The Drosophila Cystoblast Differentiation Factor, benign gonial cell neoplasm, Is Related to DExH-box Proteins and Interacts Genetically With bag-of-marbles

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ABSTRACT

Selection of asymmetric cell fates can involve both intrinsic and extrinsic factors. Previously we have identified the bag-of-marbles (bam) gene as an intrinsic factor for cystoblast fate in Drosophila germline cells and shown that it requires active product from the benign gonial cell neoplasm (bgcn) gene. Here we present the cloning and characterization of bgcn. The predicted Bgcn protein is related to the DExH-box family of RNA-dependent helicases but lacks critical residues for ATPase and helicase functions. Expression of the bgcn gene is extremely limited in ovaries but, significantly, bgcn mRNA is expressed in a very limited number of germline cells, including the stem cells. Also, mutations in bgcn dominantly enhance a bam mutant phenotype, further corroborating the interdependence of these two genes’ functions. On the basis of known functions of DExH-box proteins, we propose that Bgcn and Bam may be involved in regulating translational events that are necessary for activation of the cystoblast differentiation program.

ASYMMETRIC stem cell divisions produce a new daughter stem cell and a second daughter that will undergo specialized differentiation (Morrison et al. 1997). The germline stem cells (GSCs) of Drosophila are an excellent model system for the study of stem cell biology (de Cuevas et al. 1997; Lin 1998). When oogenesis begins in Drosophila third instar larvae, GSC division produces another GSC and a cystoblast that will divide precisely four times with incomplete cytokinesis to form the syncytial germline cyst (Brown and King 1964; Wieschaus and Szabad 1979; Lin and Spradling 1993). Topics under active investigation include identifying factors that are essential to establish/maintain stem cells and determining the changes that take place in differentiating non-stem-cell daughters. Studies from several laboratories have shown that both extrinsic and intrinsic factors are necessary for GSC and cystoblast formation (McKearin 1997; Xie and Spradling 1998; King and Lin 1999).

Establishing and maintaining oogenic GSCs requires the transforming growth factor β (TGFβ)-like signal Decapentaplegic (Dpp) since mutations in Dpp receptors or signal transducing Smad transcription factors cause stem cell loss (Xie and Spradling 1998). Likewise, inactivating mutations of the piwi gene eliminate GSCs (Cox et al. 1998). Thus, piwi-dependent signaling and Dpp signaling are implicated in maintaining GSCs. In the absence of proper signals, GSCs apparently divide as cystoblasts, thus ablating a self-renewing population of stem cells for germline replenishment.

Intrinsic GSC maintenance factors include Pumilio (Pum) and perhaps Nanos (Nos; Forbes and Lehmann 1998) that were initially studied as key regulators of embryonic polarity (St Johnston and Nüsslein-Volhard 1992). Biochemical and molecular studies have shown that Pum and Nos are translational repressors of transcripts that carry a sequence-specific element known as the NRE (Curtis et al. 1997; Zamore et al. 1997; Wharton et al. 1998). Subsequent work, using strong or complete loss-of-function nos and pum alleles, established that proper germ cell cyst development required both genes (Wang et al. 1994; Lin and Spradling 1997; Forbes and Lehmann 1998). For example, GSCs carrying inactivating pum mutations divided symmetrically and produced only apparent cystoblasts; nos inactivation produced a range of phenotypes including the failure to establish GSCs and defective cyst formation. If Nos and Pum functions are the same in embryos and GSCs, we can infer that establishing and maintaining GSC identity requires translational repression of specific RNA targets. Presently, the germ cell targets of such regulation are unknown.

