (plates were lacking in tryptophan and uracil).

β-Catenin::Gal4 activation domain fusion protein plasmids were made by PCR on appropriate cDNA templates and insertion of PCR products into the plasmid pACT (gift of S. Elledge). pDE280 (BAR-1) was made using ODE61 and ODE62; pSP12 (HMP-2) was made using ODE177 and ODE170; pLN2 (WRM-1) was made using ODE126 and ODE127; pAS1CYH2-β-cat (mouse β-catenin) was a gift of S. Kaech and S. Kim. The interacting protein fusion plasmids were the following: pAH8 (APR-1) and pAH10 (NT APR-1, residues 1–486, gifts of A. Hajnal); pRL182 (POP-1, gift of R. Lin); pCCM369 (LIT-1, gift of T. Shin and C. Mello); pAS2-1N (NT EGL-27, residues 1–637) and pAS2-1C (CT EGL-27, residues 628–1129, gifts of T. Ratliff and M. Herman); pACT2-α-cat (mouse α-catenin, gift of S. Kaech and S. Kim); pVB10LN (LIN-25 residues 116–565), pVB11LN (LIN-25 residues 473–938), and pVB12LN (LIN-25 residues 732–1139, gifts of S. Tuck); and pDE268 (HMP-1 residues 8–514) and pDE290 (delNTPOP-1, residues 56–487). pDE290 was made by PCR on pRL182 (contains *pop-1* cDNA) with ODE176 and ODE143 and insertion of the product into pAS1. pDE268 was made by PCR on *hmp-1* cDNA (gift of B. Raich and J. Hardin) with ODE86 and ODE87 and insertion of the product into pAS1. pSE1111, encoding Snf4::Gal4 activation domain, and pSE1112, encoding Snf1::Gal4DBD, served as controls (gifts of S. Elledge).

**BAR-1 structure/function analysis in *C. elegans*:** pDE204 contains the *bar-1* genomic region (5.1 kb upstream and 0.8 kb downstream of the open reading frame) and can rescue a *bar-1(ga80)* mutation (Eisenmann *et al.* 1998). In the *bar-1* coding region there is a *Bam*HI site in the first exon at amino acid 4 and in the last exon at amino acid 761. Portions of the *bar-1* cDNA were isolated by PCR and inserted between these *Bam*HI sites to create the following plasmids. The rescuing plasmid pDE248 contains a full-length *bar-1* cDNA (Eisenmann *et al.* 1998). pDE282 [ΔNTBAR-1, amino acids (aa) 1–4, 90–811] was made using ODE49 and ODE40. pDE291 (BAR-1ΔCT1, aa 1–758) was made by combining a *Pvu*II-*Spe*I fragment made using ODE32 and ODE148 with an *Xba*I-*Pvu*II fragment from

pDE219. pDE292 (BAR-1ΔCT2, aa 1–667) was made by combining a *Pvu*II-*Spe*I fragment made using ODE32 and ODE149B with an *Xba*I-*Pvu*2 fragment from pDE219. Both pDE291 and pDE292 contain two stop codons at the 3′ *Bam*HI site, so the resulting *bar-1* ORF terminates prematurely but still has the rest of the *bar-1* final exon and 3′ untranslated region sequences intact. pDE293 (BAR-1ΔNTΔCT, aa 1–4, 90–667) was made by combining a 0.35-kb *Bam*HI/*Xho*I fragment from pDE282 with a 2.3-kb *Xho*I/*Pst*I fragment from pDE292 and ligating into pDE204 digested with *Bam*HI and *Pst*I.

Each construct was injected into *bar-1(ga80)* mutants and transgenic lines were identified as described above. To score the vulval phenotype of these strains, >100 non-Unc L4 hermaphrodites were picked to a separate plate and scored the next day as adults for the retention of older embryos or larvae (egg-laying defective/Egl phenotype) or for a protruding vulva (Pvl phenotype). To examine vulval morphology in these strains >50 non-Unc L4 hermaphrodites were placed on a slide in M9 plus 10 mM sodium azide and the number of induced cells was determined by examining the morphology of the vulval induction in each animal. Animals with fewer than three induced vulval precursor cells (VPCs) were considered to have an Underinduced phenotype.

**HMP-2/WRM-1/Armadillo rescue of *bar-1(ga80)*:** pDE249 (*bar-1p::arm*) was made by PCR with ODE50 and ODE54 on an *arm* cDNA (gift of R. Nusse) and insertion of the product between the *Bam*HI sites of pDE204. The resulting ORF encodes residues 1–4 of BAR-1 and 5–843 of Armadillo. For *wrm-1* and *hmp-2* constructs, internal *Bam*HI sites in the cDNAs required the use of pDE204NBX, which contains *Not*I, *Bgl*II, and *Xho*I restriction sites introduced between the *Bam*HI sites of pDE204, destroying the second *Bam*HI site. pJBH2 (*bar-1p::hmp-2*), was made by PCR on a *hmp-2* cDNA with ODE106 and ODE107. The PCR product was inserted into pDE204NBX digested with *Not*I. The resulting ORF encodes residues 1–5 of BAR-1, the amino acids RRP, and residues 2–678 of HMP-2. pJBW1 (*bar-1p::wrm-1*) was made by PCR on the *wrm-1* cDNA with ODE126 and ODE127. The product was ligated into pDE204NBX digested with *Bam*HI and *Xho*I. The resulting ORF encodes residues 1–4 of BAR-1 and residues 2–796 of WRM-1 with a V3L change. The resulting plasmids were injected into *bar-1(ga80)* mutant animals and transgenic lines were analyzed for their Egl and Underinduced phenotypes as described above.

**Oligonucleotides:** The following oligonucleotides were used in this work: ODE32, GCCACCAAAGAGATGGGTGACTTGA GAG; ODE40, CATATGAATGTGCAACAAATATCCGACTGG; ODE49, GGATCCGATCCCCACTCTTTCAGATCAGC; ODE50, GGATCCAGCCCAGAATCGAACCATGTCGC; ODE54, GATTC CGGGATCCGGGATGGAATCAAAGC; ODE57, GGTCATATG AGCATGACCACCCCACGAAGC; ODE61, GGACCTAGATCTG AACCTAGTTATTAACC; ODE62, AGATCTAATCVACTATTC CTAGAAGGATAATAATCAGACG; ODE86, GGATCCATGCGT ATTTCAACATCGACGAAGTGC; ODE87, GGATCCCCCGCTC ACAGTCTTCAACAATATGTGC; ODE106, GCGGCCGCTTCT TCACTCTACCAACTCTTATTCG; ODE107, GCGGCCGCTTA CAAATCGGTATCGTACCAATTGTG; ODE126, ATAGATCTG GATTGCGCAGAAAC (causes V3L change); ODE127, CTCGA GATTCATTCACATTAGTTGTCG; ODE143, CCGGAGAAGAT CTTTAAATAGTACACATCG; ODE148, GGATCCTACTACGT CTCGGGAGGTCCAATTG; ODE149, AGATCTATTCTCGCTT CTCATACATCATCTTG; ODE149B, GGATCCTTGTCTATTC TCGCTTCTCATACATC; ODE157, CATATGGATCTTCTAACC TACGAAG; ODE158 CCATGTGTGGATTGCGCAGAAAC; OD-E160, AGATCTATTCAGACATTAGTTGTCGATGATG; ODE1 69, CATATGCTTCTTCACTCTACCAACTCTTATTCG; ODE1 70, GTCGACTTACAAATCGGTATCGTACCAATTGTG; ODE 172, CCATGGGCGAATATGTCAGAAATGACCG; ODE173, GT CGACTTCAAATTGACAAAATGAATGCTAGC; ODE174, CAT ATGAATGTGCAAGAATCTATCGAAGG; ODE176, CCATGGA TGTGTTAAAAAGTGCATTTCC; and ODE177, GTCGACTTC TTCACTCTACCAACTCTTATTCG.

