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# Multiple Members of the UDP-GalNAc: Polypeptide *N*-Acetylgalactosaminyltransferase Family Are Essential for Viability in *Drosophila*[*][S]

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**Background:** Protein *O*-glycosylation is an evolutionarily conserved modification that is initiated by a family of enzymes.
**Results:** RNA interference to the genes encoding each enzyme of the family identified 4 genes that are essential for viability.
**Conclusion:** Protein *O*-glycosylation is required for eukaryotic development and viability.
**Significance:** Certain members of this enzyme family serve unique and essential functions in specific tissues during eukaryotic development.

**Mucin-type *O*-glycosylation represents a major form of post-translational modification that is conserved across most eukaryotic species. This type of glycosylation is initiated by a family of enzymes (GalNAc-Ts in mammals and PGANTs in *Drosophila*) whose members are expressed in distinct spatial and temporal patterns during development. Previous work from our group demonstrated that one member of this family is essential for viability and another member modulates extracellular matrix composition and integrin-mediated cell adhesion during development. To investigate whether other members of this family are essential, we employed RNA interference (RNAi) to each gene *in vivo*. Using this approach, we identified 4 additional *pgant* genes that are required for viability. Ubiquitous RNAi to *pgant4*, *pgant5*, *pgant7*, or the putative glycosyltransferase *CG30463* resulted in lethality. Tissue-specific RNAi was also used to define the specific organ systems and tissues in which each essential family member is required. Interestingly, each essential *pgant* had a unique complement of tissues in which it was required. Additionally, certain tissues (mesoderm, digestive system, and tracheal system) required more than one *pgant*, suggesting unique functions for specific enzymes in these tissues. Expanding upon our RNAi results, we found that conventional mutations in *pgant5* resulted in lethality and specific defects in specialized cells of the digestive tract, resulting in loss of proper digestive system acidification. In summary, our results highlight essential roles for *O*-glycosylation and specific members of the *pgant* family in many aspects of development and organogenesis.**

Post-translational modifications provide added levels of complexity and diversity to the encoded genome by influencing many aspects of protein structure, function, and stability. In the post-genomic era, understanding how these protein modifications operate is essential for a comprehensive understanding of all factors involved in various developmental events. Glycosylation, or the addition of sugars to proteins, is an abundant and diverse set of co- and post-translational modifications that imparts a multitude of effects *in vivo* (reviewed in Refs. 1–8). Various types of glycosylation have been shown to influence protein processing, stability, and transport, as well as modulate protein interactions with other molecules, thereby affecting diverse cellular processes, including signaling events, proteolytic cascades, and the unfolded protein response (UPR).

Mucin-type *O*-linked glycosylation is an evolutionarily conserved form of glycosylation found across diverse species and in most developing organ systems (6, 9–13). This type of glycosylation is initiated by UDP-GalNAc: polypeptide *N*-acetylgalactosaminyltransferase family of enzymes (PGANTs in *Drosophila* or GalNAc-Ts in mammals) (Fig. 1) whose members transfer a GalNAc sugar onto serine or threonine residues in protein substrates. The genes encoding members of this enzyme family have unique developmental expression patterns (10, 11) (Fig. 1*B*), suggesting specific functional roles in certain developing tissues. *In vitro* biochemical assays have revealed unique peptide preferences for certain family members that are conserved across species (14, 15). Additionally, members of this family form 2 subgroups: 1) those that perform the initial addition of GalNAc to unmodified substrates (peptide transferases) and 2) those that will only add GalNAc to previously glycosylated substrates (glycopeptide transferases) (Fig. 1, *A* and *B*) (see Refs. 16–18 and reviewed in Refs. 6 and 12). In summary, the unique expression patterns and substrate specificities of the many members of this family suggest unappreciated biological complexity associated with this type of glycosylation.

The importance of mucin-type *O*-linked glycosylation in human health has been demonstrated by the discovery that defects in *O*-glycosylation are responsible for Tn syndrome (19) and familial tumoral calcinosis, a disease characterized by hyperphosphatemia and the development of subdermal calcified tumors (20, 21). Additionally, genome wide association studies and linkage studies have identified the genes controlling

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**TABLE 1**
**Gal4 driver lines and expression patterns**

| Gal4 driver line | Expression pattern | Source | Reference |
|---|---|---|---|
| Tub | Ubiquitous | Bloomington No. 5138 | Flybase[a] |
| MS1096 | Dorsal wing disc | Bloomington No. 25706 | Flybase |
| 6-4 | Larval hemocytes | Bloomington No. 6396 | Flybase |
| c381 | Amnioserosa, PNS | Bloomington No. 3734 | Flybase |
| 69B | Embryonic epidermis, imaginal discs | Bloomington No. 1774 | Flybase |
| 48Y | Embryonic endoderm | Bloomington No. 4935 | Flybase |
| 24B | Embryonic mesoderm | Bloomington No. 1767 | Flybase |
| c179 | Embryonic mesoderm, larval muscles, wing imaginal discs | Bloomington No. 6450 | Flybase |
| btl | Tracheal cells/respiratory system | D. Andrew | Shiga *et al.* (36) |
| c135 | Proventriculus, midgut, salivary gland, fat body, reproductive tract | Bloomington No. 6978 | Flybase |
| elav | Nervous system | Bloomington No. 8765 | Flybase |

[a] Flybase.org.