Studies focused on how asymmetric division produces the cystoblast have identified two intrinsic factors, bag-of-marbles (bam; McKearin and Spradling 1990) and benign gonial cell neoplasm (bgcn; Gateff 1982; Lavoie et al. 1999). Mutations in either gene produce tumorous egg chambers that are caused by symmetric GSC divisions that produce only more GSCs (McKearin and Spradling 1990; Gateff 1994; Lavoie et al. 1999). Misexpression of Bam is sufficient to eliminate GSCs, appar-
ently by converting them into cystoblasts, which is remi- niscent of the phenotypes produced by abrogation of Dpp or piwi-dependent signaling (Ohlstein and McKearin 1997). Bam is a novel protein and is a compo- nent of the germ-cell-specific fusome organelle where it is required for proper formation of the endoplasmic reticulum (ER)-like fusome cisternae (McKearin and Ohlstein 1995; León and McKearin 1999). However, the low abundance of the Bam protein and its novelty has frustrated efforts to discover its biochemical function.

Previously, we have shown that Bgcn is an essential cystoblast differentiation factor that is required for Bam function and can regulate Bam localization (Lavoie et al. 1999). Mutations that inactivated the bgcn gene blocked the ability of misexpressed Bam to eliminate GSCs, implying that Bgcn was essential for Bam to exert its cystoblast-converting effect on wild-type GSCs. We also noted that Bam fusome localization was blocked by a strong bgcn allele. On the basis of these findings, we concluded that Bam and Bgcn action was interdepen- dent (Lavoie et al. 1999). Furthermore, we postulated that bgcn may act permissively in wild-type GSCs since Bam misexpression converted GSCs into apparent cystoblasts (Ohlstein and McKearin 1997). We have therefore cloned the bgcn gene to extend our studies of factors controlling cystoblast differentiation. Bgcn is a large protein related to the DEXH-box ATP-dependent RNA helicase proteins (Aubourg et al. 1999; de la Cruz et al. 1999) although Bgcn lacks signature motifs essential for ATP binding and unwinding activity (Gorbalenya and Koonin 1993; de la Cruz et al. 1999). We speculate instead that Bgcn may share RNA-binding activity with proteins of the DExH family. Consistent with its genetic role as a highly specific regulator of cystoblast formation, bgcn transcripts are restricted to a small num- ber of cells including GSCs. The activity of bgcn as a dominant enhancer of bam phenotype provides addi- tional evidence that Bam and Bgcn function interdepen- dently.

MATERIALS AND METHODS

Drosophila stocks and culture: Flies were maintained on standard molasses agar media as described in Ashburner (1989). Stocks carrying chromosomal deficiencies were ob- tained from the Drosophila Stock Centers at Bloomington and Umea.

Chromosomes carrying deficiencies of the 60A region were used to map the position of the bgcn gene. The distal breakpoint for Df(2R)OVI1 is in chromosomal locus 60A1 while the distal breakpoint of Df(2R)b23 extends an additional 15 kb (Wharton et al. 1999). Appropriate matings were estab- lished to show that Df(2R)OVI complements bgcn while Df(2R)b23 fails to complement.

Sequencing the bam(BW) allele and candidate genes from bgcn alleles: DNA corresponding to candidate genes was recov- ered from wild-type genomes and mutant bgcn alleles by PCR and the products were sequenced at the Department of Molecu- lar Biology Sequencing Facility at U.T. Southwestern. The products from at least four parallel PCR reactions were pooled for sequencing to minimize the potential for mistaking PCR errors for in vivo mutations. When these experiments revealed mutations in relevant DNA fragments, the appropriate frag- ment was recovered again from multiple, parallel PCR reac- tions and sequenced to confirm the mutation.

Recovering bgcn candidates from cDNA and genomic DNA libraries: Genomic clones including the bgcn locus were ob- tained as cosmids from the European Genome Consortium. wibg cDNA clones were recovered from an ovarian cDNA li- brary (Stroombakis et al. 1994) and bgcn cDNA clones from a testis cDNA library (gift from T. Hazelrigg) by standard hybridization procedures. The longest bgcn cDNA clone started 19 nucleotides from a predicted initiating Met codon and extended 3886 nucleotides, stopping adjacent to a consen- sus polyA addition signal. The sequence was extended at the 5’ end by RT-PCR to a position that corresponded to ~92 nucleotides from the putative initiating Met. A termination codon in frame with the predicted bgcn open reading frame (ORF) lies at position ~78; the next Met in frame with the longest ORF was selected as the most likely translational start codon and designated position +1.

bgcn alleles: bgcn191a and bgcn191b were obtained from Drs. A. Mahowald and R. Steward; originally they were recovered from EMS screens by Dr. T. Schüpbach (Schüpbach and Wieschaus 1991). Both of these alleles carried the same transition mutation that produced a termination codon. For the purposes of correlating the bgcn gene with a particular segment of genomic DNA, we considered these alleles as nonindepen- dent events.