## RESULTS

**C. *elegans* contains three β-catenin homologs and a single p120 homolog:** Genes encoding β-catenin proteins have been found in many metazoan phyla and the encoded proteins share strong conservation at the amino acid level. For example, Drosophila Armadillo is 67% identical to human β-catenin (Table 1; Peifer and Wieschaus 1990) and a β-catenin homolog from the cnidarian *Hydra magnipapillata* is 54% identical to the human protein (Hobmayer *et al.* 1996). Although the *C. elegans* HMP-2, WRM-1, and BAR-1 proteins have the overall structure of β-catenin proteins they show a surprisingly low percentage of amino acid identity when compared to β-catenins from other species (Table 1). BAR-1 and HMP-2 are only 23 and 27% identical to human β-catenin, and WRM-1 is even less so (17%). In fact WRM-1 is only slightly more identical to β-catenin than it is to other β-catenin-related proteins such as mouse p120 and plakophilins (Table 1; see Anastasiadis and Reynolds 2000). Each *C. elegans* protein shares more amino acid identity with vertebrate β-catenin than with the other two nematode proteins (BAR-1:HMP-2, 18%, BAR-1:WRM-1, 17%, WRM-1:HMP-2, 15%). The low similarity of *C. elegans* β-catenins to those of other species is surprising, given eukaryotic phylogenies that indicate that nematodes shared a more recent common ancestor with arthropods and chordates than do phyla such as cnidarians (Holland 1999; Knoll and Carroll 1999).

Given the low percentage identity of the *C. elegans* proteins to β-catenin and the presence of three putative β-catenin homologs in *C. elegans,* we asked whether any protein more similar to β-catenin was encoded in the *C. elegans* genome and whether any of the three *C. elegans* proteins was more similar to one or more vertebrate β-catenin-related proteins than it is to β-catenin. The *C. elegans* genome was searched for predicted proteins most similar to β-catenin, plakoglobin, and the following β-catenin-related proteins: p120 catenin (Reynolds *et al.* 1992), ARVCF (Sirotkin *et al.* 1997), NPRAP (Tanahashi and Tabira 1999), p0071 (Hatzfeld and Nachtsheim 1996), plakophilin 1 (Hatzfeld *et al.* 1994), plakophilin 2 (Mertens *et al.* 1996), and plakophilin 3 (Schmidt *et al.* 1999). No predicted protein more homologous to β-catenin and plakoglobin than HMP-2 was found in the current nearly complete *C. elegans* genomic sequence (*C. elegans* Sequencing Consortium 1998), and no predicted proteins similar to β-catenin and plakoglobin, other than HMP-2, BAR-1, and WRM-1, were found. A single predicted pro-

**TABLE 1**

**Percentage identity of β-catenin family members across species**

| | β-Catenin | HMP-2 | BAR-1 | WRM-1 | p120 | Cep120 |
|---|---|---|---|---|---|---|
| β-Catenin | — | 27 | 23 | 17 | 16 | 15 |
| Armadillo | 67 | 27 | 22 | 15 | 17 | 15 |
| Plakoglobin | 65 | 28 | 22 | 17 | 15 | 15 |
| p120 | 16 | 15 | 15 | 15 | — | 23 |
| ARVCF | 14 | 15 | 14 | 14 | 45 | 24 |
| NPRAP | 16 | 14 | 13 | 14 | 30 | 24 |
| p0071 | 16 | 14 | 15 | 15 | 29 | 23 |
| Plakophilin 1 | 15 | 13 | 16 | 13 | 24 | 17 |
| Plakophilin 2 | 15 | 15 | 12 | 13 | 23 | 18 |
| Plakophilin 3 | 15 | 15 | 14 | 14 | 23 | 17 |

Percentage amino acid identity between β-catenin family members and other related proteins from vertebrates, Drosophila, and *C. elegans* based on sequence alignments using CLUSTAL W. All proteins are from *H. sapiens* except Armadillo (*D. melanogaster*), p120 (*M. musculus*), and HMP-2, WRM-1, BAR-1, and Cep120 (*C. elegans*). Cep120 is predicted protein Y105C5B.21.

tein related to the p120/ARVCF/NPRAP/p0071 subfamily of β-catenin-related proteins was found. This predicted protein, Y105C5B.21, is 23–24% identical to those vertebrate proteins and 23% identical to a predicted p120 homolog found in the Drosophila genome (data not shown).

These results suggest that while vertebrates appear to have two conserved β-catenin family members and *D. melanogaster* has only one, *C. elegans* has three β-catenin proteins that have widely diverged in primary sequence from β-catenins in other species. We previously hypothesized on the basis of the mutant phenotypes of *bar-1*, *wrm-1*, and *hmp-2* that *C. elegans* has increased its number of β-catenin proteins by gene duplication and that these proteins may have segregated the Wnt signaling and cell adhesion functions of β-catenin and Armadillo among themselves (Eisenmann *et al.* 1998). Therefore we sought to characterize these proteins in more detail and to determine whether the three *C. elegans* proteins have distinct or overlapping functions.

**BAR-1 contains transcription activation domains in its amino- and carboxyl-terminal domains:** *bar-1* activity is required to maintain expression of Hox target genes during two Wnt-mediated postembryonic developmental processes, suggesting that, like β-catenin, BAR-1 may function to regulate transcription in the nucleus (Eisenmann *et al.* 1998; Maloof *et al.* 1999; Eisenmann and Kim 2000). Consistent with this, BAR-1 protein and a BAR-1::GFP fusion were both found in the nucleus when overexpressed (Eisenmann *et al.* 1998). To determine if BAR-1 can activate transcription directly, BAR-1 was analyzed in the yeast *Saccharomyces cerevisiae*, since neither cadherin-catenin complex proteins nor Wnt signaling pathway proteins are found in this organism.