*O*-glycosylation among those associated with alterations in HDL cholesterol and triglyceride levels (22–24), congenital heart disease (25), and increased risk of colon cancer (26). However, the specific roles of *O*-glycosylation in these instances remains unknown.

Studies investigating the role of mucin-type *O*-glycosylation in *Drosophila melanogaster* provided the first demonstration that one member of this gene family (*pgant35A*) is essential for viability (27, 28). Indeed, *pgant35A* was shown to affect the establishment of apical-basal polarity and diffusion barrier formation in the developing respiratory system (29). Another member, *pgant3*, was found to affect integrin-mediated cell adhesion during *Drosophila* development by influencing the secretion of an extracellular matrix protein (30–31). In addition, studies in mice demonstrated roles for *O*-glycosylation and the gene *Galnt1* in lymphocyte homing and blood coagulation (32). Thus, the use of genetically tractable model organisms can provide crucial insight into the diversity of cellular and biological functions of this complex post-translational modification.

*In vivo* RNA interference (RNAi) has been used successfully in *Drosophila* to decipher the biological role of many genes by specifically targeting the gene of interest and faithfully reproducing phenotypes seen with conventional gene mutations (31, 33–35). Here we demonstrate that 4 additional *pgant* family members are essential for viability in *Drosophila* by employing RNAi *in vivo*. Additionally, we show that individual family members are required in specific organs and tissues by employing tissue-specific RNAi. Finally, we expand upon our RNAi results by examining the effect of conventional mutations in *pgant5* on digestive system function. Our results suggest that the PGANT family of enzymes serve diverse functions throughout *Drosophila* development and provide further insight into the roles of *O*-glycosylation in mammalian development and disease.

## EXPERIMENTAL PROCEDURES

*Fly Strains Used*—The Gal4 driver stocks used in this study are listed in Table 1 (36). The wild type stocks used were either Oregon-R or $w^{1118}$ (VDRC 60000). Stocks from the Vienna *Drosophila* RNAi Center (VDRC)[2] (37) that were used to perform RNAi *in vivo* are listed in Table 2. Additionally, VDRC 60008 ($w^{1118}$; *P{UAS-dicer2*$^{w+}$*}*) was used to recombine with *UAS-pgant35AIR#7* to generate the line *UAS-pgant35AIR#7,dicer2*$^{D}$ (to enhance levels of *pgant35A* knock-down). VDRC 60009 ($w^{1118}$; *P{UAS-dicer2*$^{w+}$*}*) was used to recombine with VDRC 26163 to generate the line VDRC 26163,*dicer2* (to enhance knock-down of *pgant2*). Bloomington stocks 8283 ($w^{1118}$; *CyO, P{w*$^{+mc}$ *= FRT (w*$^{+}$*)Tub-PBac/T}2/wg*$^{sp-1}$), 8674 ($w^{1118}$; *Df(2L)BSC109/CyO*), and 1522 (*w**; *P{UAS-GFP.S65T}T10*) were used. The transposon insertion in *pgant5* (*PBacpgant5*$^{c03193}$*/CyO*) was from the *Drosophila* Exelixis Collection (38). The *w*; *Dr/TM3, Sb*$^{1}$, *twi-2XGFP* stock was the kind gift of Dr. D. Andrew. The following stocks were the kind gifts of Dr. J. Kennison: *w*; *TM6C, cu, Sb, e, ca/Su(Tpl)*$^{s1}$, *red, e* stock; *w*; *Sco/SM6a* stock, and *cn*$^{1}$ *bw*$^{1}$ *sp*$^{1}$ stock.

*Construction of Gal4-inducible pgantIR Vectors and Transgenic Lines*—The pWIZ plasmid (39) was used to generate Gal4-inducible constructs expressing double-stranded RNA (dsRNA) to each *pgant,* which were then used to create transgenic flies. To generate each Gal4-inducible *pgant* construct (UAS-*pgantIR*), sense and antisense primers (supplemental Table S1) were used to amplify an ~500 bp fragment from each *pgant*. The PCR product was then cloned stepwise into the AvrII and NheI sites on either side of the *white* intron in the vector pWIZ (39) to generate a vector containing two inversely oriented fragments or inverted repeats (IR) from the gene of interest flanking the *white* intron. Transformants were produced by Genetic Services Inc. (Cambridge, MA) using methodology based on the procedure described previously (40, 41).