The P( lacW) transposon (Bier et al. 1989) was mobilized and 19,000 second chromosome insertions were tested for new female sterile or lethal insertions that were not com- plemented by Df(2R)b23. One of these lines failed to comple- ment the bgcn1 allele and was designated bgcn191a.

The bgcn1 allele was recovered from an EMS screen of w; b flies. Alleles bgcn1231a, bgcn1231b, and bgcn1231c were recovered from a mutagenesis screen of cn bw flies for male sterile muta- tions (B. Wakimoto, D. Lindsay, E. Koundakjian and C. Zuker, personal communication).

Germline transformation: Germline transformation was car- ried out essentially as described by Rubin and Spradling (1982). Three different wibg transgenes were constructed and used for germline transformation rescue experiments. Two transgenes utilized a full-length cDNA clone recovered from an ovarian cDNA library (Stroombakis et al. 1994) fused to either a heat-shock-70-gene promoter (pCaSpeR; Thummel and Pirrotta 1991) or an actin gene promoter (pcOG; Robinson and Cooley 1997). The third wibg transgene was con- structed by inserting a 4.2-kb EcoRI fragment of genomic DNA, recovered from cosmids 64H6 (Siden-Kiamo et al. 1990), into pCaSpeR. The bgcn transgene was constructed in cloning vec- tor pCaSpeR-Hs (Pirrotta 1988) using the full-length cDNA described above. Expression of the transgene was induced by 1 hr heat shocks at 37° repeated twice/ day for 2 days. We tested the rescuing activity of the bgcn transgene by inducing expression in newly eclosed P(w+); hs-bgcnl+/−; bgcn1/−bgcn1 animals and examining their ovaries on days 5–8 posteclosion.

Molecular biology: PCR, Northern blots, Southern blots, cloning, etc., were all carried out essentially as described in Sambrook et al. (1989). RNA in situ hybridization was per- formed as described in Christianson and McKearin (1994). Digoxigenin-labeled single-stranded DNA probes were pre- pared by cyclic reactions using a single antisense primer and double-stranded DNA template using reaction conditions de- scribed by the manufacturer (Boehringer Mannheim, India- napolis).
Germline Stem Cell-Cystoblast Differentiation Factors

Figure 1.—bgcn maps to a 20-kbp region at chromosomal position 60A3. Deficiency chromosomes Df(2R)b23 and Df(2R)OV1 were used to map bgcn to a region of ~20 kbp. Position of the gene was further refined by a bgcn P-allele, which failed to complement female sterility when opposite Df(2R)b23. The position of the genomic fragment that was used as a molecular probe to recover cDNAs from the region around the P element inserted in bgcn

RESULTS

Mapping the bgcn locus: Previous meiotic mapping placed bgcn in the 60A region (Lindsley and Zimm 1992). We mapped the bgcn gene onto a 20-kb segment of the 60A1 region using combinations of chromosomal deficiencies placing the bgcn locus between positions shown on the map in Figure 1.

The P-allele bgcn was used to refine the position of the bgcn gene. Chromosomal in situ hybridization showed that bgcn flies (materials and methods) carried a P-element insertion at 60A. Homozygous and hemizygous bgcn flies were male and female sterile and had tumorous gonads like canonical bgcn mutations. Finally, excision of the P element from bgcn restored fertility to both males and females. Thus we concluded that the transposon in bgcn was inserted in or very close to the bgcn gene.