A chimeric protein containing the entire BAR-1 open reading frame fused to the DNA-binding domain of the yeast Gal4 transcription factor (Gal4DBD) was expressed in a yeast strain containing Gal4-binding sites upstream of the yeast *HIS3* and *Escherichia coli lacZ* genes (James *et al.* 1996). Yeast expressing the full-length BAR-1 fusion protein were able to grow on plates lacking histidine and containing high concentrations of the *HIS3* competitive inhibitor 3AT and turned dark blue on plates containing the substrate X-Gal, indicating that BAR-1 can function as a transcription activator in yeast (Figure 1). To delineate the region(s) within BAR-1 capable of activating transcription, fusion proteins containing different portions of the BAR-1 protein fused to the Gal4DBD were expressed (Figure 1). BAR-1 lacking both the amino-terminal domain and the last 50 amino acids (residues 85–762) was able to activate transcription nearly as well as the full-length BAR-1 protein. However, a fusion containing Armadillo repeats 1–9 from the central domain showed no ability to activate transcription (residues 85–540), indicating that a transcription activation domain is present in residues 541–762 of BAR-1, which encompasses the last three Armadillo repeats and the beginning of the carboxyl-terminal domain. Although the region containing Armadillo repeats 9–12 (residues 539–668) was not able to activate transcription, the carboxyl-terminal region of BAR-1 (residues 685–811) was able to activate transcription about as well as the full-length BAR-1 protein on the basis of assays of β-galactosidase activity. A smaller part of this region, containing sequences immediately following the repeat region (residues 685–762), was still able to activate transcription, although less well than the full carboxyl-terminal domain. These results indicate that the carboxyl-terminal region of BAR-1 contains one or more transcription activation domains. The region amino-terminal to the Armadillo repeats (residues 1–85) was also able to activate transcription. Loss of this region had little effect on transcription activity when the carboxyl-terminal region was still present (residues 85–762), suggesting that the

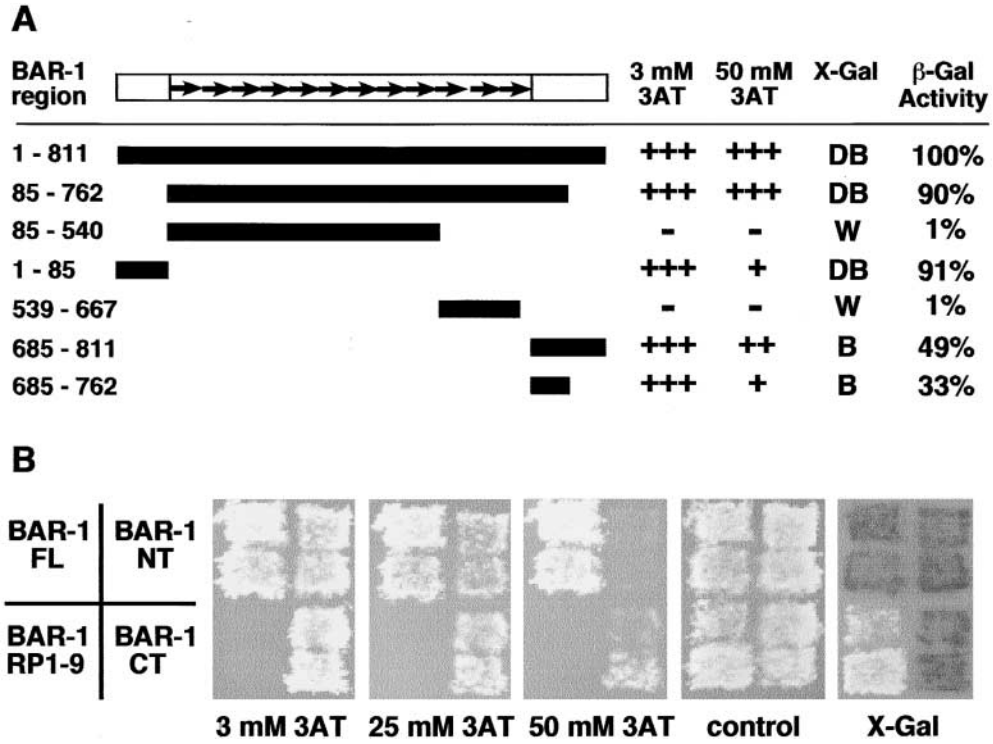


FIGURE 1.—Ability of different BAR-1 domains to activate transcription in yeast. (A) Proteins encoding the indicated regions of BAR-1 fused to the Gal4 DNA-binding domain were expressed in yeast and analyzed after 4 days for activation of expression of *HIS3* and *lacZ* genes containing upstream Gal4 binding sites. +++, strong growth; ++, moderate growth; +, weak growth; and −, no growth. DB, dark blue; B, medium blue; LB, pale blue; and W, no blue color. Strains were assayed for β-galactosidase activity. The activity due to full-length BAR-1 was set to 100% and the activity of other proteins is shown relative to that value. (B) Growth of selected strains on plates containing the indicated concentrations of 3AT or X-Gal. BAR-1FL, 1–811; BAR-1NT, 1–85; BAR-1RP1-9, 85–540; BAR-1(CT), 685–811.

amino-terminal and carboxyl-terminal transcription activation domains are redundant in yeast. Since transcription activation domains have been found in the amino- and carboxyl-terminal domains of vertebrate β-catenin (HSU *et al.* 1998; SIMCHA *et al.* 1998; HECHT *et al.* 1999), these results indicate a conservation of function and domain structure between the nematode protein BAR-1 and β-catenin.

**The BAR-1 transcription activation domains are functionally redundant in *C. elegans*:** Although both the amino- and carboxyl-terminal regions of BAR-1 contain transcription activation domains, it is possible that these regions might contain other functional domains required for BAR-1 activity in *C. elegans*. To test this we introduced mutated *bar-1* cDNAs into a rescuing *bar-1* minigene construct (Table 2; EISENMANN *et al.* 1998) and asked whether BAR-1 proteins lacking the amino- or carboxyl-terminal domains could rescue the mutant phenotypes of a *bar-1(ga80)* mutant. The *ga80* mutation creates a truncated BAR-1 protein, and the *bar-1(ga80)* phenotype is likely due to complete loss of BAR-1 function (EISENMANN *et al.* 1998). In wild-type animals three vulval precursor cells adopt induced fates and give rise to the cells that form the vulval opening (GREENWALD 1997). In 59% of *bar-1(ga80)* mutants fewer than three vulval precursor cells adopt induced fates, which results in defective vulval formation (Table 2; EISENMANN *et al.* 1998). Forty-five percent of *bar-1(ga80)* animals show one of several vulval mutant phenotypes when observed as adults on plates using a dissecting microscope (Table 2).