*Fly Crosses—Drosophila* crosses were performed as described previously (30, 31). Briefly, crosses to generate expression of dsRNA were performed using flies from a UAS-*pgantIR* transgenic line and the Gal4-driver stocks described herein (Table 1). Crosses to generate RNAi to each *pgant* gene ubiquitously were performed by crossing UAS-*pgantIR* transgenic lines to a tubulin-Gal4-driver line (*P{tubP-GAL4}LL7/TM3, Sb*$^{1}$, *twi-2XGFP*); viability of flies expressing dsRNA to each *pgant* was determined by quantitating the number of progeny with and without *Sb*$^{1}$. Tissue-specific knockdown of each *pgant* was performed by crossing each UAS-*pgantIR* transgenic line to the tissue-specific Gal4 driver lines listed in Table 1 and quantitating the percentage of surviving progeny that express dsRNA to the *pgant* of interest. Crosses to the wing-specific driver (MS1096-Gal4) were performed using homozygous $w^{1118}$, *P{w*$^{+mW.hs}$ *= GawB}Bx*$^{MS1096}$ females crossed to UAS-*pgantIR* transgenic males; wing blistering was quantitated in

[2] The abbreviations used are: VDRC, Vienna *Drosophila* RNAi Center; GalNAc-T or PGANT or *pgant*, UDP-GalNAc:polypeptide *N*-acetylgalactosaminyltransferase; qPCR, quantitative PCR; dsRNA, double-stranded RNA; IR, inverted repeat; PNA, peanut agglutinin; TRITC, tetramethylrhodamine isothiocyanate.

progeny expressing dsRNA to each *pgant*. All *Drosophila* crosses were kept on MM media (KD Medical, Inc.) at 25 °C unless specified otherwise.

*Quantitative RT-PCR*—Quantitative RT-PCR (qPCR) to determine expression levels of all *pgant* family members when RNAi was induced was performed using the PCR primers listed in supplemental Table S2. cDNA prepared from first instar larvae from *Tub-Gal4>pgantIR* or the controls *Tub-Gal4>VDRC60000* ($w^{1118}$) or Oregon-R was used in qPCR. Briefly, RNA was isolated using the FastRNA Pro Green kit (Q-BIOgene). cDNA synthesis was performed using the iScript cDNA Synthesis Kit (Bio-Rad). PCR primers were designed using Beacon Designer software (Bio-Rad). qPCR was performed on a CFX96 real time PCR thermocycler (Bio-Rad) using the SYBR Green PCR Master Mix (Bio-Rad). RNA levels were normalized to 18S rRNA.

*Western Blotting*—Protein extracts were prepared from midguts dissected from third instar wandering larvae. Tissues were dissected in PBS and immediately transferred to lysis buffer (PBS, 1% Triton X-100, 5 mM EDTA) containing protease inhibitors (Thermo Scientific). Tissues were then homogenized on ice. Protein extracts from an equivalent number of wild type, *c135>pgant5IR*, $pgant5^{c03193}/Df$ (*2L*) *BSC109*, or $pgant5^{c03193(excision)}/Df(2L)BSC109$ midguts were electrophoresed under reducing conditions in 4–12% SDS-PAGE gradient gels and then transferred to nitrocellulose membranes. The membranes were blocked with Odyssey Blocking Buffer (LI-COR) and incubated with IRDye 680LT-conjugated peanut agglutinin (PNA) (1:5000) and rabbit α-tubulin antibody (Cell Signaling, 1:1000). IRDye 800CW-conjugated goat anti-rabbit secondary antibody was used (1:10,000). Membranes were scanned using a LI-COR Odyssey Infrared Imaging System.

*Tissue Fixation, Staining, and Immunofluorescence*—Midguts from third instar wandering larvae were dissected in PBS and immediately transferred to 4% paraformaldehyde in PBS. Midguts were fixed for 20 min at 4 °C. Following fixation, samples were washed three times in PBST (PBS, 0.1% Triton X-100) and transferred to blocking buffer (2% goat serum/PBS, 0.1% Triton X-100) for 1 h on a rocker at room temperature. To visualize actin staining in copper cells, samples were incubated with either TRITC-conjugated phalloidin (Sigma, 1:100) or Alexa 488-conjugated phalloidin (Invitrogen, 1:100) for 3 h at room temperature. Samples were incubated with fluorescein- or rhodamine-labeled PNA (Vector Laboratories, 1:100) for 3 h at room temperature to visualize core 1 *O*-glycans (Galβ1–3GalNAcα1-S/T). Samples were imaged using a Zeiss Axiovert microscope.