A fragment of genomic DNA adjacent to the P element in bgcn was recovered by plasmid rescue (Pirrotta 1986). When the sequence of the genomic fragment was aligned with the DNA sequence of the 60A1 region (Berkeley Drosophila Genome Project, unpublished result; http://www.fruitfly.org), we found that the transposon insertion site corresponded to the position on the genomic map (Figure 1) that fell within the 20-kb fragment between the Df(2R)OV1 and Df(2R)b23 breakpoints (materials and methods).

bgcn is a complex gene: The genomic fragment recovered by plasmid rescue was used to identify cDNA clones that corresponded to a 1.0-kb transcript, which included the insertion site for the bgcn transposon. However, several results suggested that this transcript did not belong to the bgcn gene. The most compelling data were that a 4.2-kb genomic fragment containing the coding sequence for the 1.0-kb transcript could not rescue bgcn mutant flies and the coding sequence was wild-type in four EMS-induced bgcn alleles. While this manuscript was in preparation, an article describing transcription units in the 60A region appeared (Lukasovitch et al. 1999). Comparison of the 1.0-kb transcript to mRNAs identified in that study showed that the 1.0-kb transcript corresponded to their anonymous mRNA, UD3. We have subsequently designated the 1.0-kb transcript within bgcn (wibg).

We concluded that the authentic bgcn gene must be near the bgcn transposon insertion site and considered the possibility that a larger gene encompassed the region. Indeed, we found that probes derived from either side of the wibg gene recognized the same ~4100 nucleotide transcript of very low abundance in females and higher abundance in males (Figure 2). Thus we concluded that the wibg gene was located within the intron of a larger transcript that was a candidate for the bgcn gene.

cDNA clones for the larger transcript were recovered from a testis cDNA library and sequenced. The assembled transcript is ~3900 nucleotides in length and predicts a protein of 1215 amino acids with M, 1.39 × 105. The intron-exon structure and features of the cDNA and predicted protein sequence are shown in Figures 1 and 3. As we had observed with genomic probes for mRNAs, the candidate bgcn transcript is abundant in samples of male poly(A+), mRNA but exceedingly rare in female poly(A+) samples (Figure 2).

We used the genomic DNA sequence (Berkeley Drosophila Genome Project, unpublished results; http://www.fruitfly.org) to design primers for sequencing the candidate bgcn ORF from EMS-induced bgcn alleles (Lavoie et al. 1999) since we expected to find mutations that would alter the predicted ORF. Four of five alleles sequenced contained point mutations that introduced a nonsense codon into the conceptual ORF while the fifth allele contained a G-to-A transition that altered a 3'-acceptor splice site and would produce a frameshift mutation in the mature transcript. The position of these mutations and affected amino acids are shown in Figure 3. All of these alleles show the same phenotype; males and females are viable but sterile with germline tumors in their gonads. We predict that this is the null pheno-
Figure 2.—bgcn mRNA is expressed in adult males and females. Poly-A+ RNA was transferred from an agarose gel to nylon membrane and hybridized with a probe from the bgcn coding region. Expression of the 4-kb female transcript is very low compared with expression of the 4.2-kb male transcript and the female sample was therefore overloaded to improve the detection of the female transcript. Detection of mRNA encoding the ubiquitous translation factor eIF4A was used as a loading control (Cooly et al. 1992).

Figure 3.—bgcn mutants encode truncated protein products. DNA from bgcn mutant flies was amplified, sequenced, and compared to wild-type genomic sequence (materials and methods). Alleles bgcn\textsuperscript{05}, bgcn\textsuperscript{2295}, and bgcn\textsuperscript{1} carry G-to-A transitions that introduce premature termination codons. Allele bgcn\textsuperscript{2295} carries a G-to-A mutation at the 3'-donor splice site and causes a predicted frameshift of one nucleotide. Allele bgcn\textsuperscript{2295} carries a C-to-T mutation that introduces a stop codon. The five alleles tested were isolated from three different mutagenic screens.