The amino-terminal region of BAR-1 contains consensus GSK3β phosphorylation sites (EISENMANN *et al.* 1998) and a transcription activation domain. A protein lacking this region (ΔNT BAR-1) was still able to rescue the vulval induction defects of a *bar-1(ga80)* strain (Table 2), indicating that no sequences in the amino-terminal region are necessary for BAR-1 activity. In these experiments the *bar-1* plasmid is present on a mitotically unstable extrachromosomal array, so full rescue of the *bar-1(ga80)* mutant phenotype is not observed. The ΔNT BAR-1 construct was also introduced into wild-type animals to look for a dominant positive phenotype, since deletion of the amino terminus or mutation of the GSK3β phosphorylation sites leads to a stabilized β-catenin protein capable of activating the transcription of Wnt-responsive genes in vertebrates and Drosophila (YOST *et al.* 1996; MORIN *et al.* 1997; PAI *et al.* 1997). No obvious defect in vulval development was seen in wild-type animals expressing ΔNT BAR-1 from the *bar-1* promoter (data not shown). However, expression of ΔNT BAR-1 from the heat shock promoter can cause defects in vulval development (J. GLEASON and D. EISENMANN, unpublished results).

BAR-1 proteins lacking the last one-half of the carboxyl-terminal domain (ΔCT1 BAR-1; residues 1–759) or the entire region carboxyl terminal to the Armadillo repeats (ΔCT2 BAR-1; residues 1–667) were also analyzed. Both of these truncated proteins could rescue the *bar-1* vulval mutant phenotype as well as the full-length *bar-1* construct (Table 2). Together these results suggest that neither the amino- nor the carboxyl-terminal portion of BAR-1 is necessary for *bar-1* activity in *C. elegans*.

**TABLE 2**

**Structure/function analysis of BAR-1 in *C. elegans***

| Strain | % mutants (plate) | *N* | % mutants (DIC) | *N* |
|---|---|---|---|---|
| *bar-1(ga80)* | 45 | 200 | 59 | 120 |
| *bar-1(ga80) Ex[bar-1(+)]* | 10 | 267 | 27 | 100 |
| *bar-1(ga80) Ex[ΔNTbar-1]* | 4 | 200 | 9 | 111 |
| *bar-1(ga80) Ex[ΔCT1bar-1]* | 11 | 200 | 22 | 104 |
| *bar-1(ga80) Ex[ΔCT2bar-1]* | 8 | 200 | 10 | 126 |
| *bar-1(ga80) Ex[ΔNTΔCT2bar-1]* | 56 | 212 | 50 | 301 |

Plasmids expressing the full-length BAR-1 and BAR-1 deletion derivatives from the *bar-1* promoter were injected into *bar-1(ga80)* mutant animals and multiple transgenic lines were obtained. Animals from two to four independent lines were analyzed for the percentage of animals displaying a vulval mutant phenotype (Egl, Pvl) as adults on plates or an Underinduced phenotype (fewer than three induced Pn.p cells) when examined by Nomarski (DIC) microscopy at the L4 stage.

As both regions are able to activate transcription in yeast and a known function of β-catenin proteins is the activation of target gene expression, this suggests that BAR-1 may have transcription activation domains that are functionally redundant *in vivo*. To address this, a protein truncated at both its amino- and carboxyl-termini (ΔNTΔCT2BAR-1) was tested. Fifty-six percent of *bar-1(ga80)* animals carrying this transgene displayed vulval mutant phenotypes when examined by dissecting microscope, compared to 45% for *bar-1(ga80)* alone, and 50% of animals displayed defects in vulval induction when examined by Nomarski microscopy, compared to 59% for *bar-1(ga80)* alone (Table 2). The fact that ΔNTΔCT2BAR-1 does not rescue the *bar-1(ga80)* vulval mutant phenotype, while both ΔNTBAR-1 and ΔCT2 BAR-1 do, indicates that the transcription activation domains identified in the yeast experiments are necessary and redundant for BAR-1 function *in vivo*.

**The WRM-1 and HMP-2 proteins can also activate transcription in yeast:** We wished to address whether each of the three *C. elegans* β-catenin proteins retains all the functions of β-catenin and Armadillo or whether these functions have been segregated among the three proteins. For example, it could be the case that HMP-2, which functions in a process with *C. elegans* cadherin and α-catenin homologs (Costa *et al.* 1998), might no longer function in Wnt signaling and would not retain the ability to activate transcription. Therefore, we asked whether HMP-2 and WRM-1 also have the ability to activate transcription in yeast. Fusion proteins containing WRM-1 or HMP-2 fused to the Gal4 DNA-binding domain were expressed in the yeast strain used previously. Both WRM-1 and HMP-2 proteins are able to activate transcription in yeast on the basis of the growth of these strains on plates lacking histidine and containing the HIS3 competitive inhibitor 3AT and on plates containing X-Gal (Figure 2). Both proteins were less effective activators of transcription than BAR-1, and HMP-2 was more effective than WRM-1, based on the growth of these strains on plates containing increasing concentrations of 3AT and on β-galactosidase assays (Figure 2). We did not specifically localize the transcription activation domains in these proteins; however, deleting the amino- and carboxyl-terminal domains and last three repeats of HMP-2 reduced the ability of HMP-2 to activate transcription, as was the case for BAR-1. Deletion of the regions flanking the Armadillo repeat domain in WRM-1 did not abolish the ability of WRM-1 to activate transcription but decreased it by ~50% (Figure 2). Thus, this analysis shows that all three *C. elegans* β-catenin proteins can activate transcription in a heterologous system and therefore retain this function of β-catenin/Armadillo.

**BAR-1, WRM-1, and HMP-2 make specific protein-protein interactions:** Although the three *C. elegans* proteins retain the transcription activation function of β-catenin, it remains possible that other functions of β-catenin/Armadillo are dispersed among the proteins, a possibility suggested by the different phenotypes of *hmp-2*, *bar-1*, and *wrm-1* mutants. To address this issue we used two-hybrid analysis in yeast (Fields and Song 1989) to study the interactions of HMP-2, BAR-1, and WRM-1 with several other proteins known to function in Wnt signaling or cellular morphogenesis in *C. elegans*. Specifically, proteins containing full-length sequences of BAR-1, HMP-2, WRM-1, and mouse β-catenin fused to the transcription activation domain of the yeast Gal4 protein were tested for interactions with the TCF/LEF protein POP-1 (Lin *et al.* 1995), the APC homolog APR-1 (Rocheleau *et al.* 1997), the α-catenin HMP-1 (Costa *et al.* 1998), and the MAP kinase-related serine/threonine kinase LIT-1 (Meneghini *et al.* 1999; Rocheleau *et al.* 1999; Shin *et al.* 1999). Each of these factors was previously shown by genetic analysis to function in at least one process with BAR-1, HMP-2, or WRM-1. We also tested for interactions with (1) *C. elegans* EGL-27, which is homologous to a component of a mammalian complex possessing histone deacetylase and chromatin remodeling activities and which functions in a Wnt-mediated process (Herman *et al.* 1999; Solari *et al.* 1999); (2) *C. elegans* LIN-25 protein, a putative downstream target of Ras signaling in vulval development (Tuck and Greenwald 1995); and (3) vertebrate α-catenin. Interaction between two fusion proteins in this yeast assay leads to transcription from the *HIS3* and *lacZ* reporter genes, as shown by growth on plates lacking histidine and plates containing X-Gal, respectively.

