Direct Inhibition of Pumilio Activity by Bam and Bgcn in Drosophila Germ Line Stem Cell Differentiation*

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The fate of stem cells is intricately regulated by numerous extrinsic and intrinsic factors that promote maintenance or differentiation. The RNA-binding translational repressor Pumilio (Pum) in conjunction with Nanos (Nos) is required for self-renewal, whereas Bam (bag-of-marbles) and Bgcn (benign gonial neoplasm) promote differentiation of germ line stem cells in the Drosophila ovary. Genetic analysis suggests that Bam and Bgcn antagonize Pum/Nos function to promote differentiation; however, the molecular basis of this epistatic relationship is currently unknown. Here, we show that Bam and Bgcn inhibit Pum function through direct binding. We identified a ternary complex involving Bam, Bgcn, and Pum in which Bam, but not Bgcn, directly interacts with Pum, and this interaction is greatly increased by the presence of Bgcn. In a heterologous reporter assay to monitor Pum activity, Bam, but not Bgcn, inhibits Pum activity. Notably, the N-terminal region of Pum, which lacks the C-terminal RNA-binding Puf domain, mediates both the ternary protein interaction and the Bam inhibition of Pum function. These studies suggest that, in cystoblasts, Bam and Bgcn may directly inhibit Pum/Nos activity to promote differentiation of germ line stem cells.

Stem cells divide asymmetrically to yield a self-renewed daughter stem cell as well as a differentiated daughter cell. Molecular mechanisms underlying asymmetric stem cell division have been largely unearthed from studies of Drosophila germ line stem cells, which generate an unlimited number of germ cells, either eggs or sperm (1, 2). Germ line stem cells are located in the gerarium at the anterior tip of the ovariole and are connected to the surrounding somatic niche cells via adherens junctions (3). When a stem cell divides, one cell in contact with the niche remains as a stem cell, whereas the more distant cell becomes a differentiated cell or cystoblast, which further divides four times with incomplete cytokinesis to give rise to a cyst containing 16 germ cells (4).

Two important intrinsic factors, Bam (bag-of-marbles) and Bgcn (benign gonial neoplasm), play critical roles in stem cell differentiation (5, 6). Loss-of-function mutations in either Bam or Bgcn cause stem cell differentiation to arrest (5–7).

Conversely, ectopic expression of Bam in stem cells overrides stem cell self-renewal capabilities and promotes differentiation (8). Genetic analyses have shown that Bam and Bgcn require each other for function. Bgcn is present in stem cells as well as cystoblasts and early mitotic cysts (6–9), whereas Bam is not expressed in stem cells but is expressed in cystoblasts and early mitotic cysts (9–12). Bam silencing in stem cells is governed by the BMP2/4 homolog decapentaplegic signal emanating from the niche cells (13–15).

In addition to the extrinsic factors emanating from niche cells, stem cell maintenance requires intrinsic stem cell factors. Pumilio (Pum)2 and Nanos (Nos) are such intrinsic factors (16–19). Pum is an RNA-binding protein with a C-terminal Puf (Pum and Fem3-binding factor) domain, which binds the Nos response element (NRE) sequences at the 3′-untranslated region of its target mRNAs (20–23). Binding of the Puf domain to Nos recruits Nos to this complex, resulting in the repression of the translation of the target mRNAs (20, 24). Because Pum and Nos are required for repression of differentiation in germ line stem cells (17, 18), it is conceivable that this complex targets a suite of genes that are required for differentiation, although the identities of these genes are unknown.

Genetic epistasis analysis of double mutants of Bam and Pum indicated that Bam antagonizes Pum function to promote differentiation of stem cells (10, 12). For the differentiating cystoblasts to begin differentiation, the Pum/Nos activity must be inhibited in the cystoblast. We explored the possibility that the Bam-Bgcn complex may inhibit Pum-Nos activity at the protein level and discovered a direct interaction between Bam and Pum. Notably, the Bam-Pum interaction is greatly increased in the presence of Bgcn, and this interaction allows for the formation of a strong ternary complex involving Bam, Bgcn, and Pum. Consistent with this physical interaction, Bam inhibits Pum activity in a heterologous reporter assay, which monitors the activity of Pum. On the other hand, no ternary interaction between Bam, Bgcn, and Nos was detected, suggesting that Bam and Bgcn specifically target Pum directly to negatively regulate Pum/Nos activity and promote stem cell differentiation.

