Genetic Analysis of the Bone Morphogenetic Protein-Related Gene, *gbb*, Identifies Multiple Requirements During Drosophila Development

Kristi A. Wharton, James M. Cook, Sonia Torres-Schumann, Katherine de Castro, Emily Borod and Deborah A. Phillips

Department of Molecular Biology, Cell Biology and Biochemistry, Brown University, Providence, Rhode Island 02912

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ABSTRACT

We have isolated mutations in the *Drosophila melanogaster* gene *glass bottom boat* (*gbb*), which encodes a TGF-β signaling molecule (formerly referred to as *60A*) with highest sequence similarity to members of the bone morphogenetic protein (BMP) subgroup including vertebrate BMPs 5–8. Genetic analysis of both null and hypomorphic *gbb* alleles indicates that the gene is required in many developmental processes, including embryonic midgut morphogenesis, patterning of the larval cuticle, fat body morphology, and development and patterning of the imaginal discs. In the embryonic midgut, we show that *gbb* is required for the formation of the anterior constriction and for maintenance of the homeotic gene *Antennapedia* in the visceral mesoderm. In addition, we show a requirement for *gbb* in the anterior and posterior cells of the underlying endoderm and in the formation and extension of the gastric caecae. *gbb* is required in all the imaginal discs for proper disc growth and for specification of veins in the wing and of macrochaete in the notum. Significantly, some of these tissues have been shown to also require the Drosophila BMP2/4 homolog *decapentaplegic* (*dpp*), while others do not. These results indicate that signaling by both *gbb* and *dpp* may contribute to the development of some tissues, while in others, *gbb* may signal independently of *dpp*.

THE TGF-β superfamily of secreted signaling molecules consists of three evolutionarily related groups, the TGF-βs, bone morphogenetic proteins (BMPs), and activins, which have been shown to regulate many developmental events, from cell proliferation to cell fate specification and apoptosis (for review see Kingsley 1994; Massagué *et al.* 1994; Hogan 1996). These structurally similar molecules are synthesized as proproteins, and they are processed to release the mature ligand. The ligand binds to a heterotetrameric receptor complex made up of type I and type II integral membrane proteins, each with a cytoplasmic serine/threonine kinase domain (reviewed in ten Dijke *et al.* 1996; Massagué 1998). The signal is transduced to the nucleus through the action of a complex of proteins belonging to the Smad family of transcriptional regulators (reviewed in Massagué *et al.* 1997; Cho and Blitz 1998; Padgett *et al.* 1998).

TGF-β ligands can exist as hetero- or homodimers, but the extent to which heterodimers *vs.* homodimers form and function *in vivo* is poorly understood. In the few cases analyzed, heterodimers have been shown to have a qualitatively different function or activity. In one case, the activin homodimer and the inhibin heterodimer have been shown to be mutually antagonistic (reviewed in Sporn and Roberts 1990), and, in hepatoma cells, this antagonism appears to occur at the level of receptor binding (Xu *et al.* 1995). In other cases, heterodimers behave cooperatively, as has been shown with Xenopus BMP4 and BMP7, where the BMP4/7 heterodimer has a higher activity than either homodimer (Aono *et al.* 1995; Suzuki *et al.* 1997). The qualitatively different responses elicited by heterodimers *vs.* homodimers most certainly contribute, at least partially, to the diveristy of biological processes attributed to the TGF-β/BMP family members (Massagué *et al.* 1994; Simin *et al.* 1998).

In recent years, many different TGF-β superfamily members have been identified and their expression patterns defined. While genetic approaches have begun to address the function of these ligands as well as identify and establish relationships between potential components of their signaling pathways, our understanding of the role of these molecules in development and in the progression of disease is still incomplete. Studies of TGF-β superfamily members in vertebrates have revealed that, in many tissues, more than one TGF-β- or BMP-type ligand are often expressed, and that while their overall patterns of expression are usually distinct, in some cells they often overlap whereby two ligands may be coexpressed (*e.g.*, Lyons *et al.* 1995; Dudley and Robertson 1997). These types of data suggest that the development of some tissues may be influenced by the action of multiple ligands. Currently, we understand very little about the potential combinatorial action of different ligands. Does each ligand have a unique or

Corresponding author: Kristi A. Wharton, Division of Biology and Medicine–MCB, Brown University, Box G-J160, Providence, RI 02912. E-mail: kristi_wharton@brown.edu

similar function? If the functions are the same, do multiple ligands work together to amplify the signal? Given that different ligands are often expressed in overlapping patterns during development, it is likely that a single cell may be exposed to multiple ligands simultaneously and must respond accordingly. To fully appreciate the impact of signaling by multiple ligands, it is essential that we have a complete understanding of the contribution of individual ligands and the interplay between their different signaling pathways. As a first step toward understanding the importance of signaling by multiple related ligands, we identified a new Drosophila BMP, the *60A* gene (Wharton *et al.* 1991), to characterize its function and examine potential interactions between 60A signaling and that of other Drosophila BMPs. Here we report the isolation of both null and hypomorphic alleles of *60A* and describe the mutant phenotypes associated with these mutations.

To date, three TGF-β superfamily members have been identified in Drosophila, *decapentaplegic* (*dpp*), *60A*, and *screw* (*scw*; Padgett *et al.* 1987; Wharton *et al.* 1991; Doctor *et al.* 1992; Arora *et al.* 1994), all of which belong to the BMP group. The *dpp* gene is the best characterized of the three, and it has been shown to function in a number of different developmental processes throughout the life cycle of Drosophila (Spencer *et al.* 1982; Segal and Gelbart 1985; Gelbart 1989; Twombly *et al.* 1996). The requirement for *scw* appears to be limited to embryogenesis, where it acts in combination with *dpp* to specify dorsal cell fates (Arora *et al.* 1994). The *60A* gene has been renamed *glass bottom boat* (*gbb*) to reflect the null phenotype (see also Khalsa *et al.* 1998). Our phenotypic analysis of *gbb* mutants indicates that *gbb*, like *dpp*, is required throughout development for a number of diverse developmental processes. Many of the *gbb* mutant phenotypes resemble those displayed by *dpp* alleles, suggesting that for at least some developmental processes, both *gbb* and *dpp* signaling are required. Consistent with this common requirement in the development of certain tissues or structures, we have recently shown that *gbb* and *dpp* signal together to pattern the wing (Khalsa *et al.* 1998) and that these signals are mediated by *tkv* and *sax* type I receptors (Haerry *et al.* 1998; Khalsa *et al.* 1998). Other *gbb* mutant phenotypes have not been reported for alleles of *dpp*, suggesting that in these cells or tissues, *gbb* may act independently of *dpp* or that *gbb* in some way elicits a qualitatively different response. Future studies detailing *gbb* signaling in specific developmental processes will reveal the nature of its relationship to signaling by other members of the TGF-β superfamily and delineate the contribution made by each ligand to that process as a whole.

MATERIALS AND METHODS

Fly strains and culture conditions: All mutations and chromosomes are described in Lindsley and Zimm (1992) or Flybase (1996), except where noted. Enhancer trap line P-1 (Sun *et al.* 1995) was kindly provided by H. Sun. P[ry^+; lacZ]0331 was obtained from S. Wasserman. Flies were reared on standard Drosophila cornmeal/sucrose/yeast medium at 25° unless otherwise noted.

F_2 lethal screen and complementation analysis: Males isogenic for the second chromosome bearing the markers *dp cn* and *bw* were mutagenized with ethyl methanesulfonate (Sigma, St. Louis) as described in Lewis and Bacher (1968). Mutagenized males were crossed to $dpp^{d\text{-}ho}$ *Bc Elp*/*CyO* females. Male progeny of the genotypes *dp cn bw*/$dpp^{d\text{-}ho}$ *Bc Elp* or *dp cn bw*/*CyO* were mated individually to *Df(2R)*bw^{S46}/*SM6a* females. A total of 7000 fertile crosses were scored for the presence of Cy^+ progeny. Any line identified as lethal *in trans* to *Df(2R)*bw^{S46} was retested against *Df(2R)G10-7-5* and *Df(2R)HB-132*. In addition to the 100 lethals recovered from our screen, 66 lethals were isolated in an independent screen (Reed 1992) and mapped to the interval between the proximal breakpoint of *Df(2R)G10-7-5* and the distal breakpoint of *Df(2R)*bw^{S46}.