The results of this analysis are shown in Figure 3 and show that the three *C. elegans* β-catenin proteins make distinct protein-protein interactions that are consistent

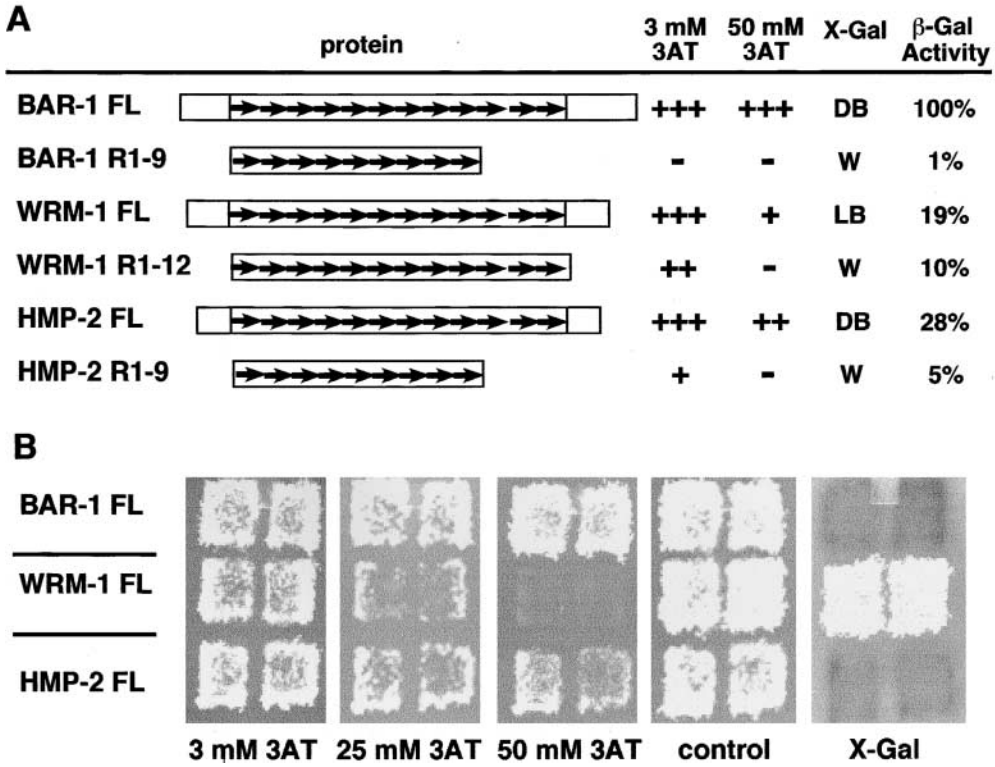


FIGURE 2.—Transcription activation by WRM-1 and HMP-2 in yeast. (A) Proteins encoding the indicated regions of BAR-1, WRM-1, and HMP-2 fused to the Gal4 DNA-binding domain were expressed in yeast and analyzed as in Figure 1. Data for BAR-1 and BAR-1 (R1-9) are from Figure 1. (B) Growth of selected strains on plates containing the indicated concentrations of 3AT or X-Gal.

with previous genetic data. For example, HMP-2 interacts with the *C. elegans* α-catenin homolog HMP-1, and *hmp-1* and *hmp-2* mutants have similar phenotypes (COSTA *et al.* 1998). HMP-2 also interacts well with a vertebrate α-catenin, indicating that although HMP-2 is only 27% identical to vertebrate β-catenin, it has retained the sequences necessary for a functional interaction with vertebrate α-catenin. In a similar assay, HMP-2 was also shown to physically interact with the cadherin homolog HMR-1 (KORSWAGEN *et al.* 2000). HMP-2 also showed a weak interaction with APR-1, suggesting HMP-2 retains the ability to interact with an APC homolog. However, we did not see an interaction with POP-1 or LIT-1, two other known Wnt signaling components. Conversely, neither WRM-1 nor BAR-1 showed any interaction with HMP-1 or the vertebrate α-catenin.

BAR-1 and WRM-1 did interact with known Wnt signaling pathway components. Both interacted with the TCF/LEF homolog POP-1, although the interaction with BAR-1 was stronger based on the ability of the BAR-1 + POP-1 strain to grow on plates containing 50 mM 3AT, while the WRM-1 + POP-1 strain could grow only on plates containing 3 mM 3AT (3AT is a competitive inhibitor of the *HIS3* reporter gene). The physical and functional interaction of BAR-1 with POP-1 was described recently in yeast and vertebrate culture cells (KORSWAGEN *et al.* 2000). WRM-1 also interacted strongly with LIT-1, which is consistent with previous genetic data indicating that these factors function together in embryonic endoderm specification (ROCHELEAU *et al.* 1999; SHIN *et al.* 1999). Curiously, an interaction was not observed between WRM-1 and APR-1, which is also known to function in the process of endodermal specification (ROCHELEAU *et al.* 1997). Conversely, BAR-1 did interact strongly with APR-1 but did not interact with LIT-1. BAR-1 also interacted with both a full-length APR-1 fusion protein (1186 amino acids) and a truncated version containing only the first 486 amino acids, indicating that the region required for interaction is in the amino-terminal half of APR-1 (see DISCUSSION). Therefore, BAR-1 and WRM-1 both interact with Wnt pathway components but display qualitative and quantitative differences in the their interactions. As a control for these experiments, vertebrate β-catenin was shown to interact with α-catenin in this assay. No interaction was detected between vertebrate β-catenin and any *C. elegans* protein, nor did any of the β-catenin proteins interact with any region of EGL-27 or LIN-25.

TCF family members interact with β-catenin through a conserved region at their amino terminus (BEHRENS *et al.* 1996; VAN DE WETERING *et al.* 1997); therefore a version of POP-1 in which the amino-terminal 55 amino acids have been deleted (ΔNTPOP-1) was tested for interactions with BAR-1 and WRM-1. This deletion completely abolished the ability of POP-1 to interact with BAR-1, indicating that POP-1, like other TCF/LEF proteins, interacts with β-catenins via its amino-terminal domain (see also KORSWAGEN *et al.* 2000). However, the weak interaction observed between POP-1 and WRM-1 was still present with ΔNTPOP-1, suggesting that WRM-1 and BAR-1 may interact with POP-1 in different manners.

| | HMP-2 | BAR-1 | WRM-1 | β-CAT | SNF4 |
|---|---|---|---|---|---|
| POP-1 | - | +++ | + | - | - |
| ΔNTPOP-1 | - | - | + | - | - |
| APR-1 FL | + | +++ | - | - | - |
| APR-1 NT | + | +++ | - | - | - |
| LIT-1 | - | - | +++ | - | - |
| HMP-1 | +++ | - | - | - | - |
| α-CAT | +++ | - | - | +++ | - |
| SNF1 | - | - | - | - | +++ |

FIGURE 3.—HMP-2, BAR-1, and WRM-1 interactions in yeast two-hybrid assay. The first column lists fusions of various cadherin/catenin or Wnt signaling components to the Gal4 DNA-binding domain. All fusions are to the full-length test protein except for APR-1NT (residues 1–486), and HMP-1 (residues 628–1129). The top row indicates fusions of full-length HMP-2, BAR-1, WRM-1, and mouse β-catenin to the Gal4 activation domain. The Snf1 DNA-binding domain and Snf4 activation domain fusions serve as positive controls. Each cell of the table indicates the growth of a yeast strain containing the two different fusions on SC − His plates containing 3 mM, 25 mM, or 50 mM 3AT. +++, growth in the presence of 50 mM 3AT; +, growth on 3 mM 3AT but not higher concentrations; and −, no growth on plates with 3 mM 3AT.