Figure 4.—Expression of bgcn cDNA from a heterologous promoter rescues the tumorous bgcn\textsuperscript{1} phenotype. (A) Ovaries in homozygous bgcn females are filled with tumorous egg chambers. (B) Flies transformed with a transgene containing bgcn cDNA under the control of the hsp70 promoter were treated with heat shock for 2 days and ovaries dissected on the sixth day contained organized germaria (regions 1-3 as R1, R2, and R3) and maturing egg chambers. Bar, 20 μm.
we have also been unable to rescue spermatogenesis in bgcn^−/− bgcn^− males with heat-induced expression from the P[w^−; hs-bgcn] transgene.

Since rescue of the oogenic tumorous phenotype verified that transgenic Bgcn was active in early germ cells, we could examine the effects of bgcn misexpression during early stages of the germ cell lineage. Previous experiments had demonstrated that bam and bgcn phenotypes were sufficiently similar to suggest a common function (Lavoie et al. 1999) and that misexpression of bam from a heat-shock promoter caused GSC ablation (Ohlstein and McKearin 1997). We therefore tested if ectopic bgcn expression also affected GSC maintenance. Induction of bgcn in either bgcn/ bgcn or wild-type genotypes did not produce any of the phenotypes characteristic of GSC ablation. Even 8 days after the initial heat shocks, when P[h-s-bam] transgenic animals have empty germaria (Ohlstein and McKearin 1997), germaria from P[h-s-bgcn] females contained a normal array of developing cysts and GSCs could be identified by single, spherical fusomes (not shown) when reacted with anti-Hts antibodies (Zaccai and Lipshitz 1996).

Comparative analysis of the bgcn gene: The bgcn sequence was compared to sequences in the GenBank database using the BLAST algorithm available at NCBI (Altschul et al. 1997; http://www.ncbi.nlm.nih.gov/BLAST). The conceptual Bgcn protein showed a highly significant similarity to many members of the DExH family of RNA helicases (Figure 3); the top 65 scores were either DExH-box or DEAD-box proteins from diverse organisms. Members of the DExH/DEAD family contain seven highly conserved signature motifs (Figure 5, part 1; de la Cruz et al. 1999) that occur colinearly over a core region of ~675 amino acids that are more weakly conserved. Bgcn was ~25–30% identical to helicase proteins over the conserved core of 675 amino acids but, notably, showed little conservation in the hallmark helicase motifs.

Figure 5, part 1 shows the alignment of Bgcn and Arabidopsis thaliana HVT1 protein, the highest scoring BLAST match (~10^{-42}). A. thaliana HVT1 is a member of the DExH helicases based on sequence comparison although its specific function is unknown (Wei et al. 1997). The alignment shows that Bgcn does not match any of the helicase signature motifs (Walker motifs and domains 1a-VI in red in Figure 5, part 1). For example, the sequence from positions 184–188 (VILDD; Figure 5, part 2) in Bgcn matches the sequence and position of a Walker B site for Mg^{2+}-ATP binding commonly found in many RNA-dependent helicases (Walker et al. 1982; de la Cruz et al. 1999). However, Bgcn sequence at amino acids 171–178, which is the expected position of the Walker A site (phosphate-binding "P-loop"), lacks critical features of the ATP-binding P-loop (Saraste et al. 1990), making it very unlikely that Bgcn could bind any nucleotide triphosphate. In the DExH-box motif (DEIH in HVT1), Bgcn conserves the His residue at the fourth position but diverges in invariant D and E positions (Gorbalenya and Koonin 1993; de la Cruz 1999). In the case of the other signature helicase motifs, which are somewhat more flexible in sequence conservation, Bgcn does not have any previously recognized motif variant. Taken together, these divergences make it unlikely that Bgcn has either ATP-binding/hydrolysis or helicase activities (Gorbalenya and Koonin 1993).

Many residues outside of the canonical helicase family motifs are also conserved in helicases but they have not been associated with biochemical activities (Aubourg et al. 1999; de la Cruz et al. 1999). Figure 5, part 2 shows alignment of Bgcn fragments with the equivalent fragments from several diverse helicases. This method of comparison illustrated that Bgcn conservation was more extensive than would be concluded from simple pairwise alignments. It was especially striking that the sequences immediately surrounding several canonical motifs showed higher levels of conservation than within the signature motifs (Figure 5, part 2); the significance of conservation in these sequences is not known.