EXPERIMENTAL PROCEDURES

Anti-Pum Antibodies—Peptides (795PRPLpTPSQQ807, labeled as PumT803, and 978LGAP1pTPPPS984, labeled as PumT803, and 978LGAP1pTPPPS984, labeled as

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2 The abbreviations used are: Pum, Pumilio; NRE, Nanos response element; X-gal, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside; Nos, Nanos; HA, hemagglutinin; FCA, fragment complementation analysis; TAD, transcription activation domain; GFP, green fluorescent protein.
Bam Binding to Pum

PumT980 were synthesized and conjugated to the keyhole limpet hemocyanin carrier protein and immunized to rabbits by four injections at 3-week intervals. The antibodies were purified by affinity chromatography on a peptide-conjugated column. In brief, 2 mg of peptide was coupled to resin for affinity purification and packed in a column. 5 ml of the rabbit sera were loaded onto the column. The bound antibody was eluted.

Co-immunoprecipitation Assay—FLAG (5'-ATGGATTA-CAAGGATGACAGGATAAG-3'), HA (5'-ATGGCCCTC-CTACCCCTTAGTGTGCGAGATTATGCCTTCCC-3'), and Myc (5'-ATGGAGCGAAGAATCCTGATAGAGGAGGATCTG-3') tags were cloned into the Kpnl/EcoRI sites of pAc5.1/V5-His A vector (Invitrogen), generating pAc5.1-FLAG, pAc5.1-HA, and pAC5.1-Myc, respectively. The BAG, BGCN, and PUM coding sequences were cloned into the BamHI/XhoI sites of pAc5.1-FLAG, EcoRI/XhoI sites of pAc5.1-HA, and BamH/XhoI sites of pAc5.1-Myc, respectively.

Drosophila S2 cells were maintained in Schneider’s medium without serum on the day prior to transfection. The cells were transiently transfected with the appropriate set of expression plasmids using LipofectamineTM 2000 (Invitrogen) according to the manufacturer’s instructions. Forty-eight hours after transfection, the cells were washed in phosphate-buffered saline and lysed with radioimmune precipitation assay buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% Nonidet P-40, 5 mM EDTA, and 1 mM phenylmethylsulfonfyl fluoride; ELPLIS-Biotech. Inc.) containing protease inhibitor mixture. The lysates were clarified by centrifugation at 13,000 rpm (Eppendorf centrifuge) for 10 min at 4 °C. The cleared extracts (1–3 mg) were precipitated by Eppendorf PumT980 antibody, or control IgG were added and incubated at 4 °C overnight. The beads were precipitated by Eppendorf centrifugation and washed three times with 20 mM HEPES (pH 7.4) and 1 mM dithiothreitol containing protease inhibitor mixture. The bound proteins were eluted using 0.1M glycine–trifluoroacetic acid. The pellets were resuspended in 200 μl of anti-FLAG M2-conjugated agarose beads (Sigma) and rotated at 4 °C overnight. For antibody interference assay, 6 μg of anti-PumT803 antibody, 6 μg of anti-PumT980 antibody, or control IgG were added and incubated at 4 °C overnight. The beads were precipitated by Eppendorf centrifugation and washed three times with 20 mM HEPES (pH 7.7), 150 mM NaCl, 2.5 mM MgCl2, 0.05% Nonidet P-40, 10% glycerol, and 1 mM dithiothreitol containing protease inhibitor mixture. The bound proteins were eluted using 0.1 M glycine-acetate (pH 3.0), and the eluates were precipitated with the trichloroacetic acid. The pellets were resuspended in 2× SDS loading buffer, and Western blot analysis was performed using anti-HA (Roche Applied Science), anti-Nos (gift from R. P. Wharton), anti-Pum 1637 (gift from P. M. Macdonald), anti-Myc (Cell Signaling), and anti-FLAG (M2; Sigma) antibodies. The band intensity of the Western blot analysis was quantified with the Scion Image program (Scion Corp., Frederick, MD) (25).