In summary, these results indicate that each *C. elegans* β-catenin protein has a pattern of protein interactions distinct from that of the other two, and the pattern of interactions suggests that the three *C. elegans* β-catenin proteins may have segregated the functions of β-catenin among themselves, with HMP-2 functioning only in a cadherin-catenin complex and BAR-1 and WRM-1 functioning only in Wnt signaling but with different modalities.

**WRM-1, HMP-2, and Armadillo can substitute for BAR-1 *in vivo*:** To examine the relevance of the yeast data *in vivo* in *C. elegans*, we asked whether HMP-2 or WRM-1 could substitute for the function of BAR-1 *in vivo* and rescue the phenotype of a *bar-1* mutant strain. To perform this experiment, full-length *hmp-2* or *wrm-1* cDNAs were inserted into the same vector previously used for *bar-1* structure/function analysis *in vivo* (pDE-248), resulting in the production of HMP-2 or WRM-1 protein under the same temporal and spatial controls as BAR-1. The results of this analysis are shown in Table 3. Expression of either WRM-1 or HMP-2 from the *bar-1* promoter from a multicopy extrachromosomal array rescued the vulval defects of a *bar-1* mutant strain and did so nearly as well as a *bar-1*(+) control. The Drosophila Armadillo protein was also able to substitute for *bar-1* activity, indicating that although these proteins show significant divergence in primary sequence, the insect and nematode proteins retain common functions and interactions.

WRM-1 and Armadillo are known to function in Wnt signaling, so their ability to substitute for BAR-1 was not completely unexpected; however, the result with HMP-2 was surprising since the yeast two-hybrid analysis suggests that HMP-2 and BAR-1 make different protein interactions. It is clear that the rescue is not due to artifactual rescue by *bar-1* promoter sequences or coinjection marker DNA, since the ΔNTΔCT2BAR-1 protein expressed in the same manner was not able to rescue a *bar-1(ga80)* mutant (Table 2). Since injected transgenic DNAs in *C. elegans* are present as high copy extrachromosomal arrays, we reasoned that the ability of HMP-2 to rescue *bar-1(ga80)* might be dependent on a high level of expression of the protein. We therefore injected *bar-1(ga80)* animals with 10-fold less of the BAR-1- and HMP-2-expressing constructs. Under these conditions, the BAR-1-expressing plasmid could still rescue the *bar-1(ga80)* vulval phenotype fairly well (35% mutant *vs.* 27% mutant when injected at 100 μg/ml), while the HMP-2-expressing plasmid rescued poorly if at all (53% mutant *vs.* 27% when injected at 100 μg/ml; Table 3). This suggests that HMP-2 may be able to substitute for *bar-1* function *in vivo*, but only when overexpressed. Therefore, although the yeast two-hybrid data suggest a segregation of protein-protein interactions among the three *C. elegans* β-catenins, both WRM-1 and HMP-2 retain the ability to substitute for BAR-1 *in vivo* during vulval development when expressed at high levels in the appropriate temporal and spatial pattern, indicating that the three *C. elegans* proteins display functional redundancy *in vivo*.

**TABLE 3**

**Rescue of *bar-1(ga80)* mutant by other β-catenin proteins**

| Strain | % mutants (plate) | *N* | % mutants (DIC) | *N* |
|---|---|---|---|---|
| *bar-1(ga80)* | 45 | 200 | 59 | 120 |
| *bar-1(ga80) Ex[pbar-1::bar-1]* | 10 | 267 | 27 | 100 |
| *bar-1(ga80) Ex[pbar-1::wrm-1]* | 15 | 253 | 34 | 111 |
| *bar-1(ga80) Ex[pbar-1::hmp-2]* | 15 | 212 | 27 | 110 |
| *bar-1(ga80) Ex[pbar-1::arm]* | 17 | 252 | 37 | 109 |
| *bar-1(ga80) Ex[pbar-1::bar-1]* 10 μg/ml | ND | ND | 35 | 210 |
| *bar-1(ga80) Ex[bar-1::hmp-2]* 10 μg/ml | ND | ND | 53 | 232 |

Plasmids expressing full-length BAR-1, WRM-1, HMP-2, and Armadillo proteins from the *bar-1* promoter were injected into *bar-1(ga80)* mutant animals at 100 μg/ml (except where indicated) and multiple transgenic lines were obtained. Animals from two to four independent lines were analyzed for the percentage of animals displaying a vulval mutant phenotype (Egl, Pvl) as adults on plates or an Underinduced phenotype (fewer than three induced Pn.p cells) when examined by Nomarski (DIC) microscopy at the L4 stage. ND, not determined.

## DISCUSSION

β-Catenin proteins function in cell fate determination via their transduction of Wnt signals and in cell adhesion and morphogenesis via their participation in the cadherin/catenin complex (Kemler 1993; Miller and Moon 1996; Yap *et al.* 1997; Willert and Nusse 1998). Drosophila has a single β-catenin family member, Armadillo (Peifer and Wieschaus 1990), while vertebrates have two β-catenin family members, β-catenin and plakoglobin (Butz *et al.* 1992; Peifer *et al.* 1992). The nematode *C. elegans* expresses three β-catenin family members, BAR-1, WRM-1, and HMP-2 (Rocheleau *et al.* 1997; Costa *et al.* 1998; Eisenmann *et al.* 1998). All three have the structure of β-catenin proteins but are much less well conserved in primary sequence than β-catenins from other species. We asked whether these three β-catenins still retain all of the functions of β-catenin (and Armadillo) or whether the functions of β-catenin have been segregated to different molecules in *C. elegans*.

We have shown that (1) the essentially complete *C. elegans* genome encodes only these three β-catenin homologs and a single less-related p120-like protein; (2) all three *C. elegans* β-catenins retain the ability to activate transcription when assayed in yeast, with BAR-1 the strongest activator of transcription (three- to fivefold better than HMP-2 and WRM-1); (3) BAR-1 contains transcription activation domains in its amino-terminal and carboxyl-terminal regions, which are functionally redundant *in vivo*; (4) the three *C. elegans* β-catenins make distinct protein-protein interactions on the basis of yeast two-hybrid analysis, with WRM-1 and BAR-1 making contacts with Wnt signaling factors and HMP-2 making contacts with a component of the cadherin-catenin complex; and (5) HMP-2 and WRM-1 (and Drosophila Armadillo) can partially rescue the *bar-1* vulval mutant phenotype when expressed from the *bar-1* promoter, suggesting that these proteins retain the ability to function in a manner similar to BAR-1 *in vivo*.