Bgcn alignment with HVT1 also includes sequences that are not typically part of the DExH/DEAD family core. Bgcn and HVT1 share limited sequence similarity in the N-terminal 150 amino acids and C-terminal 300 amino acids but these regions are not similar to any other proteins in the GenBank database. The N terminus of some DexH/DEAD-box proteins contains divergent versions of the double-stranded RNA-binding domain (DSR-BD; Aubourg et al. 1999) but the N-terminal sequences of Bgcn and HVT1 do not match the consensus for DSR-BD.

The region between residues 320 and 475 in Bgcn does not align with other helicase proteins except HVT1. The SMART algorithm, which recognizes common protein domains based on likely secondary structure (http://smart.embl-heidelberg.de/SMART; Schultz et al. 1998), predicts tandem ankyrin repeats at positions 407 to 436 and 440 to 472 in Bgcn and the first of these aligns with an ankyrin domain in HVT1 (Figure 5). Ankyrin repeats have been recognized in diverse proteins and are probably protein-protein interaction domains (Sedgewick and Sedgwick 1999). The ankyrin repeat at amino acids 407–436 (AD1) is most closely related to one of the ankyrin domains found in black widow spider lactroinsectotoxin, a protein containing many tandemly repeated ankyrin domains (Kiyatkin et al. 1993). Despite the prediction of the SMART algorithm, AD2 (amino acids 440 to 472) is a divergent ankyrin repeat and does not show significant amino acid conservation with known ankyrin domains. Finally, the SMART algorithm also identified two possible transmembrane domains at positions 228 to 248 and 926 to 946 in the conceptual Bgcn sequence (http://smart.embl-heidelberg.de/SMART; Schultz et al. 1998).

bgcn mRNA pattern confirms predicted GSC expres-
The predicted Bgcn translation product is related to many members of the DExH family of RNA helicases. (A) Of all members of the DExH family, Bgcn shares the highest level of homology over its entire sequence with the HVT1 protein, a putative RNA helicase from A. thaliana. In order, the helicase consensus motifs are the following: Walker A [aa 210–217], Walker B (aa223–227), Ia (aa243–249), DExH-box (aa313–316), domain III (aa345–347), domain IV (aa556–559), domain V (aa615–624), and domain VI (aa672–679), which are shown in red type. (B) Analysis of Bgcn sequence against subdomains of family members from humans (Hhlx), Arabidopsis (Hvt), and Drosophila (Maleless) reveals that Bgcn lacks homology within domains shown to be necessary for RNA helicase activities (asterisks). The predicted Bgcn sequence has two putative ankyrin repeats. One of these is shown aligned with ankyrin domains from mouse Notch4 (Notch4), fly Cactus (Cact), black widow spider latroinsectotoxin (Toxin), and the Hvt helicase from Arabidopsis.
We had previously observed that bgcn mutant GSCs were not eliminated by Bam misexpression (Lavoie et al. 1999). This suggested two conclusions about bgcn: (1) Bam requires bgcn for full activity and (2) bgcn gene product could be expressed in wild-type germline stem cells and therefore might not be a limiting factor for cystoblast differentiation. We examined the distribution of bgcn expression by RNA in situ hybridization against wild-type ovaries (Figure 6). Like bam expression (McKearin and Spradling 1990), bgcn mRNA was detectable in very few cells (5–8 cells) at the anterior tip of the germarium; the small number of bgcn-positive cells explained the low signal on Northern blots. However, careful comparison of bgcn and bam in situ hybridizations revealed that the patterns were distinct; bgcn-positive germ cells included those immediately adjacent to the terminal filament. Thus it appeared that GSCs, the bgcn gene dosage was reduced by half in transallelic animals (bgcn/1; bamz3-2884/bamD86), females became sterile and produced only tumorous cysts similar to those found typically in bamD86/bamD86 flies (Figure 7). This can be contrasted with bam mRNA that is detectable in cystoblasts but not GSCs. The bgcn expression pattern interaction was tested for five bgcn alleles derived from four separate mutagenic screens and all behaved identically as dominant enhancers of the “sensitized” bam phenotype.