Genomic walk and deficiency breakpoint mapping: Genomic clones corresponding to the 60A chromosomal region were isolated from a λDASH II (Stratagene, La Jolla, CA) genomic library constructed from a strain isogenic for *dp cl cn bw* (from R. W. Padgett). A genomic walk was constructed, and phage subclones were used to identify deficiency breakpoints. Genomic DNA was isolated from various mutant strains by grinding 50–100 adult flies in 0.1 m Tris-HCl, pH 9.0, 0.1 m EDTA, 1% SDS, and 1% DEPC. After a 30-min incubation at 70°, the samples were made 1 m potassium acetate and incubated on ice for 30 min. After centrifuging for 15 min at 4°, the DNA was precipitated from the supernatant by adding 0.5 volumes of isopropanol at room temperature. The DNA isolated from deficiency strains was analyzed by Southern analysis with specific subclones from the genomic walk as probes. Restriction-digested genomic DNA isolated from wild-type flies (Oregon-R) and flies heterozygous for *Df(2R)HB132*, *Df(2R)b23*, and *Df(2R)*egl^2 were probed with the phage from our genomic walk.

Quantitative Southern analysis: To determine which deficiencies deleted the *gbb* gene, we quantitated the signal produced by a *gbb* coding region probe on total genomic blots of DNA from wild type (+/+) and flies heterozygous for a deficiency (*Df*/+). Genomic DNA was isolated as described above from stocks heterozygous for *Df(2R)*bw^{S46}, *Df(2R) HB132*, *Df(2R)*egl^2, *Df(2R)b23*, *Df(2R)*bw^{DRa}, *Df(2R)106*, *Df(2R)G10-CD14*, *Df(2R)G10-7-5*, and Oregon-R (OR). The DNA was digested with *Eco*RI, and the amount of DNA in each lane was quantitated by hybridizing each blot with a control probe. The blot was also hybridized with a 9.5-kb *Eco*RI fragment derived from the *gbb* gene. The intensity of each band was measured using a densitometer (LKB, Piscataway, NJ). The data for each deficiency line (*Df*/+), normalized for the amount of DNA loaded in each lane, were then compared to those of the wild type (+/+).

Constructs, *P*-element transformation, and rescue analysis: A 6.8-kb genomic *Sal*I fragment from λT3-6a was subcloned into pCasper 2 (Pirotta 1988). This P[w^+; 60A S6.8, gbb^+] construct was transformed into w^{1118}, and eight independent insertions were isolated. Lines Tn6.6 and Tn55.2 on the X chromosome and Tn1.2, Tn6.3, and Tn55.4 on the third chromosome were used in this study. The *gbb* gene does not contain intronic sequences (Wharton *et al.* 1991); therefore, this rescue construct contains 3.7 kb of genomic DNA 5′ to the start of *gbb* transcription and 1.5 kb 3′ of the polyadenylation site. Representative alleles of five different complementation groups (J, F, K, L, or M) were tested for rescue of lethality *in trans* to *Df(2R)b23* by crossing each stock (lethal/SM6a) to *Df(2R)b23 If* /+; P[w^+; 60A S6.8, gbb^+]/+ and scoring for the presence of *If* Cy^+ flies.

The *gbb* knockout construct was made by inserting a linker into the *gbb* coding region, generating a stop codon at residue 38 (Wharton *et al.* 1991). Two 17-mer oligonucleotides, 623: CTAG**TCTAGA**CTAGTTG and 622: CTAG**TCTAGA**CTAG CAA, were annealed and ligated into the *Sfi*I site (nucleotide 510) of the 6.8-kb *Sal*I genomic subclone (see Figure 2). Clones were identified by the loss of the *Sfi*I site and the presence of the *Xba*I site introduced by the linker (in boldface above). The insertion of a stop codon in the correct translational frame was verified by sequencing. The *gbb* knockout (KO) fragment was cloned into pCasper 2, and a single transformant, P[w^+; 60A S6.8, gbb^{KO}], which was inserted on the second chromosome, was obtained. Attempts to transpose this insertion to another chromosome were unsuccessful, so it was recombined onto a *Df(2R)b23* chromosome marked with the dominant marker *Irregular facets* (*If*). Males of the genotype *w*/Y; P[w^+; 60A S6.8, gbb^{KO}] *Df(2R)b23 If*/*CyO* were crossed to alleles of the F, J, and M complementation groups (*/*SM6a*). The ability of P[w^+; 60A S6.8, gbb^{KO}] to rescue the lethality associated with these alleles was determined by scoring the progeny for the presence of Cy^+ *If* flies. The percentage rescue was calculated as the number of Cy^+ *If*/half the number of *Cy* × 100. Pupal lethality was scored by the presence of uneclosed, desiccated, or black pupae 5–6 days after the normal time of eclosion.

Four-cutter analysis and sequencing: Genomic DNA isolated from stocks heterozygous for alleles of the F, M, and J complementation groups was digested with a number of four- and five-cutter restriction enzymes, and Southern blots of digested DNA were probed with a genomic fragment containing the *gbb* gene. To verify the P6-103 restriction-site polymorphism, P6-103/+ genomic DNA containing the aberrant restriction site was amplified by PCR from a single adult and sequenced. The lesions associated with the other three alleles of the J complementation group (ac-17, Ab-4, and An-4) were also determined by sequence analysis. As expected, due to the isogenic nature of the mutagenized chromosomes, no other changes or polymorphisms were detected.

Lethality studies: More than 1000 embryos from the crosses gbb^*/*SM6a* × *Df(2R)b23*/+ and gbb^*/*SM6a* × gbb^*/+ (* = allele 1, 2, 3 or 4) were collected on apple juice plates. The number of individuals that hatched, pupated, and eclosed was compared to the number of individuals that survived the previous developmental stage and served as a measure of embryonic, larval, or pupal lethality, respectively.

Germ-line clones: Germ-line clones were produced in females as described in Chou *et al.* (1993). Cy^+ females produced in the cross *w* P[ry^+; FLP]/Y; P[w^+; FRT]G13 P[w^+; ovo^{D1}]32X9 × +/+; P[w^+; FRT]G13 *L bw* gbb^1/*CyO, S cn bw* were tested for their ability to lay viable eggs. A total of 10 different FRTG13 gbb^1 recombinant chromosomes were tested in this way. Fertile females were recovered from all 10 lines and, in all cases, each produced >90% viable eggs.

Immunohistochemistry and microscopy: For antibody incubations, dechorionated embryos were fixed in 1:1 heptane:4% formaldehyde (in 100 mm Pipes, 2 mm $MgSO_4$, 1 mm EGTA) for 17 min. Fixed embryos were devitellinized in 1:1 heptane:methanol, washed in methanol + 0.3% hydrogen peroxide for 2 min, 1:1 methanol:PBT (PBS + 0.1% Triton-X) and PBT + 0.2% BSA (PBTB) for 3 hr. Embryos were blocked in PBTB + 5% normal goat serum for 30 min and incubated with preadsorbed primary antibody overnight at 4°. Horseradish peroxidase (HRP)-conjugated secondary antibodies (Jackson Immunological Research) were used at 1:500 for 2–3 hr at room temperature. HRP was detected with 0.5 mg/ml diaminobenzidine and 0.03% hydrogen peroxide. Stained embryos were mounted in 2 parts Permount (Fisher, Pittsburgh):1 part methyl salicylate (Sigma, St. Louis). Rabbit anti-β-galactosidase (Cappel) was used at 1:1000. Rabbit anti-Antp (T. Kaufman) was used at 1:100 after X-Gal staining of gbb^1/*CyO, ftz lacZ* embryos. Wings were mounted as described previously (Khalsa 1998). Images were obtained with a photomicroscope (FXA; Nikon, Garden City, NY). For scanning electron microscopy, adult flies were dehydrated through an ethanol series and immersed in hexamethyldisilazane (Electron Microscopy Sciences, Fort Washington, PA) that was then allowed to sublime at room temperature. Flies were sputter coated and examined on a Hitachi 2700 SEM microscope.