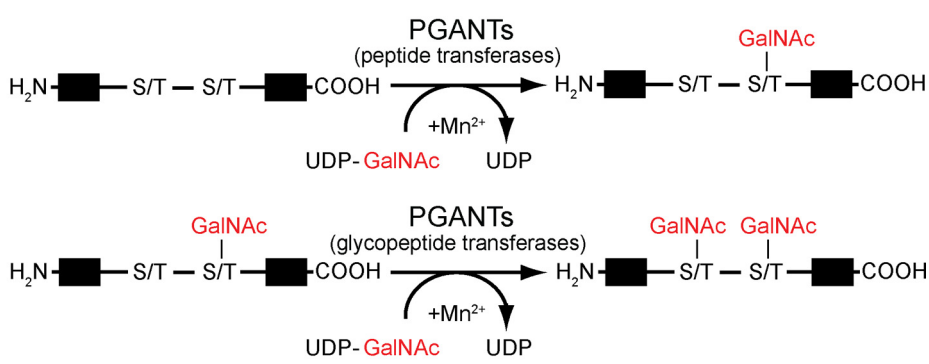
## RESULTS

*Additional pgant Family Members Are Essential for Viability*—The *Drosophila* family of *pgant*s consists of 9 genes encoding enzymes that have been biochemically confirmed to function as glycosyltransferases, along with 3 putative family members based on sequence conservation (10, 18) (Fig. 1*B*). Previous studies have identified unique developmental expression patterns for each family member (10) (summarized in Fig. 1*B*). To comprehensively investigate the *in vivo* role of each remaining member of this multigene family, we performed RNAi *in vivo* by crossing transgenic lines containing Gal4-inducible IR for each gene to a line expressing the Gal4 transcriptional activator under the control of the ubiquitously expressed tubulin promoter (Tub-Gal4). Progeny from these crosses will express dsRNA to the gene of interest in all cells and tissues throughout development, thus inducing RNAi ubiquitously. RNA from progeny expressing dsRNA was collected and qPCR was performed to verify that the expression of the targeted gene was specifically decreased. As shown in Fig. 2, induction of dsRNA for *pgant2*, *pgant4*, *pgant5*, *pgant6*, *pgant7*, *pgant35A*, or the putative *pgant*, *CG30463*, resulted in a specific and significant decrease in expression of the targeted gene. Specific knockdown of *pgant3* expression via RNAi was also seen and has been reported previously (31). Expression levels of *CG10000* and *CG31776* (2 putative members of the *pgant* family) were below levels of detection in all wild type tissues and stages examined, thus we were unable to quantitate specific knockdown of these putative glycosyltransferase genes. Finally, we were unable to see a specific decrease in gene expression for *pgant1* or *pgant8* with multiple different RNAi lines, so these genes were not pursued further in this study.

Progeny from each cross was then scored to determine the effects on viability (Table 2). Interestingly, RNAi to *pgant4*, *pgant5*, *pgant7*, or *CG30463* using the Tub-Gal4 driver resulted in complete loss of viability, indicating an essential role for these genes in *Drosophila* (Table 2). As expected, RNAi to *pgant35A* (an essential gene in *Drosophila* (27–29)) also resulted in lethality. Additionally, whereas RNAi to *pgant6* did not cause complete lethality, it did result in reduced viability. No effect on viability was seen after RNAi to *pgant2, pgant3, CG10000*, or *CG31776*.

*pgant Genes Are Required in Specific Tissues during Development*—To investigate the specific role of the newly identified essential *pgant*s, we performed tissue-specific RNAi to each gene using the Gal4 driver lines listed in Table 1. Lines expressing Gal4 specifically in the endoderm, mesoderm, ectoderm, respiratory system, nervous system, digestive system, salivary gland, amnioserosa, and hemocytes were used. Interestingly, tissue-specific requirements were seen for each essential *pgant* (Table 3). RNAi to *pgant4* or *pgant5* in the digestive system resulted in almost complete lethality, indicating an essential role for each in the developing gut. *pgant5* was also required in the embryonic mesoderm and the respiratory system. RNAi to *pgant35A* in the respiratory system resulted in a dramatic reduction in viability, supporting previous studies indicating a role for this transferase in tracheal system development (29). Interestingly, *pgant35A* was also found to be required in the mesoderm. Tissue-specific RNAi to *pgant7* revealed a role for it in respiratory system development. And finally, knockdown of the putative family member, *CG30463*, in the mesoderm, the respiratory system, or the amnioserosa resulted in lethality. RNAi to *CG30463* in the digestive system also resulted in a reduction in viability. These *in vivo* results further suggest that this conceptual gene may encode a functional glycosyltransferase that is performing unique roles in specific developing tissues. Taken together, our results indicate that multiple members of the *pgant* family are essential for viability and are performing functions in specific developing tissues. Additionally,