**DISCUSSION**

**bgcn is a dominant enhancer of bam phenotypes:** Previously, we have presented evidence that Bgcn and Bam might be interdependent cystoblast differentiation factors (Lavoie et al. 1999). Since bgcn RNA in situ hybridization results indicated that Bam and Bgcn were coexpressed in cystoblasts, we reasoned that bam and bgcn mutations might show synergistic effects in genetic tests of cystoblast differentiation. However, bgcn/++; bam/++; and wild-type flies were equally fertile. We next compared the effects of partial loss of bgcn activity in a genetic background with decreased bam activity. Recently we have characterized a weak bam allele, designated bam22884. Drs. B. Wakimoto and D. Lindsley first recognized this allele as a male sterile mutation that induced a tumorous gonial cell phenotype and we determined subsequently that it failed to complement the null bamD86 allele (McKearin and Ohlstein 1995). The bam22884/bamD86 genotype causes females to be weakly fertile with small ovaries that contain mostly wild-type germ cells but occasionally produce cysts of normal size that contain mostly wild-type germ cells (Figure 7). This interaction was tested for five bgcn alleles derived from four separate mutagenic screens and all behaved identically as dominant enhancers of the “sensitized” bam phenotype.
assembly was rescued by an inducible bgcn transgene, oogenesis was incomplete in rescued females. Possible explanations include that the P[hst-bgcnn] transgene does not provide Bgcn product at all appropriate times for full oogenic rescue or misexpression of bgcn in somatic ovarian cells may interfere with proper oogenic progress. These same reasons, applied to spermatogenesis, could explain the failure of the P[hst-bgcnn] transgene to rescue fertility in bgcn mutant males.

Ovarian in situ hybridization with bgcn revealed that the major site of bgcn accumulation was in a small number of cells at the most anterior tip of the gerarium. Although the signal-to-noise ratio was reliable in these assays, we cannot exclude that bgcn is expressed at a low level elsewhere. The most significant aspect of bgcn expression was that, unlike bam mRNA, GSCs were positive for bgcn transcripts. At first, GSC expression appeared counterintuitive since bgcn is required for cystoblast, but not GSC, development (Gateff 1982; Lavoie et al. 1999). We had, however, considered it likely that bgcn would be expressed within GSCs because of the consequences of Bam misexpression. Since expression of the P[H-S-Bam] transgene had ablated wild-type GSCs and apparently converted them to cystoblasts (Ohlstein and McKearin 1997), we expected that Bam accumulation would be limiting in wild-type GSCs while other cystoblast factors would be expressed in GSCs (Lavoie et al. 1999). From this perspective GSCs, which have no detectable bam mRNA (McKearin and Spradling 1990), are primed to become cystoblasts and lack only a higher expression level of the bam gene.

Northern blot analysis revealed that bgcn was expressed at very low levels in ovaries but at significantly higher abundance in testes. In addition, the female transcript was reproducibly smaller than the male. The very low abundance of bgcn mRNA in female poly-A+ samples can be explained by the very restricted pattern of expression in ovaries although we do not yet know what factors account for the sexually dimorphic expression levels. Preliminary in situ hybridization suggested that bgcn was expressed throughout the testis but a more clear understanding of Bgcn expression will emerge when antibodies are available.