All of the protein interactions we observed using the yeast two-hybrid assay are consistent with genetic results in *C. elegans*. For example, HMP-2 clearly functions in a cadherin-catenin complex during embryonic development with the α-catenin homolog HMP-1 and the cadherin homolog HMR-1, and we observed an interaction between HMP-2 and HMP-1 (Costa *et al.* 1998). There is no evidence to date of a role for HMP-2 in any Wnt-mediated process in *C. elegans*, and we did not detect an interaction between HMP-2 and any Wnt pathway component, except APR-1. However, APC, which functions in the regulation of Wnt signaling, has also been shown to function in the regulation of cell adhesion and migration in vertebrates (Nathke *et al.* 1996). Indeed, *apr-1* mutants have defects in epithelial cell migration during embryogenesis and APR-1 localizes to cell junctions (Hoier *et al.* 2000). Therefore, the HMP-2-APR-1 interaction may not indicate a role for HMP-2 in Wnt signaling in *C. elegans*. Korswagen *et al.* (2000) also reported the interaction of HMP-2 with the cadherin homolog HMR-1 but not with the TCF homolog POP-1.

APR-1 also interacts strongly with BAR-1. Consistent with the observed interaction, loss of *apr-1* activity in the VPCs causes *bar-1*-like defects in cell fate specification and *lin-39* expression, suggesting that APR-1 functions in a Wnt signaling pathway with BAR-1 during vulval development (Hoier *et al.* 2000). Both BAR-1 and HMP-2 were able to interact with both a full-length APR-1 fusion (residues 1–1186) and a fusion containing only residues 1–486. Vertebrate APC interacts with β-catenin via multiple 15- and 20-amino-acid repeat motifs located in the central region of the molecule (Rubinfeld *et al.* 1993; Su *et al.* 1993). No sequences obviously homologous to these β-catenin-binding motifs can be found in APR-1, suggesting APR-1 may contact β-catenin in a different manner from its vertebrate homolog. Further work will be needed to delineate the regions of APR-1 utilized for interaction with the *C. elegans* β-catenin proteins. Curiously, we did not observe an interaction between APR-1 and WRM-1, even though loss of *apr-1* activity by RNA interference leads to a defect in endoderm specification like that caused by loss of *wrm-1* activity (Rocheleau *et al.* 1997). Perhaps additional factors are required to form a complex between WRM-1 and APR-1, but not between BAR-1 and APR-1. Alternatively, the functional relationship between WRM-1 and APR-1 may be different from that of their vertebrate counterparts.

BAR-1 also interacts with the TCF/LEF homolog POP-1. POP-1 acts in Hox gene regulation and cell fate specification in the progeny of the Q neuroblasts, a Wnt-mediated process that utilizes *bar-1* (Maloof *et al.* 1999; Korswagen *et al.* 2000; Herman 2001). POP-1 functions in a positive manner in this process since expression of a dominant-negative version of POP-1 resulted in a $Q_L$ migration phenotype like that caused by loss of *egl-20 (Wnt)* or *bar-1* (Korswagen *et al.* 2000). In addition, we have found that loss of zygotic *pop-1* function by RNA interference results in defects in vulval formation (S. Peyrot and D. Eisenmann, unpublished results). Together these results suggest POP-1 functions in two postembryonic functions with BAR-1, consistent with their observed interaction in the yeast assay. BAR-1 and POP-1 were recently shown to physically and functionally interact in yeast and in vertebrate culture cells (Korswagen *et al.* 2000). This interaction was dependent on the POP-1 amino terminus, consistent with our yeast two-hybrid result.

We also observed a weak interaction between POP-1 and WRM-1, an interaction that has been reported previously (Rocheleau *et al.* 1999) and that is consistent with their known genetic functions in embryogenesis (Lin *et al.* 1995; Rocheleau *et al.* 1997). We found that the WRM-1-POP-1 interaction was weaker than that of

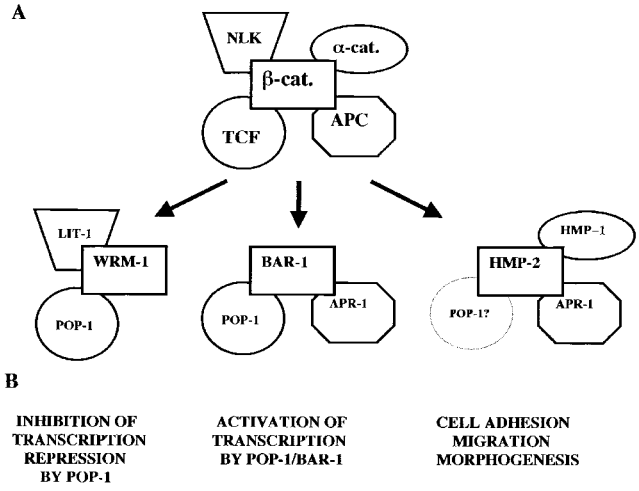


FIGURE 4.—Model. (A) Vertebrate β-catenin is known to make protein-protein interactions with the four proteins indicated (α-catenin, APC, TCF, and NLK). Not all protein interactions of β-catenin are indicated here. The three *C. elegans* β-catenin homologs each display only a subset of the protein interactions displayed by vertebrate β-catenin. A possible HMP-2 and POP-1 interaction, suggested by rescue experiments in *C. elegans*, is indicated. (B) The three *C. elegans* β-catenins may function in distinct ways *in vivo*. WRM-1 functions as a part of a Wnt pathway that acts to disrupt the ability of the TCF homolog POP-1 to repress target genes. In this process WRM-1 serves to bring LIT-1 into the proximity of POP-1 so that LIT-1 can phosphorylate POP-1 and inhibit its function. BAR-1 functions in a canonical Wnt pathway as part of a bipartite transcriptional activator with POP-1. HMP-2 functions in a cadherin/catenin complex with HMP-1 and HMR-1 (not shown) to regulate cell migration and morphogenesis during embryogenesis.

BAR-1 and POP-1 and was not abolished by deletion of the amino-terminal region of POP-1. This result suggests that WRM-1 and BAR-1 may interact with POP-1 in qualitatively different manners. This may be reflected by the observation that in embryonic Wnt signaling mediated by WRM-1, POP-1 acts in a negative manner that is inhibited by Wnt signaling (THORPE *et al.* 1997), whereas, in $Q_L$ migration, POP-1 appears to act in a positive manner to transduce the Wnt signal (KORSWAGEN *et al.* 2000; HERMAN 2001).