A




B

| Gene | Biochemical activity[a] | Embryonic expression[b] | Mammalian orthologue[c] |
|---|---|---|---|
| *pgant1* | pep / glycopep | s.g., digestive tract | *GalNt5* |
| *pgant2* | pep / glycopep | n.s., respiratory system | *GalNt2* |
| *pgant3* | pep / glycopep | foregut, epidermis | *GalNt1* |
| *pgant4* | glycopep | proventriculus | *GalNt10* |
| *pgant5* | pep / glycopep | s.g., digestive tract, epidermis | *GalNt1* |
| *pgant6* | glycopep | s.g., digestive tract, epidermis | *GalNt10* |
| *pgant7* | glycopep | s.g., digestive tract, epidermis | *GalNt7* |
| *pgant8* | pep / glycopep | digestive tract | *GalNt17* |
| *pgant35A* | pep / glycopep | s.g., digestive tract, epidermis | *GalNt11* |
| *CG30463* | ND | s.g., amnioserosa | *GalNt1* |
| *CG10000* | ND | midgut (weak) | *GalNt2* |
| *CG31776* | ND | digestive tract (weak), epidermis | *GalNt10* |

pep= peptide transferase
glycopep= glycopeptide transferase
ND= none detected
s.g.= salivary gland
n.s.= nervous system

[a] of the encoded enzyme; from Ten Hagen et al. 2003 (18)
[b] of *Drosophila pgants* beginning at stage 12; from Tian and Ten Hagen, 2006 (10)
[c] orthologue or most closely related mammalian isoform based on amino acid similarity and alignments within the conserved catalytic region as described previously (18)

FIGURE 1. **Protein *O*-linked glycosylation.** *A*, the enzymatic addition of *N*-acetylgalactosamine (GalNAc) to serine or threonine of protein substrates is catalyzed by the PGANT family of enzymes. The PGANT family is divided into 2 subgroups: peptide transferases, which catalyze the addition of GalNAc to unmodified substrates; and glycopeptide transferases, which only add GalNAc to previously glycosylated substrates. *B*, summarized is the *pgant* gene family from *Drosophila,* their embryonic expression patterns, the biochemical activity of the encoded enzymes, and the most closely related mammalian isoforms.

certain tissues (the respiratory system, digestive system, and mesoderm) require more than one *pgant*.

*pgant5 Is Required for Proper Gut Acidification*—To follow up on the RNAi results presented above, we examined conventional mutations in *pgant5*. For these studies we utilized a piggyBac transposon insertion line from the Exelixis *Drosophila* stock collection that contains a transposon in the second intron of the *pgant5* gene (*PBacpgant5*$^{c03193}$, hereafter designated as *pgant5*$^{c03193}$) (Fig. 3*A*). *pgant5*$^{c03193}$ flies were crossed to a deficiency line that uncovers the *pgant5* gene (*Df(2L)BSC109*) to assess the effect of the transposon on viability and *pgant5* gene expression. Indeed, *pgant5*$^{c03193}$/*Df(2L)BSC109* progeny were not viable when compared with either the heterozygous transposon insertion (*pgant5*$^{c03193}$/+) or heterozygous deficiency (*Df(2L)BSC109*/+) (Fig. 3*C*). qPCR confirmed that the transposon in *pgant5* abrogated *pgant5* expression, but did not decrease the expression of the flanking genes (*CG5828* and *RpL37A*) (Fig. 3*B*). To confirm that the transposon insertion lethality is specifically due to its effect on *pgant5* gene expression, we precisely excised the transposon from the *pgant5* gene using the piggyBac transposase line (Bloomington number 8283), as described previously (38). Excision of the transposon (*pgant5*$^{c03193(excision)}$) resulted in restoration of *pgant5* expression and viability (Fig. 3, *B* and *C*). These results confirm that *pgant5* is required for viability and provide an independent validation of our *in vivo* RNAi results.

Building upon our tissue-specific RNAi results that indicated a role for *pgant5* in the digestive system, we next examined the larval digestive tracts of *pgant5* mutants. Interestingly, both conventional *pgant5* mutations (*pgant5*$^{c03193}$/*Df(2L)BSC109*) as well as RNAi to *pgant5* (*c135>pgant5IR*) resulted in midguts with an unusually basic pH, suggesting a role for *pgant5* in the proper digestive system acidification (Fig. 4*A*). Western blots of midguts probed with the lectin PNA, which detects core 1 *O*-glycan structures (Gal$\beta$1–3GalNAc$\alpha$1-S/T), revealed the loss or reduction in intensity of many bands in *pgant5* mutants