Bgcnn is distantly related to DExH-box proteins: The predicted Bgcn sequence revealed two specific similarities; one to the superfamily of ATP-dependent RNA helicases and a second to ankyrin domains. RNA helicases are a very large family of proteins that are primarily involved in mRNA or in translation control (Aubourg et al. 1999; de la Cruz et al. 1999). Bgcn showed position alignment and sequence conservation with dozens of helicase family members. RNA helicases have been recognized and catalogued on the basis of seven conserved domains (Gorbalenya and Koonin 1993; de la Cruz et al. 1999). Four motifs have been implicated in ATP binding and hydrolysis while two others have been implicated in nucleic acid unwinding. The final helicase motif, GRAGR, was implicated in RNA interaction in eIF4A (Pause et al. 1993) but was required for ATP hydrolysis and RNA unwinding, but not RNA interaction, in the NPH-II protein (Gross and Schuman 1996). Alignment of helicase family members illustrates that sequence conservation extends well beyond the short canonical motifs but no biochemical functions have been associated with conserved sequences outside of domains I–VII. A reasonable hypothesis is that some of the sequences conserved in DEXH proteins are involved with RNA interactions especially since many DEXH-box family members lack recognizable RNA-binding motifs (Nagai and Mattaj 1994; Zhang and Gross 1997).

The degree of conservation predicts that Bgcn and helicases share some biochemical activities. Since Bgcn does not have the motifs required for ATP binding and
ATP hydrolysis and RNA helicase catalysis. An alternative allowed limited female fertility and created "sensitized" genotypes. In previous studies we demonstrated restricted to the plant's vascular and tapetum tissue (nutritionally interdependent and suggest that Bam and Bgcn action interfaces (et al. 1999). The function of both proteins is unknown. Bgcn as a member of the DEIH-box subfamily of helicases is that HVT1 protein and contains ankyrin repeats of their expression, we have presented evidence (this article; Lavoie et al. 1999). More recently we discovered a weak bam allele that allowed limited female fertility and created flies that are sensitive to even small reductions in bam activity. We observed that these flies became sterile and produced tumorous, "bam-like" egg chambers when these "sensitized" bam females were made heterozygous for bam. Bgcn and HVT1 proteins may interact. This hypothesis predicts that Bgcn action would be cytoplasmic and its role as DEIH-box protein would more likely be involved with translational control rather than splicing regulation. Studies of the key role that Pum plays in GSC maintenance implicate translational regulation in the transition between GSC and cystoblast fate (Lin and Spradling 1997; Forbes and Lehmann 1998). Perhaps a Bgcn-Bam protein complex acts as a translational regulator of cystoblast-promoting transcripts that would be translationally repressed in GSCs. We note that our previous

Figure 7.—bgcn interacts genetically with the cystoblast differentiation factor bam. (A) Ovaries dissected from bgcn+/; bam^{32289} bam^{336} adults display germ cell hyperplasia that is indistinguishable from bgcn or bam homozogous mutant ovaries. DAPI staining these germaria (D) revealed that none of the bgcn+/; bam^{32289} bam^{336} germ cells contained the polyploid DNA complement that would be diagnostic of nurse cell formation. Bar, 20 μm.

bgcn and bam gene function are closely related and dosage sensitive: In previous studies we demonstrated that bgcn was necessary for proper Bam function and proposed that Bam and Bgcn may act together in a complex to accomplish cystoblast differentiation (Lavoie et al. 1999). More recently we discovered a weak bam allele that allowed limited female fertility and created flies that are sensitive to even small reductions in bam activity. We observed that these flies became sterile and produced tumorous, "bam-like" egg chambers when these "sensitized" bam females were made heterozygous for bgcn. The simplest interpretation for this observation is that decreasing bgcn dosage by half can effectively decrease bam activity and alter phenotype in the sensitized genetic background. This implies that Bam and Bgcn work together closely in the molecular pathway leading to cystoblast differentiation.
data suggest association between Bam and the fusome reticulum (McKearin and Ohlstein 1995; Leon and McKearin 1999), a structure resembling a germ cell reticulum (Ohlstein and Cooley 1970). We also thank B. Wakiimo and K. Wharton for sharing flies and unpublished information. Many thanksto E. Matunis, S. Wasserman, and L. Cooley for comments over the course of this work. Mary Kuhn provided expert technical assistance with genetic and cytological work for many of the experiments described; Erika Jost provided valuable technical assistance during the isolation of the bgm<sup>ts1</sup> allele. This work was supported by National Institutes of Health grant GM 45820 to D.M.

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