RESULTS

F_2 lethal screen in 60A chromosomal region: To identify mutations in the TGF-β/BMP family member *gbb*, we carried out an F_2 lethal screen. *In situ* hybridizations to polytene chromosomes indicated that *Df(2R)*bw^{S46} deletes the *gbb* gene while *Df(2R)HB132* does not (data not shown). Thus, *Df(2R)*bw^{S46}/*SM6a* was used as a tester strain for our screen, as described in materials and methods. A total of 175 chromosomes that failed to complement *Df(2R)*bw^{S46} were recovered; 100 of these also failed to complement *Df(2R)G10-7-5*, but complemented *Df(2R)HB132*. Thus, the lethal in each of the 175 lines mapped to the 59F8;60A7 chromosomal interval. Of the 66 lethal lines obtained from B. Reed and M. Ashburner, 57 were assigned to the 59F8;60A7 interval on the basis of their ability to complement *Df(2R)HB132* and failure to complement *Df(2R)G10-7-5*. In total, 157 mutagenized chromosomes were isolated that could potentially contain a mutation in the *gbb* gene.

Deficiency mapping and complementation analysis: Each mutagenized chromosome was tested *in trans* to six different deficiencies [*Df(2R)G10-CD14*, *Df(2R)106*, *Df(2R)*bw^{DRa}, *Df(2R)*egl^2, *Df(2R)b23*, and *Df(2R)*bw^{DRj}] that break within the 59F8;60A7 interval (Table 1) and form a series of nested deletion breakpoints defining eight

TABLE 1

Deficiencies of 59D;60A

Deficiency	Cytology[a]
Df(2R)bw^{s46}	59D8;60A8-A16
Df(2R)HB132	59D8-11;59F6-8
Df(2R)bw^{DRj}	59C5;59F6-8
Df(2R)bw^{DRa}	59E1;60A4-5
Df(2R)bw^{DRt}	59D4;60A1
Df(2R)egl^9	59E;60A1
Df(2R)G10-9-1	59F3;60A1
Df(2R)G10-CD14	59F3;60A3-7
Df(2R)G10-7-5	59F3;60A8-16
Df(2R)or^{BR-11}	59F6-8;60A8-16
Df(2R)b23	59F8;60A1
Df(2R)106	59F6;60A7
Df(2R)OVI	59F5-6;60A1

[a] Cytology is as reported in Flybase (1996), except for *Df(2R)G10-9-1*, *Df(2R)G10-CD14*, *Df(2R)G10-7-5*, and *Df(2R)106*, which are reported in Reed (1992).

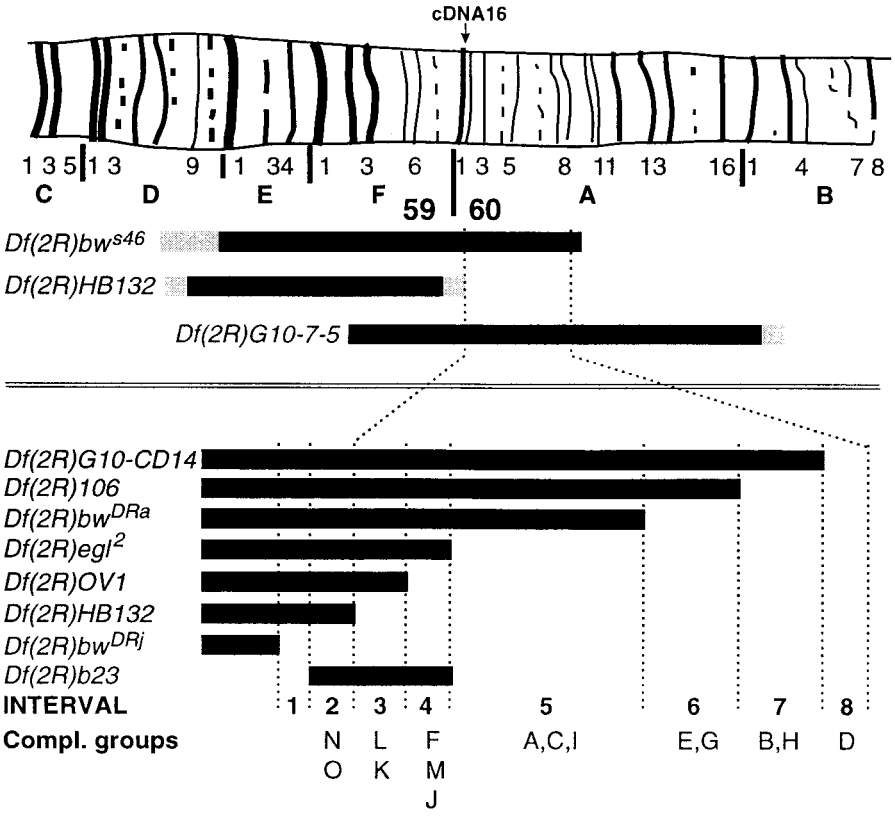

Figure 1.—Genetic map of the 60A chromosomal region. The extents of deficiencies used to define intervals in the 60A region are indicated by thick black lines. Eight chromosomal intervals (1–8) are defined by the distal breakpoints of the overlapping deficiencies. Complementation groups (A–O) identified in the screen are indicated below the interval to which they map. The polytene chromosome hybridization site for cDNA16, the original cDNA clone described in Wharton *et al.* (1991), is shown at the top of the figure.

chromosomal intervals (Figure 1). Each lethal was thus assigned to a specific interval (Figure 1). *Inter se* crosses were performed with approximately one-third of the total 175 lines, and out of these, at least 15 complementation groups within the 59F6-8;60A7 interval were established (Table 2).

A number of deficiencies were tested for the presence of the *gbb* locus by quantitative Southern analysis. This analysis corroborated our polytene *in situ* hybridization data that *Df(2R)bw*^{S46} deletes the *gbb* gene while *Df(2R)HB-132* does not. In addition, it demonstrated that *Df(2R)-G10-CD14*, *Df(2R)106*, *Df(2R)bw*^{DRa}, *Df(2R)egl*^{2}, and *Df(2R)-b23* also delete the *gbb* gene, thus placing *gbb* within the region defined by the distal breaks of *Df(2R)b23* and *Df(2R)HB132* (Figure 1, intervals 3 and 4). We mapped the distal breakpoints of *Df(2R)b23* and *Df(2R)egl*^{2} on our genomic walk, further verifying our results from the quantitative Southern analysis (Figure 2). A total of 21 different lines have lethal mutations that map within the interval defined by the distal break of *Df(2R)b23* and *Df(2R)HB132* and that constitute five separate complementation groups (K, L, F, M, or J; Table 2). Subsequently, we have shown that alleles of complementation groups K and L fail to complement *Df(2R)OV1* and thus map to interval 3, while groups F, M, and J map to interval 4.