Yeast Two- and Three-Hybrid Assays—The full-length sequences of Bam, Pum, and BgcN and their derivatives were cloned into the BamHI/Xhol sites of pLEXa (Clontech), the EcoRI/Xhol sites of pB42 (Clontech), and the BamHI/Xhol sites of pRS325GU, which is a modified version of the pRS 325 vector (26) such that the gal1 and ara3 promoters are inserted in the flanking multi-cloning site, thus allowing expression of genes either constitutively or under galactose induction. A combination of plasmids including plasmids expressing the LexA-fused protein, B42-fused protein, NRE-expressing vector pII/NRE/MS2 (NRE +) or its NRE mutant (NRE −), BgcN-expressing vector pRS325URA-BgcN, and pRS325URA vector alone, were co-transformed into yeast strain YPH500 (MATa, ade2, his3, leu2, lys2, trpl, ura3) harboring the pSH18–34 plasmid (lexAop–LacZ reporter) (27) by the standard lithium acetate method (28). Independent transformants were patched either onto galactose plates to activate the galactose-inducible promoter of the B42 vector or onto glucose plates containing X-gal. Pictures were taken 48 h after incubation.

Protein Fragment Complementation Analysis (FCA)—A Bam sequence fragment was cloned into the BamHI/Xhol sites of pcDNA3 (Invitrogen) and pshKGN-MN vector (CoralHue® Fluo-chase kit; Medical and Biological Lab Co.). A Pum fragment was cloned into the EcoRI/Xhol sites of pcDNA3 (Invitrogen) and the EcoRI/Sall sites of the pshKGC-MN vector. A BgcN fragment was cloned into the pcDNA3 vector (Invitrogen) using the NotI/Xhol sites. P50-mKG_N and P65-mKG_C were provided with the CoralHue® Fluo-chase kit as control plasmids.

The N- or C-terminal fragment of the monomeric Kusabira-Green (mKG) was fused to either Bam, BgcN, or Pum. To examine the binary protein interaction, HEK 293 cells were transiently expressed with the mKG_N and mKG_C fusion proteins in all combinations. To examine the ternary protein interactions, Bam and Pum fusion proteins were co-expressed with intact BgcN. For imaging, the cells were plated on gelatin-coated glass coverslips and then allowed to grow in medium. Green fluorescence signals were examined 24 h after transfection. GFP signals from either p50-mKG_N or p65-mKG_C alone or from the combination of these two were used as negative and positive controls, respectively. For fluorescence microscopy, the cells were grown on gelatin-coated glass slides in 6-well plates. Approximately 24 h after transfection, the cells were washed twice with phosphate-buffered saline and incubated with 3.7% formaldehyde for 10 min. After incubation, the cells were washed with phosphate-buffered saline and mounted on microscope slides. Images of cells were acquired using a fluorescence microscope. Representative images of single optical sections are shown.

Luciferase Reporter Assay—For the construction of pcDNA3-LUC/NRE, the luciferase gene was obtained from the pGL3-Basic vector (Promega) by PCR and cloned into the HindIII/BamHI sites of pcDNA3 (Invitrogen), generating pcDNA3-LUC. The hunchback NRE fragment (5'-ATTATTTTGTGGTCCAAAATTGTACATAAGCCGAATTC-3') was generated by oligomer dimerization and inserted into the BamHI/Xhol sites of pcDNA3-LUC. All of the constructs were verified by DNA sequencing. HEK 293 cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum for 24 h and then transiently transfected with the appropriate set of reporter and expression plasmids using SuperFect reagent (Qiagen). The plasmid DNA used for transfection included the pcDNA3-LUC/NRE reporter (100 ng/well) and the pSV-β-gal plasmid, pcDNA3-Bam, pcDNA3-BgcN,
pcDNA3-Puf, or pcDNA3-Pum (200 ng/well). For the reporter assays, the cells were harvested 24 h after transfection and assayed for luciferase activity as described previously (29). Luciferase activities were measured as described (30). The results from triplicate samples were averaged and normalized to *LacZ* expression from pSV-β-gal to account for transfection efficiency.