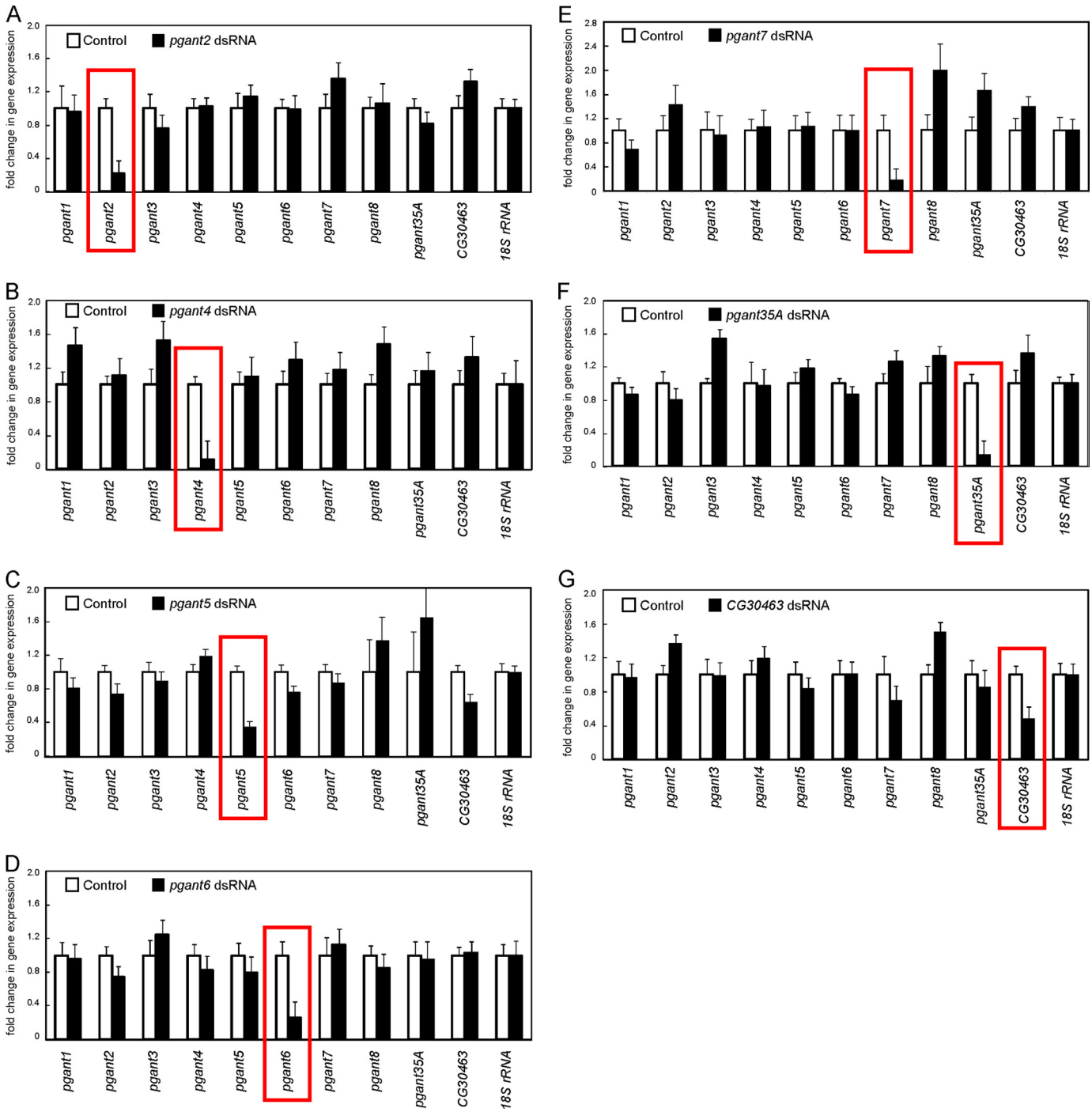



FIGURE 2. **Quantitative PCR reveals specific decreases in gene expression of each *pgant* in progeny expressing dsRNA.** Progeny expressing dsRNA to (*A*) *pgant2*, (*B*) *pgant4*, (*C*) *pgant5*, (*D*) *pgant6*, (*E*) *pgant7*, (*F*) *pgant35A*, and (*G*) *CG30463* are shown. Total RNA was isolated and qPCR was performed for all *pgant* family members to verify specific knockdown of gene expression (*red boxes*). RNA levels were normalized to 18 S rRNA. Standard deviations are shown.

(Fig. 4*B*), indicating that *pgant5* is responsible for the glycosylation of numerous proteins within the larval midgut.

We next examined the effects of *pgant5* on copper cell *O*-glycosylation and morphology. Copper cells are specialized cells of the *Drosophila* midgut that regulate gut acidification (42, 43). These cells have a unique morphology, with an invaginated apical surface rich in actin-based microvilli (Fig. 4*C*) (42, 43). Copper cells from wild type and *pgant5* mutants were stained with fluorescently labeled PNA to detect *O*-glycoproteins, and with phalloidin to detect the actin-rich apical surface (Fig. 4*D*). Interestingly, wild type copper cells displayed abundant *O*-glycoproteins throughout the apical and luminal regions of copper cells (Fig. 4*D*). However, loss of *pgant5* (*c135>pgant5IR* or $pgant5^{c03193}/Df(2L)BSC109$) was accompanied by a dramatic loss of *O*-glycosylation normally found on copper cells (Fig. 4*D*). Although phalloidin staining of the actin-rich apical surface was normal in *c135>pgant5IR* copper cells (Fig. 4*D*), it was irregular in shape and size in $pgant5^{c03193}/Df(2L)BSC109$ copper cells (Fig. 4*D*), indicating a more severe apical/cytoskeletal phenotype for the conventional *pgant5* mutants. Copper cell