Genomic rescue and functional identification of *gbb* alleles: A 6.8-kb genomic *Sal*I fragment containing the 1.67-kb *gbb* transcription unit was used to make a *gbb*

TABLE 2

Complementation groups in 59F8;60A7

Interval[a]	Complementation group	Number[b]
1	Unassigned	10
2	*l(2)60A-O*	2
	l(2)60A-N	4
	Unassigned	3
3	*l(2)60A-L*	1
	l(2)60A-K	4
4	*l(2)60A-F*	6
	l(2)60A-M	6
	l(2)60A-J	4
5	*l(2)60A-A*	4
	l(2)60A-C	8
	l(2)60A-I	3
	P[ry+; lacZ]0331	
	Unassigned	58
6	*l(2)60A-E*	3
	l(2)60A-G	5
	Unassigned	13
7	*l(2)60A-B*	3
	l(2)60A-H	2
	Unassigned	18
8	*l(2)60A-D*	3
	Unassigned	16

[a] Chromosomal interval as depicted in Figure 1.
[b] Number of alleles or lethals isolated within that interval.

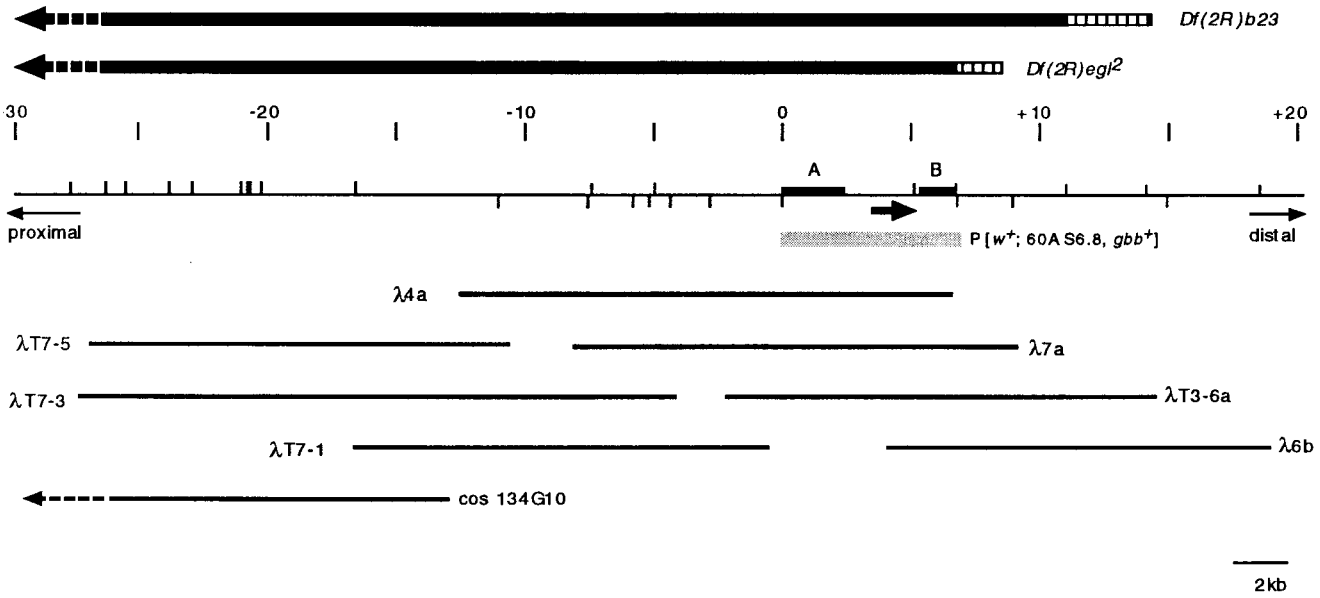

Figure 2.—Molecular map of the 60A chromosomal region. At the top of the figure, black lines denote the regions deleted by *Df(2R)b23* and *Df(2R)egl*^{2}, and the vertically hatched bars indicate regions of uncertainty around the distal breakpoints. Molecular coordinates are indicated in 10-kb intervals above a restriction map of the *gbb* region. Lines extending above the restriction map correspond to *Eco*RI sites, those below to *Sal*I sites. The position of the *gbb* transcription unit is denoted by a thick arrow below the restriction map. The 6.8-kb *Sal*I genomic fragment used for constructs to generate P[w^+; 60A S6.8, *gbb*$^+$] and P[w^+; 60A S6.8, *gbb*^{KO}] is denoted by the stippled gray bar below the restriction map. Probes A and B used in the transcriptional analysis are indicated. The positions of phage and cosmid clones along the molecular map are shown at the bottom of the figure.

rescue construct (Figure 2). Five transgenic lines with insertions of the 6.8-kb genomic fragment were tested for their ability to rescue the lethality associated with alleles of complementation groups F, J, K, L, and M *in trans* to *Df(2R)b23* (Table 3). All five transgenic lines rescued alleles from complementation groups F, M, and J. We did not observe consistent rescue of the lethality associated with alleles in the F complementation group. However, as some F alleles that are not rescued with a single copy of P[w^+; 60A S6.8, *gbb*$^+$] are rescued with two copies, we presume that this variability is caused by position effect. A transcriptional analysis of the genomic region surrounding *gbb* provided an explanation for the rescue of all three complementation groups, F, M, and J. At least four distinct RNAs (2.4, 1.55, 1.4, and 1.2 kb) are derived from sequences immediately proximal to *gbb* (Figure 2, fragment A), while a 6-kb RNA is derived from sequences immediately distal (Figure 2, fragment B; data not shown). Thus, *gbb* is one of at most six genes that map to this 6.8-kb genomic fragment should each transcript represent one gene.

To determine which of the three complementation groups corresponds to the *gbb* gene, we generated a *gbb* knockout construct that is derived from P[w^+; 60A S6.8, *gbb*$^+$]. A translational stop was introduced into the *gbb* gene in the context of the 6.8-kb genomic fragment,

TABLE 3

A genomic 6.8-kg *SalI* fragment rescues three complementation groups

Complementation group (allele)	Rescue to adulthood with single copy (%)[a]					Rescue with two copies (%): Tn55.2/Tn55.2
	Tn6.6	Tn55.2	Tn1.2	Tn6.3	Tn55.4	
J (P6-103)	49	57	33	53	41	nd
F (P23-180)	42	0[b]	40	0	36	60
F (16-198)	47	0	19	4	45	nd
M (Aa-3)	41	67	33	39	44	nd
K (At-4)	0	0	nd	0	0	nd
L (P4-111)	0	0	nd	nd	0	nd

[a] Percentage rescue is the percentage expected for individuals in the genotypic class */*Df(2R)b23*; P[w$^+$; 60A S6.8, *gbb*$^+$]Tn**/+, where * is an allele from the J, F, M, K, or L complementation group and ** denotes the transgenic line number.

[b] The lethality of P23-180/*Df(2R)b23* was rescued from larval to pupal lethality.

TABLE 4

Knockout construct fails to rescue J alleles

Genotype	Rescue to adult (%)[a]
J	
P6-103 / *Df(2R)b23*	0
Ab-4 / *Df(2R)b23*	0
An-4 / *Df(2R)b23*	0
ac17 / *Df(2R)b23*	0
M	
2-124 / *Df(2R)b23*	27.5
9-50 / *Df(2R)b23*	38.7
17-63 / *Df(2R)b23*	41.4
18-6 / *Df(2R)b23*	75.1
Aa-3 / *Df(2R)b23*	95.0
F	
16-198 / *Df(2R)b23*	38.2
P23-180 / *Df(2R)b23*	0[b]
Ad / *Df(2R)b23*	0[b]
Ae-3 / *Df(2R)b23*	0[b]

[a] Percentage rescue is calculated as the percentage expected for */P[w^+; 60A S6.8, gbb^{KO}] *Df(2R)b23*, where * represents alleles that belong to complementation group J, M, or F.