**RESULTS**

**Bam, Bgcn, and Pum Form a Ternary Complex**—To examine direct interactions between Bam, Bgcn, Pum, and Nos, we performed a yeast two-hybrid assay where either Bam, Bgcn, Pum, or Nos was fused to either the LexA DNA-binding domain or the B42 transcription activation domain (TAD). No Bam-Bgcn interaction was detected with this assay (Table 1). In addition, no direct interaction between Bgcn and Pum was found (Table 1). However, a very weak interaction was detected between Bam and Pum; no blue colors appeared in 48 h of incubation of yeast patches on X-gal plates (Fig. 1B), but a faint blue color appeared in a longer incubation (Table 1). Importantly, a strong ternary interaction involving Bam, Bgcn, and Pum was detected (Fig. 1B and Table 1). The N-terminal region of Pum, but not the C-terminal Puf domain, mediates the ternary complex formation (Fig. 1 and Table 1). Interestingly, neither a binary complex involving Bam or Bgcn and Nos nor a ternary complex involving Bam, Bgcn, and Nos was detected (Fig. 1 and Table 1). On the contrary, an interaction between the C-terminal Puf domain and Nos was detected in the presence of the NRE sequence (Table 1), which is consistent with previous observations that the C-terminal Puf domain mediates Pum binding to Nos in an NRE-dependent manner (24).

To verify these observed interactions, we used the protein FCA to detect *in situ* protein-protein interactions via a fluorescent signal in mammalian HEK 293 cells. In this assay, when a fusion protein harboring the N-terminal region of the monomeric Kusabira-Green (designated as mKG_N) and another fusion protein containing the C-terminal region of the fluorescent protein (mKG_C) form a complex, the two fragments of mKG are in close proximity and thereby emit a green fluorescence signal (31). Bam, Bgcn, and Pum were fused to either the N or C terminus of mKG. Individual expression of the Bam, Bgcn, or Pum fusion proteins yielded no fluorescence (Fig. 2A). In addition, binary expression of the Bam and Bgcn or Bgcn and Pum fusion proteins failed to produce a fluorescent signal; however, co-expression of the Bam and Pum fusion proteins yielded weak fluorescence in a few cells (Fig. 2A). This signal was

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**TABLE 1**

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<tr>
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<th>Bam</th>
<th>Bgcn</th>
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<th>Puf + Bgcn</th>
<th>Bam + Puf + Bgcn</th>
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<tr>
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**FIGURE 1. A ternary complex involving Bam, Bgcn, and Pum.** A yeast three-hybrid assay revealed a ternary complex involving Bam, Bgcn, and Pum. A, a schematic diagram depicting a yeast three-hybrid assay where Bam is fused to the LexA DNA-binding domain (DBD), Pum or its derivatives (Puf or the N terminus of Pum (Pum_N)) is fused to the B42 TAD, Nos is fused to the Gal4 TAD, and Bgcn is added in a non-fusion form. Interaction was monitored with LacZ expression. B, left panel, schematic diagrams of Pum, the C-terminal Puf domain, and the N-terminal region lacking the Puf domain used for yeast three-hybrid assay. Right panel, yeast patches yielded a blue color on X-gal plates only when Bgcn was co-expressed with Bam and Pum or the N terminus of Pum. Co-expression of Bam with either Nos or Puf did not result in a blue color with or without Bgcn.

**FIGURE 2. A weak Bam-Pum interaction and a strong ternary complex involving Bam, Bgcn, and Pum.** A, Bam and Pum were fused to the mKG_N and mKG_C fragments of the monomeric Kusabira-Green, respectively (Bam-mKG_N and Pum-mKG_C), which alone do not yield fluorescence (left panel). Interaction between these proteins yielded fluorescent green signal in the cytoplasm in the absence of Bgcn, but co-expression of intact Bgcn with these Bam and Pum constructs resulted in a great increase in signal (center panels). The cells expressing the control p50-mKG_N and p65-mKG_C plasmids yielded fluorescence in the nucleus (right panel). B, cells emitting GFP signals after transfection were counted. The number of GFP-positive cells co-expressing Bam-mKG_N and Pum-mKG_C was compared with the number of GFP-positive cells co-expressing Bam-mKG_N, Bgcn, and Pum-mKG_C, which is normalized to 100%.
detected in the cytosol, which is consistent with the fact that Bam and Pum are subcellularly located in cytoplasm (8, 9, 19, 32), whereas the signal generated by the control proteins NF-κB subunit, p50, and p65 occurred in the nucleus (Fig. 2 and Table 2). Notably, when intact Bgcn was co-expressed together with the Bam and Pum fusion, the signal was greatly increased and was observed in most cells (Fig. 2). This result is in agreement with the data from the yeast two- and three-hybrid assays.