**TABLE 2**

**Viability of flies expressing dsRNA to each *pgant* under the control of the Tub-Gal4 driver**

Crosses are described under "Experimental Procedures."

| Gene knocked down | CG number | IR lines used | Viability[a] | *n*[b] |
|---|---|---|---|---|
| *pgant2* | *CG3254* | VDRC 26163, *dicer2* | 99% | 257 |
| *pgant3* | *CG4445* | UAS-*pgant3IR2#2* | 97% | 298 |
| *pgant4* | *CG31956* | UAS-*pgant4IR#8* | 0% | 174 |
| *pgant5* | *CG31651* | VDRC 2629 | 0% | 226 |
| *pgant6* | *CG2103* | VDRC 106203 | 41% | 207 |
| *pgant7* | *CG6394* | VDRC 105160 | 0% | 528 |
| *pgant35A* | *CG7480* | UAS-*pgant35AIR#7,dicer2*$^{D}$ | 0% | 224 |
| *CG30463* | *CG30463* | UAS-*CG30463IR#5* | 0% | 202 |
| *CG10000* | *CG10000* | UAS-*FlyKIR#2* | 92% | 232 |
| *CG31776* | *CG31776* | VDRC 7283 | 92% | 296 |

[a] Relative to siblings not expressing dsRNA.
[b] *n*, total number of flies scored.

**TABLE 3**

**Viability (relative to siblings not expressing dsRNA) of flies expressing dsRNA to essential *pgants* in specific tissues**

| Gal4 driver line (expression pattern) | IR lines used | | | | |
|---|---|---|---|---|---|
| | *pgant4IR* | *pgant5IR* | *pgant35AIR* | *pgant7IR* | *CG30463IR* |
| 6-4 (hemocytes) | 100% ($n$ = 271)[a] | 100% ($n$ = 331) | 98% ($n$ = 269) | 100% ($n$ = 485) | 95% ($n$ = 393) |
| c381 (amnioserosa, PNS) | 82% ($n$ = 317) | 100% ($n$ = 291) | 67% ($n$ = 369) | 100% ($n$ = 675) | 11% ($n$ = 211) |
| 69B (epiderm/ectoderm) | 100% ($n$ = 227) | 51% ($n$ = 292) | 45% ($n$ = 635) | 97% ($n$ = 834) | 36% ($n$ = 336) |
| 48Y (endoderm) | 100% ($n$ = 327) | 100% ($n$ = 280) | 69% ($n$ = 499) | 76% ($n$ = 576) | 62% ($n$ = 281) |
| 24B (mesoderm) | 100% ($n$ = 271) | 81% ($n$ = 336) | 0% ($n$ = 275) | 69% ($n$ = 429) | 0% ($n$ = 190) |
| c179 (mesoderm) | 100% ($n$ = 308) | 0% ($n$ = 280) | 19% ($n$ = 298) | 100% ($n$ = 825) | 0% ($n$ = 203) |
| btl (respiratory system) | 100% ($n$ = 309) | 17% ($n$ = 327) | 12% ($n$ = 254) | 12% ($n$ = 444) | 0% ($n$ = 159) |
| c135 (digestive system, reproductive tract) | 2% ($n$ = 402) | 0% ($n$ = 177) | 25% ($n$ = 208) | 100% ($n$ = 721) | 23% ($n$ = 178) |
| elav (nervous system) | 100% ($n$ = 295) | 100% ($n$ = 289) | 86% ($n$ = 290) | 85% ($n$ = 636) | 97% ($n$ = 297) |

[a] *n*, total number of flies scored.

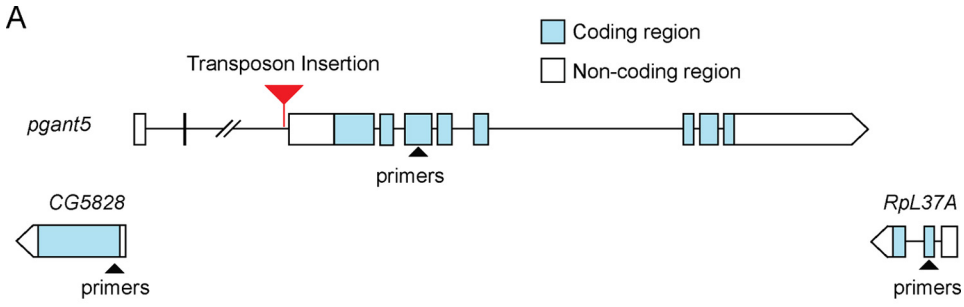
*O*-glycosylation, gut acidification, and actin staining were restored upon excision of the transposon from *pgant5* (*pgant5*$^{c03193(excision)}$/*Df(2L)BSC109*) (Fig. 4, *A*, *B*, and *D*). These results indicate that PGANT5 is responsible for glycosylating apical and luminal proteins within copper cells and that loss of PGANT5 results in loss of proper gut acidification. These results highlight a previously unknown role for *pgant5* in specialized cells of the digestive tract responsible for gut acidification.