[b] Although there is no adult survival for these genotypes, the phenotype was rescued from larval to pupal lethality (not shown).

thus creating a "*gbb* knockout construct" (P[w^+; 60A S6.8, gbb^{KO}]). A linker that introduces stop codons in all three reading frames was inserted into the *Sfi*I site at nucleotide position 508, resulting in termination of translation 38 residues after the first AUG. A transgenic line containing P[w^+; 60A S6.8, gbb^{KO}] was generated and tested for its ability to rescue alleles from complementation groups F, M, and J *in trans* to *Df(2R)b23* (Table 4). The *gbb* knockout construct rescued M and F alleles but not J alleles, thus providing functional proof that the J complementation group corresponds to the *gbb* gene.

Identification of molecular lesions associated with *gbb* alleles: To verify that the J complementation group corresponds to the *gbb* gene, genomic DNA isolated from each J line was tested for restriction fragment length polymorphisms (RFLPs). Genomic DNA was digested with four- and five-cutter restriction enzymes, and DNA from the P6-103 line revealed an RFLP when digested with *Hin*I or *Tfi*I. To verify this polymorphism, the region was PCR amplified from P6-103 genomic DNA and sequenced. In the mutant DNA, the sequence at nucleotide 1518 is altered from G**G**ATC to G**A**ATC, creating a novel *Hinf*I and *Tfi*I site at this position (Table 5). This change introduces a nonsense codon at aa 371 that results in a truncated *gbb* protein that lacks the majority of the ligand domain. The molecular lesions associated with the other three J alleles were also determined by sequence analysis, and all are point mutations in the *gbb* coding region. ac17 is a nonsense mutation at the start of the ligand domain, An-4 is a methionine-to-isoleucine change at the putative translational start of the *gbb* protein, and the Ab-4 mutation changes a conserved alanine in the ligand domain into a valine. We refer to lines P6-103, ac17, An-4, and Ab-4 as gbb^1, gbb^2, gbb^3, and gbb^4, respectively (see Table 5).

TABLE 5

Molecular lesions in *gbb* alleles

Allele[a]	Nucleotide[b]	Mutation	Change
gbb^1	1518	TGG → TG**A**	Trp 371 → Stop
gbb^2	1509	TGG → TG**A**	Trp 368 → Stop
gbb^3	408	ATG → AT**A**	Met 1 → Ile
gbb^4	1607	GCG → G**T**G	Ala 380 → Val

[a] gbb^1, gbb^2, gbb^3, and gbb^4 correspond to lethal isolates P6-103, ac-17, An-4 and Ab-4, respectively.

[b] Nucleotide numbering as in Wharton *et al.* (1991).

***gbb* is a zygotic larval lethal:** Lethal phase studies of *gbb*/*Df* or *gbb trans*-heterozygotes with *gbb* alleles gbb^1, gbb^2, and gbb^3 indicate that the lethality occurs primarily during early larval stages. Less than 10% of the individuals die as embryos. To determine if the larval lethality results from rescue of an earlier embryonic requirement by a maternal contribution, we used the FLP/FRT system (Chou *et al.* 1993) to generate germline clones of amorphic *gbb* alleles. Females with homozygous gbb^1 germline clones produced phenotypically wild-type eggs, and the embryos hatched and survived to adulthood. These results indicate that *gbb* is not required maternally if zygotic function is supplied.

gbb mutant larvae show a number of defects in morphology and cuticle patterning. They are lethargic and appear flaccid when compared to their *gbb*/+ siblings and have dramatically reduced imaginal discs. The morphology of the fat body is abnormal, and this defect is most likely responsible for the transparency of the larvae for which the gene is named *glass bottom boat* (*gbb*; Khalsa *et al.* 1998). The cuticle also exhibits a number of defects in the telson region. In the most severe cases, the posterior spiracles do not protrude from the larval body, and the stigmatophores are partially fused and more dorsally situated.

Severity of mutant alleles: gbb^1 and gbb^2 are lethal when homozygous or *in trans* to *Df(2R)b23*, and they behave as genetic nulls on the basis of the fact that the mutant phenotype of *gbb*/Df or *gbb*/*gbb* is indistinguishable. gbb^3 and gbb^4 appear to be hypomorphs. gbb^4 is a very weak hypomorphic allele and was isolated in our F_2 lethal screen because of a secondary mutation on the chromosome that enhanced the phenotype of the gbb^4 mutation. When this secondary mutation was removed by recombination, homozygous gbb^4 individuals survived to adulthood (Table 6; see also Khalsa *et al.* 1998). Crosses between gbb^4 and other *gbb* alleles or *Df(2R)b23* revealed that the number of progeny that eclose is variable, depending on the culture conditions (Table 6). In addition, we determined that the phenotype of gbb^4 is temperature sensitive, and at 18°, 100% of gbb^4 homo-

TABLE 6

Phenotypes of *gbb*^{4} transheterozygotes

Genotype[a]	Survivorship (%)[b]	Bristle defects (%) [c,d]
25°		
gbb^4/*gbb*^{4}	46	36
gbb^1/*gbb*^{4}	3	0
gbb^2/*gbb*^{4}	6	14
gbb^3/*gbb*^{4}	42	20
18°		
gbb^4/*gbb*^{4}	105	10
gbb^1/*gbb*^{4}	32	15

[a] Transheterozygous progeny were generated in a cross of *gbb*^{4}/*SM6a* females to *gbb**/*SM6a*.

[b] Survivorship is the percentage of viable *Cy*$^+$ adults expected. For all crosses at 25° $N \geq 380$ flies; at 18°, $N \geq 650$.

[c] Number refers to the percentage of *Cy*$^+$ individuals that exhibited ectopic macrochaete.

[d] In all cases, ≤3% of *Cy* siblings exhibited ectopic macrochaete ($N > 300$ in each cross).

zygotes are recovered as viable adults (Table 6). *gbb*^{3}, while completely lethal, is nevertheless a weaker mutation than *gbb*^{1} or *gbb*^{2}, as we see a greater percentage of *gbb*^{3}/*gbb*^{4} survivors when compared to the number of *gbb*^{1}/*gbb*^{4} or *gbb*^{2}/*gbb*^{4} survivors.

***gbb* mutant embryos exhibit defects in midgut morphogenesis:** While the *gbb* homozygous mutant embryos survive and hatch into larvae, we have determined that *gbb* is required during embryogenesis for the proper formation of the midgut. During wild-type midgut development, three morphological constrictions are formed during stages 15 and 16 of embryogenesis (Skaer 1993; Figure 3A). In *gbb* null mutant embryos, the anterior constriction fails to form, giving rise to a bulbous anterior midgut (Figure 3, C, E, and G). This mutant phenotype is interesting in light of previous studies that have identified a requirement for *dpp* in promoting the central midgut constriction (Panganiban *et al.* 1990; Hursh *et al.* 1993). While *dpp* mutants fail to form the central constriction, *gbb* mutant embryos appear to initiate the anterior constriction as a slight invagination, but the constriction fails to be maintained and is never completed. In *gbb* mutants the central and posterior constrictions form completely, but the position of each is somewhat abnormal. The central constriction is often shifted from its position between parasegments (PS) 7 and 8 to a more posterior position between PS 8 and 9 (Figure 3, C and G). At this stage in our analysis, it is impossible to determine whether the shift in position of the central and posterior constrictions reflects a change in the regional specification of the midgut, or if it is a secondary consequence of the absence of the anterior constriction resulting from physical constraints placed on the cells of the developing midgut.