To corroborate further the results from the yeast three-hybrid system and FCA assay, we performed co-immunoprecipitation experiments with FLAG-tagged Bam, Myc-tagged Bgcn, and HA-tagged Pum, which were expressed in various combinations in Drosophila S2 cells. FLAG-tagged Bam was immunoprecipitated with anti-FLAG antibody conjugated to agarose beads. Western blot analysis of the immunoprecipitates revealed that HA-Pum, endogenous Pum, Myc-Bgcn, and endogenous Nos were co-immunoprecipitated (Fig. 3). This result suggests that a complex including Bam, Bgcn, Pum, and Nos exists in S2 cells.

To examine whether Bam can precipitate Bgcn under conditions where the Bam interaction with Pum is disrupted, we generated affinity-purified peptide-specific antibodies against the Pum region that mediates the Bam interaction. The antibodies immunoprecipitated endogenous Pum (Fig. 4A) and were able to detect Pum by immunohistochemistry (data not shown).

We added the Pum antibodies to the S2 cell lysates containing endogenous Pum, as well as ectopically expressed Myc-Bgcn and FLAG-Bam. The Pum antibodies, but not the control antibodies (IgG), blocked Bam precipitation of Pum (Fig. 4B, lane 5), suggesting that the Pum antibodies interfered with the Bam-Pum interaction in S2 cell lysates. Intriguingly, under these conditions, Bam precipitated Bgcn (Fig. 4B, lane 5), dem-

### TABLE 2

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<th>mKG_N/mKG_C</th>
<th>Pum</th>
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<th>Bgcn</th>
<th>Bam + Bgcn</th>
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<td>+</td>
<td>ND</td>
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<td>ND</td>
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<td>Bgcn</td>
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**FIGURE 3.** A protein complex containing Bam, Bgcn, Pum, and Nos. Lysates from S2 cells expressing a combination of FLAG-tagged Bam, Myc-tagged Bgcn, and HA-tagged Pum were subjected to Western blot either directly (lanes 1–4) or after being immunoprecipitated (IP) via anti-FLAG-conjugated agarose beads (lanes 5–8) with specific antibodies as indicated.

**FIGURE 4.** Bam and Bgcn interact without Pum. A, diagram showing the regions (underlined) used to generate anti-PumT803 and anti-PumT980 antibodies. The Puf domain is indicated by a gray box. Lysates from S2 cells were immunoprecipitated (IP) with the combined anti-PumT803 and anti-PumT980 antibodies and then analyzed by Western blot with anti-Pum1637 (37). As a control, unrelated IgG was used. B, lysates from S2 cells expressing FLAG-tagged Bam and Myc-tagged Bgcn were subjected to Western blot analysis either directly (lanes 1 and 2) or after being precipitated with anti-FLAG M2 antibody-conjugated agarose beads. C, quantification of Pum and Bgcn precipitated by Bam. The Western blot bands (lanes 4 and 5 in Fig. 4B) were quantified using the Scion Image program (25). Precipitated protein levels were compared between preincubation (+) and no preincubation (−) with anti-pum antibodies, which were normalized to 100%. The results are presented as the means ± S.E. obtained from three independent experiments.
Inhibition of Pum Activity by Bam in Heterologous Cells—To examine the physiological relevance of Bam binding to Pum, we established a functional assay system for Pum activity in heterologous cells. Because Pum is a translational repressor via binding to the NRE sequence of its target mRNAs (20–22), we constructed a luciferase reporter in which the NRE sequence of hunchback is incorporated into the 3′-untranslated region of the luciferase gene (LUC/NRE, Fig. 5A). Upon Pum expression, luciferase expression was greatly reduced, whereas luciferase expression was not affected by expression of either Bam or Bgcn (Fig. 5B). To corroborate that Pum represses translation through NRE, we introduced a mutation into the NRE sequence of the reporter (LUC/NREmt) (Fig. 5A). Indeed, Pum failed to repress luciferase expression in the presence of this mutation (Fig. 5D), indicating that Pum functions through the NRE sequence. We next examined whether Bam inhibits Pum function. Indeed, Pum repression of luciferase expression is fully abrogated by Bam co-expression (Fig. 5C). By contrast, Pum repression was not affected by Bgcn co-expression (Fig. 5C).