Finally, we observed an interaction between WRM-1 and the kinase LIT-1, an interaction that was observed previously (ROCHELEAU *et al.* 1999) and that is consistent with genetic results (ROCHELEAU *et al.* 1997, 1999). We did not observe an interaction between LIT-1 and BAR-1 in the yeast two-hybrid assay, and we have not observed any defects in vulval development in *lit-1* temperature-sensitive mutants raised at the restrictive temperature after early embryogenesis (D. EISENMANN, unpublished results), suggesting that LIT-1 may not function in larval Wnt signaling mediated by BAR-1.

Our model (Figure 4) envisions that an ancestral β-catenin gene encoding a protein functioning in both adhesion and Wnt signaling was duplicated twice in *C. elegans* to generate the *hmp-2, bar-1*, and *wrm-1* loci. We hypothesize that over time, HMP-2, WRM-1, and BAR-1 may each have retained only a subset of the protein interactions of the original β-catenin and that each *C. elegans* protein may now predominantly perform only one function of β-catenin. Specifically, HMP-2 may now function only in a cadherin-catenin complex, while BAR-1 and WRM-1 function only in Wnt signaling. Further, BAR-1 appears to function as a canonical β-catenin in Wnt signaling, cooperating with a TCF/LEF transcription factor to transduce Wnt signal and activate expression of target genes, while WRM-1 appears to act in a noncanonical fashion, cooperating with LIT-1 to derepress target gene expression by inhibiting the ability of POP-1 to bind DNA and repress transcription. However, although the three *C. elegans* proteins diverged a fair amount in sequence from each and other β-catenins, they must all still retain the Wnt signaling function of BAR-1 at some level, as both WRM-1 and HMP-2 can partially provide BAR-1 activity to *bar-1* null mutants when expressed at high levels. A similar case of proteins showing distinct genetic activities but displaying functional redundancy has been demonstrated with the yeast flocculin gene products (GUO *et al.* 2000). These structurally related but diverged proteins function in different cellular processes and display distinct mutant phenotypes when inactivated, suggesting that they perform specific functions. However, when their levels and subcellular localization are altered by expression from each other's promoters, underlying functional redundancy is uncovered.

KORSWAGEN *et al.* (2000) reached similar conclusions about the segregation of β-catenin function and protein interactions among the three *C. elegans* family members. They found (1) that HMP-2 interacted with the cadherin homolog HMR-1, but neither BAR-1 nor WRM-1 did; and (2) that BAR-1 interacted with POP-1, but neither WRM-1 nor HMP-2 did. They did not observe the weak interaction between WRM-1 and POP-1 that we observed, nor did they detect an interaction of any of the β-catenin homologs with APR-1. The explanation for these different observations is unclear, but it is possible that differences in strains or conditions for the yeast two-hybrid assay could account for them.

Our results and those of KORSWAGEN *et al.* (2000) clearly suggest that the three *C. elegans* β-catenin homologs make different protein interactions, consistent with a model of segregation of β-catenin functions. However, these results are not consistent with the result that both WRM-1 and HMP-2 can partially rescue the *bar-1* vulval mutant phenotype. Specifically, we propose that BAR-1 functions in a canonical Wnt signaling pathway where it interacts with the TCF/LEF homolog POP-1 and activates expression of target genes, and genetic evidence supports this idea (KORSWAGEN *et al.* 2000; HERMAN 2001). Since WRM-1 normally interacts with POP-1 to transduce a Wnt signal, the ability of WRM-1 to substi-

tute for BAR-1 is not completely surprising. Perhaps in the absence of LIT-1, WRM-1 functions more like a canonical β-catenin. However, the HMP-2 result is surprising, since we did not see an interaction between POP-1 and HMP-2 in yeast, an interaction that presumably would be required for HMP-2 to substitute for BAR-1 *in vivo*. How can these results be reconciled?

Several explanations are possible. First, the interaction between HMP-2 and POP-1 might be sufficiently weak that it is not detected in the yeast assay, but it can still allow HMP-2 to interact with POP-1 when HMP-2 is overexpressed. It is clear that expression of BAR-1 from its own promoter on an extrachromosomal array leads to higher BAR-1 levels than normal, since an antibody to BAR-1 does not stain wild-type worms but does stain worms containing the *bar-1* gene on such an array (Eisenmann *et al.* 1998). If HMP-2 could interact with POP-1, even weakly, when expressed at high levels, presumably the transcription activation function we demonstrated in HMP-2 could then allow HMP-2 to functionally replace BAR-1. Consistent with the idea that overexpression of HMP-2 is required, a 10-fold lower concentration of *hmp-2* no longer rescued the *bar-1* phenotype. Also consistent with the suggestion that HMP-2 may retain the ability to interact with POP-1 *in vivo,* we found that five of five amino acids shown to be required for the interaction of β-catenin and LEF-1 are conserved in BAR-1 and HMP-2 (residues N426, K435, R469, H470, and K508 in β-catenin; von Kries *et al.* 2000). A second possibility is that HMP-2 might interact with a different TCF homolog in *C. elegans*, allowing it to substitute for BAR-1 without interacting with POP-1. *C. elegans* contains a more distantly related TCF homolog, SON-1 (Jiang and Sternberg 1999). *son-1* mutants do not have a vulval phenotype like that of *bar-1* mutants; however, *son-1* is expressed in all somatic cells throughout development, so it is possible that overexpression of HMP-2 in the vulval precursor cells could drive an interaction. Finally, it is possible that HMP-2 retains the ability to interact with POP-1 but requires an additional factor to do so that is not found in yeast or vertebrate tissue culture cells, so the interaction would be missed in our experiments and those of Korswagen *et al.* (2000).

If HMP-2 and WRM-1 can function in a manner analogous to BAR-1 *in vivo*, it raises the possibility that they do so normally during vulval development and that perhaps not all Wnt signaling activity in the VPCs is mediated via BAR-1. However, we found that loss of zygotic activity of *hmp-2* by dsRNA-mediated interference does not cause an obvious defect in vulval induction or any other postembryonic process nor does it enhance the penetrance of the *bar-1(ga80)* phenotype (S. Peyrot and D. Eisenmann, unpublished results). Also, while loss of *wrm-1* zygotic function does cause a defect in vulval development, the defect is different from that caused by loss of *bar-1* function and may be a secondary consequence of defects in somatic gonad development in these animals (S. Peyrot and D. Eisenmann, unpublished results). To date, there is no evidence that either WRM-1 or HMP-2 functions in wild-type vulval development in a manner similar to that of BAR-1. Therefore, although WRM-1 and HMP-2 can provide BAR-1 function *in vivo*, they may not normally function in such a manner in wild-type animals. Therefore, this result, combined with the different mutant phenotypes of *wrm-1*, *bar-1*, and *hmp-2* mutants and the distinct protein interactions seen in the yeast two-hybrid analysis, suggests that the three *C. elegans* retain some functional redundancy despite their sequence divergence but that each protein may predominantly carry out only one of the functions of vertebrate or Drosophila β-catenin.

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