The regional specification of the midgut is initiated by the action of the three homeotic genes, *Antennapedia* (*Antp*), *Ubx*, and *abd-A* (reviewed in Skaer 1993). In the visceral mesoderm cells immediately anterior to the site of the central constriction, *Ubx* has been shown to activate *dpp* expression (Immergluck *et al.* 1990; Panganiban *et al.* 1990; Hursh *et al.* 1993). In turn, *dpp* positively regulates *Ubx* to maintain its expression in these cells. In the absence of either *Ubx* or *dpp* expression, the central constriction fails to form. Given these observations and the result that *Antp* mutants fail to form the anterior constriction (Reuter and Scott 1990), we investigated the possibility that *gbb* and *Antp* may be components in a similar regulatory loop. In *gbb* mutants, the expression of *Antp* protein is altered in the visceral mesoderm overlying the site where the anterior constriction should form (Figure 3G). In the majority of embryos examined, *Antp* protein expression was absent; however, in some stage 15 *gbb* mutant embryos a few cells expressing *Antp* remain (Figure 3G). The presence of these cells suggests that *Antp* expression may be initiated, but not maintained, in *gbb* mutant embryos. It was not possible for us to establish whether *Antp* activates *gbb* expression in the visceral mesoderm, for unlike *dpp*, *gbb* is expressed broadly in the developing midgut (K. A. Wharton, unpublished data; Doctor *et al.* 1992). We were unable to detect a localized change of *gbb* expression in the thin, squamous cells of the visceral mesoderm of *Antp* mutants.

In response to *Ubx* in PS 7, *dpp* is secreted from the visceral mesoderm cells and induces the expression of *labial* in the underlying endoderm (Immergluck *et al.* 1990; Reuter *et al.* 1990; Tremml and Bienz 1992). As there is no endodermal marker analogous to *labial* that is specific to the anterior midgut, it was not possible to directly assess the effect of *gbb* on such an endodermal target. However, in *gbb* mutants we observe a change in the endodermal expression pattern of the enhancer trap P-1 (Sun *et al.* 1995), indicating that *gbb* is involved in pattern specification in the embryonic endoderm. In wild-type embryos, P-1 is expressed in the nuclei of endodermal cells within a large region of the midgut, extending from PS 6 through anterior PS 11 (Figure 3, A and B). In a *gbb* null mutant, P-1 expression is restricted to PS 7 through PS 9 (Figure 3C). These results indicate that *gbb* signaling is required in the endoderm of both the anterior and posterior midgut. Furthermore, the expression of P-1 in the endodermal cells at the very anterior of the wild-type midgut (Figure 3, B and D) is absent in *gbb* mutants (Figure 3, C and E). These cells of the ventriculus will evaginate during stage 17 and give rise to the gastric caecae. Consistent with this result, *gbb* mutants fail to extend the gastric caecae by stage 17 (Figure 3E).

***gbb* is required in imaginal disc development:** Using the hypomorphic *gbb* allele *gbb*^{4} we identified requirements for *gbb* during imaginal development. *gbb*^{4} homozygous mutant adults and *gbb*^{4}/*gbb*^{null} viable adults exhibit a number of defects. We have previously shown that *gbb* is required for wing morphogenesis (Khalsa *et al.*

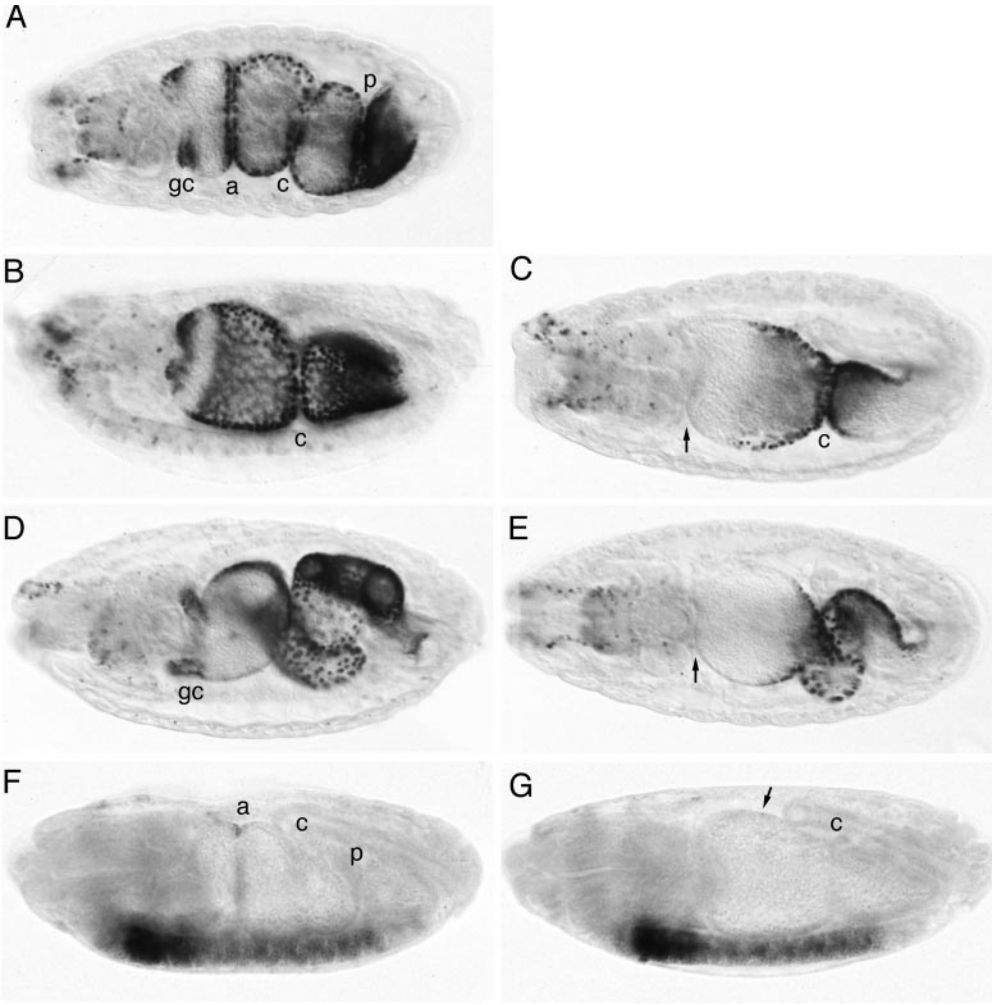

Figure 3.—*gbb* mutant embryos are defective in midgut morphogenesis. (A) A stage 16 wild-type embryo stained for expression of the enhancer trap line P-1. The three midgut constrictions, anterior (a), central (c), and posterior (p) are shown, as well as the buds of the gastric caecae (gc) forming in the ventriculus. The P-1 enhancer trap is expressed in endodermal cells throughout the midgut, with the exception of a narrow band of 8–10 cells between the ventriculus and the anterior constriction. (B–E) A comparison of P-1 expression in wild-type and *gbb* mutant embryos. (B) A stage 15 wild-type embryo in which only the central constriction has formed. P-1 expression is evident in the midgut and the ventriculus. (C) A *gbb* mutant embryo of similar stage to that in B showing loss of P-1 expression from the ventriculus and the anterior and posterior midgut. The central constriction has formed, but is shifted posteriorly with respect to its position in wild type. Also, the buds of the gastric caecae (vertical arrow) have not formed in this embryo. (D) A stage 17 wild-type embryo. At this stage, the midgut is highly convoluted, and the gastric caecae have begun to extend. (E) A *gbb* mutant embryo of similar stage to that shown in D. P-1 expression is not detected in the anterior and posterior regions of the midgut, and the gastric caecae have not formed (vertical arrow). (F and G) Expression of Antp in wild-type and *gbb* mutant embryos. (F) A stage 16 wild-type embryo showing the expression of *Antp* protein in the visceral mesoderm cells overlying the anterior constriction. (G) A *gbb* mutant embryo of similar stage to that in F. Antp expression is for the most part absent; however, in some cases, a few cells can be found that express Antp (arrow). Genotypes are as follows: (A, B, and D) gbb^1/*SM6a; P-1(lacZ)*/+, (C, E, and G) gbb^1/gbb^1; *P-1(lacZ)*/+ and (F) gbb^1/*CyO ftz-lacZ.*