In our assay, the C-terminal Puf domain repressed luciferase expression through the NRE sequence (Fig. 5D). This finding is consistent with the previous observation that the Puf domain alone represses gene expression in a NRE-dependent manner in vivo (20). Thus, we tested the significance of Bam binding to Pum by taking advantage of the fact that Puf does not contain a Bam-binding site (Fig. 1). Indeed, the Puf-dependent repression was not perturbed by Bam (Fig. 5D), indicating that Bam inhibition of Pum requires Bam binding to the N-terminal region of Pum.

DISCUSSION

Previous genetic analysis suggested that Bam and Bgcn form a complex because they require each other for function (7, 8). We therefore utilized diverse assays to probe the biochemical relevance of these genetic results. Surprisingly, both the FCA and the yeast two-hybrid assay failed to detect any interaction...
between Bam and Bgcn. However, the two assays detected a strong Bam-Bgcn-Pum complex. In contrast, the co-immunoprecipitation assay detected direct Bam-Bgcn interaction without Pum involvement, which is in accord with other recent reports (33, 34). The inability to detect direct Bam-Bgcn interaction by the FCA and the yeast two-hybrid assay may indicate that Bam-Bgcn interaction is weak in vivo.

We found that via both yeast two-hybrid and FCA, there is a weak interaction between Bam and Pum. Particularly, the interaction revealed by FCA appears authentic because the Bam-Pum interaction between Bam and Pum. The inability to detect direct Bam-Bgcn interaction with-out Pum involvement, which is in accord with other recent reports (33, 34). The inability to detect direct Bam-Bgcn interaction by the FCA and the yeast two-hybrid assay may indicate that Bam-Bgcn interaction is weak in vivo.

The ternary interaction involving Bam and Bgcn is mediated by the N terminus of Pum, which lacks the C-terminal Puf region. Consistent with this, the Puf region fails to form a ternary complex formation with Bam and Bgcn. It is known that the Puf domain mediates both NRE binding and Nos binding of Pum (24). The binding of Bam and Bgcn to the N-terminal region of Pum appears not to interfere with the binding of Nos to the Puf region, because Bam immunoprecipitates contained Bgcn, Pum, and Nos. Neither Bam nor Bgcn binds to Nos, and a ternary complex involving Bam, Bgcn, and Nos was not observed. Therefore, these results indicate that Pum can recruit both Bam/Bgcn and Nos in distinct sites and thus can account for the fact that Bam precipitates contain Bgcn, Pum, and Nos.

Using a luciferase reporter system involving the NRE sequence at the 3’- untranslated region, we addressed the relevance of Bam/Bgcn binding to Pum activity in heterologous cells. Expression of Pum repressed luciferase expression, which requires an intact NRE sequence. Bam was able to abrogate this repression by Pum, suggesting that a weak interaction between Bam and Pum is sufficient for Bam inhibition of Pum activity in this assay. The Bam inhibition of Pum function appears to require Bam binding to Pum, because Bam does not bind to Puf and failed to abrogate Puf-dependent repression. Bgcn failed to interact with Pum or affect Pum repression of the reporter gene expression (Fig. 5A).

These results yield insight into the role of Bgcn in vivo and suggest that Bgcn may be confined to facilitating Bam binding to Pum under physiological conditions where Bam protein levels may not be sufficient for the binding and inhibition of Pum.

In conclusion, following stem cell division, one daughter cell moves away from the niche cells and begins to initiate differentiation as a cystoblast. For the cystoblast to begin differentiation, Pum/Nos activity must be inhibited in the cystoblast and early dividing germ cells. One possible mechanism for this inhibition is the decrease of Pum and Nos at the protein level. In fact, these levels are gradually reduced in the cystoblasts and immediate early dividing cysts; however, not all Pum and Nos protein disappears (17–19, 35, 36). Thus, other mechanisms must exist to inhibit Pum/Nos activity in the differentiating cells. Our data suggest that Bam and Bgcn present in the cysto-blast cells (6, 8–10) play such a role by binding and inhibiting Pum directly at the protein level (Fig. 6). This notion is consistent with findings that ectopic Bam expression in stem cells triggers stem cell differentiation (8), which might occur because of direct Bam/Bgcn inhibition of Pum/Nos activity.

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