1998). Wings of *gbb* mutants are smaller and more pointed than wild type and lack the posterior cross vein (Figure 4B). Regions of longitudinal veins 4 and 5 (L4 and L5) are lost, as is the posterior portion of the anterior cross vein (ACV). The extent of longitudinal vein and ACV loss is dependent on the severity of the allelic combination. In general, vein material is lost from the distal margin, but gaps in L4 and L5 are also observed. In addition to vein loss, some *gbb* mutants also exhibit a slight thickening of distal L2 and/or ectopic vein material flanking L2 (data not shown). In addition to abnormalities in wing morphology, the eyes of gbb^1/gbb^4 individuals are reduced in size. These flies have 10–20% fewer ommatidia than observed in wild-type flies. The loss of ommatidia appears to be limited to the ventral portion of the eye (data not shown).

gbb^4/gbb^4 and gbb^1/gbb^4 individuals both exhibit ectopic scutellar and dorsocentral bristles (Table 6; Figure 5). The ectopic scutellar bristles most often occur in close proximity to the endogenous bristle; however, in some cases, extra bristles are observed between the anterior and posterior scutellars (Figure 5, B and C). Ectopic bristles are also evident in individuals raised at 18°, but are observed at a lower frequency (Table 6). The absence of bristle defects in gbb^1/gbb^4 individuals recovered at 25° (Table 6) reflects the fact that in the few individuals recovered in that particular experiment (3% of 380), none had ectopic bristles. Ectopic scutellar or dorsocentral bristles were observed in <3% of the *gbb*/+ sibs.

DISCUSSION

Identification of *gbb* mutations: Alleles of the *gbb* gene were isolated in an F_2 lethal screen for mutations in the

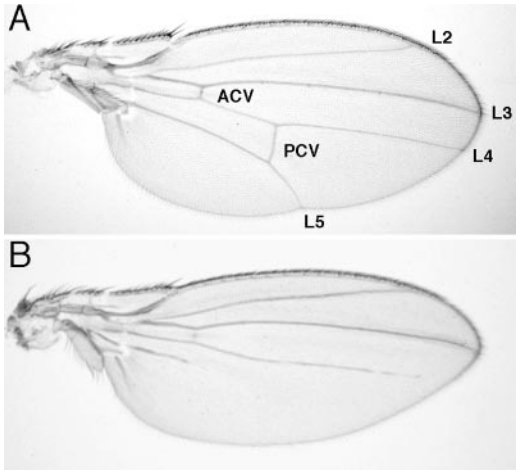

Figure 4.—*gbb* is required for wing morphogenesis. (A) Wild-type wing on which the longitudinal veins 2–5 (L2–L5) and the anterior and posterior cross veins (ACV, PCV) are noted. (B) Wings from gbb^4/gbb^2 adults are narrower and more pointed, with a complete loss of the PCV and distal portions of L5 and L4. The ACV is also often incomplete. This wing exhibits the most severe phenotype observed in viable *gbb* transheterozygotes.

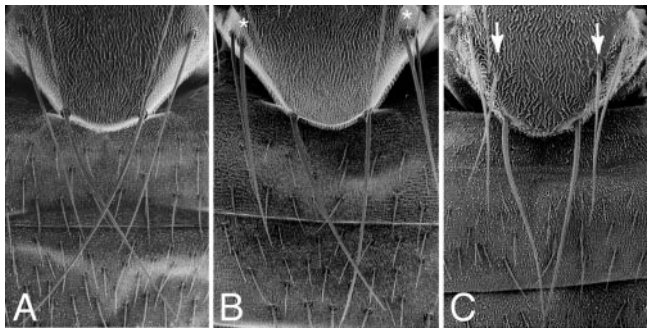

Figure 5.—*gbb* mutants exhibit ectopic macrochaete. (A) A wild-type scutellum showing the pattern of the four scutellar bristles consisting of a pair of anterior and a pair of posterior macrochaete. In *gbb* mutants (B and C), ectopic macrochaete are observed. Scutellar bristles are often "twinned" (*, B), and, in some cases, an ectopic bristle (arrow) is found between the anterior and posterior scutellars (C). We do not observe any correlation between the position of ectopic bristles and the genotype. (B) gbb^4/gbb^1; (C) gbb^4/gbb^4.

60A chromosomal region. Two null alleles, gbb^1 and gbb^2, were recovered, as well as two alleles that retain partial *gbb* function, gbb^3 and gbb^4 (Table 5). The mutations associated with gbb^1 and gbb^2 each introduce a nonsense codon that would result in a truncated *gbb* protein that lacks the majority of the ligand domain. The gbb^3 mutation changes the putative initiator methionine and, thus, we would expect this mutation to affect the initiation of *gbb* translation. However, our genetic analysis indicates that the gbb^3 allele is not null, and, therefore, it is unlikely that this lesion completely eliminates translation. While the first of four methionines found within the first 52 aa of the *gbb* ORF conforms best to the Drosophila translational consensus sequence (C/A AA C/A **ATG**; Cavener 1987), it is possible that in the gbb^3 mutant, translation begins at one of the three downstream methionines. Two of these downstream methionines (aa 21 and aa 25) fall within the signal sequence, and should translation initiate at either of these two residues, it is conceivable that some functional *gbb* protein could be produced.

The fourth *gbb* allele isolated in our F_2 lethal screen, gbb^4, results in the alteration of a conserved alanine within the ligand domain. This alanine is located within an α-helical loop thought to be conserved among all members of the TGF-β superfamily (Daopin *et al.* 1992; Schlunegger and Grutter 1992, 1993). As this region is not thought to participate in dimer formation, but rather in receptor binding (Daopin *et al.* 1992; Griffith *et al.* 1996), it is likely that the gbb^4 mutation affects ligand-receptor interactions or interactions with other extracellular proteins essential for *gbb* signaling. The gbb^4 allele is temperature sensitive, consistent with the notion that the mutation affects protein-protein interactions. The isolation of *gbb* mutations not only identifies residues essential for *gbb* function, but it allows us to determine the role of *gbb* in development and to ultimately establish the relationship between *gbb* signaling and signaling by other BMP or TGF-β superfamily members.

***gbb* is required multiple times during development:** With the isolation of both null and hypomorphic *gbb* alleles, we have determined that *gbb* has multiple requirements during development. In the embryo, *gbb* is required for midgut morphogenesis and proper telson formation. During larval stages, wild-type *gbb* function is necessary for normal fat body and imaginal disc morphology (Khalsa *et al.* 1998). *gbb* also plays a role during imaginal disc development in the attainment of normal eye and wing size as well as the establishment of wing veins and proper positioning of macrochaete on the notum. Another Drosophila BMP, *dpp*, has previously been shown to also exhibit multiple requirements during development. Interestingly, many of the tissues or structures affected by a loss in *gbb* function are also affected by mutations in *dpp*. In some cases, the resulting mutant phenotype is very similar, while in others, it is qualitatively different. For example, both *gbb* and *dpp* mutants exhibit a reduction in the size of imaginal discs and in the eyes of adults (Spencer *et al.* 1982). The severity of tissue loss differs between *dpp* and *gbb* mutants, with the animal being most sensitive to a loss of *dpp* function. Alterations in wing morphology are observed in both *gbb* and *dpp* mutants, with a reduction in the size of the wing and a loss of vein material. However, in this case the specific veins that are preferentially lost in *gbb* mutants are different from those lost in *dpp* mutants (Segal and Gelbart 1985; de Celis 1997).

***gbb* in midgut morphogenesis:** Defects in the embry-

onic midgut are also observed in both *dpp* and *gbb* mutants, but each BMP appears to play a different role in midgut morphogenesis. *gbb* is required for the formation of the anterior midgut constriction, while *dpp* is required for the central constriction. Previous work has indicated that the localized visceral mesoderm expression of homeotic genes *Antp*, *Ubx*, and *abd-A* is required for the correct positioning of the anterior, central, and posterior constrictions, respectively, in the developing midgut (Tremml and Bienz 1989; reviewed in Skaer 1993). The homeotic genes have been shown to provide regional specification through their regulation of genes encoding secreted factors, such as *dpp* and *wg*, which subsequently act on the underlying midgut endoderm (reviewed by Bienz 1994; Bienz 1996). *dpp* is activated directly by *Ubx* in a discrete band of cells in PS 7 of the visceral mesoderm from which Dpp is secreted, resulting in the induction of *labial* expression in underlying endodermal cells. It has been shown that *Ubx* expression is, in turn, maintained in the visceral mesoderm via a regulatory feedback loop through the action of *dpp*. In a manner similar to this regulation of *Ubx* by *dpp*, we have shown that the expression of the homeotic gene *Antp* is regulated by *gbb* in the visceral mesoderm cells of PS 5 and 6 (Figure 3G). However, a reciprocal regulation of *gbb* by *Antp*, as is true of the regulation of dpp by *Ubx*, is unlikely. The broad expression of *gbb* throughout the midgut indicates that *gbb* cannot be regulated exclusively by *Antp*.

gbb is expressed in both the visceral mesoderm and endoderm (Doctor *et al.* 1992; K. Wharton, data not shown), and, as indicated by the regulation of *Antp*, *gbb* signaling is required in the visceral mesoderm. *gbb* signaling is also required in specific regions of the endoderm. The absence of *gbb* function eliminates the expression of the endodermal marker P-1 from cells in both the anterior and posterior midgut, as well as from cells in the ventriculus, the site from which the gastric caecae bud. The absence of P-1 staining in the primordia of the gastric caecae in *gbb* mutant embryos is consistent with gastric caecae defects observed in *gbb* mutant first instar larvae. It appears that although no gastric caecae are evident in stage 17 *gbb* mutant embryos, gastric caecae do form, albeit abnormally, by the end of the first larval instar (R. Ray and K. Wharton, data not shown). In summary, our analysis indicates, as is true for *dpp*, that *gbb* signaling is required in both the visceral mesoderm and endoderm of the Drosophila midgut. At this time, we do not know which germ layer or layers serve as the source of the *gbb* signal.

The specification of positional identity often arises from the localized expression of genes or factors controlling that particular process. It is of interest that although *gbb* does not exhibit a localized expression pattern, it is involved in regional specification of the midgut. The role of *gbb* in this process can be explained by two different models. In one model, *gbb* acts throughout the midgut, but with a partner that provides specific positional information. This partner or cofactor could be another BMP-type ligand or some other signaling component that is specifically localized. Given that the loss of *gbb* signaling has profound effects, for example, on the formation of the anterior midgut constriction, we would predict that a *gbb* partner would be localized to the anterior region of the midgut if this model were true.

In the second model, *gbb* signaling does not specifically require a novel partner to provide positional information, but instead, cells within the midgut respond differently to varying levels of *gbb* and *dpp* signaling. This model is consistent with the paradigm we proposed for *gbb* and *dpp* signaling in the wing (Khalsa *et al.* 1998). Specification of different regions of the gut could result from the interpretation of different relative levels of *gbb* to *dpp* signaling. The total level of BMP signaling may be important, and the localized expression of *dpp* could provide a source of asymmetry necessary for the establishment of different positional information throughout the midgut. Low levels of signaling provided by *gbb* alone would specify anterior and posterior midgut *vs.* the high levels of signaling provided by both *gbb* and *dpp* that would specify the central domain of the midgut. Alternatively, differences in the responses elicited by a putative Gbb/Dpp heterodimer and Gbb and Dpp homodimers could be responsible for the assignment of different positional values. Other factors could certainly be involved in refining or elaborating the coarse pattern laid out by *gbb* and *dpp*. *wg* is an example of such a factor, as it has been shown that both *wg* and *dpp* are required to activate the expression of certain target genes in several tissues (Cohen 1990; Tremml and Bienz 1992; Campbell *et al.* 1993; Thuringer *et al.* 1993; Mathies *et al.* 1994; Bilder *et al.* 1998). At this time, it is not possible to distinguish between these two simple models, but these models provide a framework within which to investigate further the contribution of multiple BMP signaling to a specific developmental process, midgut morphogenesis.

***gbb* may signal independently of *dpp*:** In addition to the *gbb* mutant phenotypes that resemble *dpp* mutant phenotypes or those that affect tissues also affected by *dpp* mutations, we have identified several phenotypes that have not been observed in *dpp* mutants. Defects in the development of the telson and fat body of the larva have not been reported as aspects of *dpp* mutants, suggesting that in some developmental processes, *gbb* may function independently of *dpp*. It is interesting to note that mutations in the Drosophila BMP signaling components *Mad*, *Medea*, and *sax* can produce a clear larva phenotype (Raftery *et al.* 1995; Sekelsky *et al.* 1995; Das *et al.* 1998; Hudson *et al.* 1998; Wisotzkey *et al.* 1998; V. Twombly and W. M. Gelbart, personal communication). *Mad* and *Medea* encode Smad proteins shown to mediate *dpp* signaling in the midgut and the wing imaginal disc (Sekelsky *et al.* 1995; Das *et al.* 1998; Hudson *et al.* 1998; Wisotzkey *et al.* 1998). *sax* encodes

a type I receptor that has been implicated in *dpp* signaling (Brummel *et al.* 1994; Nellen *et al.* 1994; Xie *et al.* 1994) and in *gbb* signaling (Haerry *et al.* 1998). It is possible that in the formation of the telson and the fat body, *gbb* may be the only BMP signal mediated by *Mad*, *Medea*, and *sax*, and *gbb* signals independently of *dpp* in these tissues. Alternatively, the earlier requirement for *dpp* in dorsal/ventral patterning of the embryo may have precluded the identification of *dpp* involvement in fat body or telson differentiation, and in fact, both *gbb* and *dpp* signaling are required for proper development of these structures. Without the ability to bypass the early requirement for *dpp*, it is not possible at this time to distinguish between these two possibilities.

Our analysis of *gbb* alleles has also identified a requirement for *gbb* in the proper specification or positioning of bristles on the notum of the adult fly. A reduction in *gbb* activity results in the formation of ectopic macrochaete, most frequently on the scutellum. Such a phenotype has not previously been reported for *dpp* mutants. However, a recent report describing the ubiquitous activation of Tkv, a proposed Dpp receptor, results in ectopic macrochaete formation within the dorsolateral region of the notum (Tomoyasu *et al.* 1998). In this case, ectopic macrochaete formation results from the proposed activation of Dpp signaling via the Tkv receptor. In contrast, we observe ectopic macrochaete with a reduction of *gbb* function. These opposite phenotypes could reflect a fundamental difference in the role of *gbb* signaling *vs. dpp* signaling in the formation or patterning of sensory mother cells, the precursor cells to the macrochaete. Furthermore, the appearance of ectopic macrochaete in the dorsocentral *vs.* scutellar regions of the notum may reflect a different positional or spatial requirement for *dpp vs. gbb*. Further analysis will reveal whether the requirement for *gbb* in scutellar macrochaete formation is independent of the potential role for *dpp* in the dorsocentrals.

Our phenotypic analysis indicates that *gbb* and *dpp* participate in many of the same developmental processes; in some tissues the functions of *gbb* and *dpp* appear to be the same or very similar, while in others, their functions appear to be distinct. It is clear that while both *gbb* and *dpp* signaling contribute to the proper formation of the embryonic midgut and to patterning of the wing veins in the adult, the relative contribution of each BMP must be different. It is possible that overall, *gbb* and *dpp* participate in the development of certain tissues, and this could be accomplished by both cooperative or synergistic interactions and/or antagonistic interactions. As the different mutant phenotypes indicate, the mechanism by which *gbb* and *dpp* signaling each contribute to a developmental process must differ depending on the tissue. Understanding the different mechanisms by which these signals are sent and how these differences are regulated in Drosophila will provide significant insight into signaling by multiple TGF-β/BMP ligands in both invertebrates and vertebrates.

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