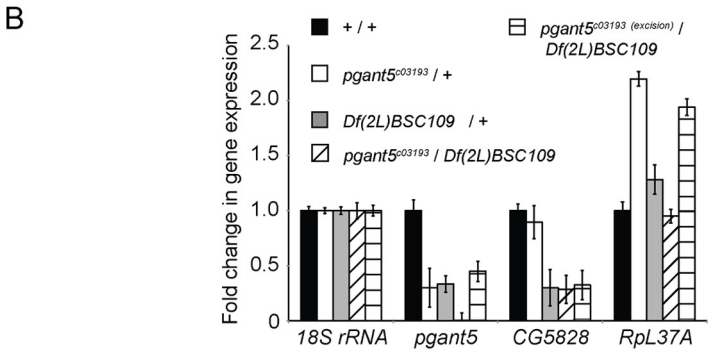
*pgant3 Is Uniquely Responsible for Wing Adhesion*—We further employed this RNAi system to address unique roles for individual *pgant* family members in a specific developmental process. Previous work from our laboratory demonstrated that loss of *pgant3* results in defects in the secretion of an extracellular matrix protein, with effects on integrin-mediated cell adhesion, resulting in wing blistering. To investigate whether other *pgant* family members are also involved in integrin-mediated cell adhesion in the developing wing, we performed RNAi to each in the developing wing using the wing-specific Gal4 driver, MS1096. Although RNAi to *pgant3* resulted in wing blistering, no blistering was seen upon induction of RNAi to the remaining family members (Table 4). These results suggest a potentially unique role for *pgant3* in specific cell adhesion processes occurring in the developing wing and are supported by previous results indicating that the expression of another family member cannot compensate for the loss of *pgant3 in vivo* (31).

## DISCUSSION

Here, we identify 4 additional members of this multigene glycosyltransferase family that are essential for viability by taking advantage of the highly efficient and specific *in vivo* RNAi system in *Drosophila*. This work serves to highlight the biological importance of *O*-linked glycosylation and of specific family members responsible for initiating this conserved post-translational modification. Additionally, these studies indicate that at least 5 family members in the fly are serving unique and nonredundant functions during development. Thus, whereas these members all catalyze the transfer of GalNAc to serine or threonine, they are doing so on specific substrates and/or at specific positions within these substrates. Previous enzymatic studies demonstrated that many mammalian GalNAc-Ts and *Drosophila* PGANTs have unique substrate preferences and sites of GalNAc addition *in vitro*, suggesting that certain substrates can only be glycosylated by certain enzymes (17, 18, 44, 45) (reviewed in Refs. 6, 12, and 15). Our *in vivo* results support this hypothesis and suggest a highly complex system for proper *O*-glycosylation of proteins.

The *in vivo* RNAi system also offers the unique advantage of allowing the knockdown of genes in specific tissues to determine exactly where a particular gene is required. We used Gal4 driver lines that induced RNAi in most major tissue types and developing organ systems to determine where each essential *pgant* was required. In agreement with previous studies demonstrating that conventional *pgant35A* mutants affect respiratory system development, we found that RNAi to *pgant35A* in the tracheal system resulted in significantly reduced viability. Interestingly, each essential gene was found to be required in a specific subset of tissues and organ systems, suggesting unique functional roles for each. Developing organs known for the abundant production of *O*-glycoproteins (digestive and respiratory systems) (11) were most significantly affected by the knockdown of *pgant* family members. Knockdown of *pgant4, pgant5, pgant35A*, or the putative transferase *CG30463* in the digestive system resulted in significant losses of viability. In addition to *pgant35A*, knockdown of *pgant5, pgant7,* or




C

| Genotype | Viability |
|---|---|
| + / + | 100% |
| *pgant5*$^{c03193}$ / + | 100% (n=110) |
| *Df(2L)BSC109* / + | 100% (n=180) |
| *pgant5*$^{c03193}$ / *Df(2L)BSC109* | 2% (n=315) |
| *pgant5*$^{c03193\ (excision)}$ / *Df(2L)BSC109* | 100% (n=228) |

FIGURE 3. **Transposon insertion mutation in *pgant5* causes lethality and confirms *pgant5* is an essential gene.** *A*, the position of the transposon insertion in intron 2 of *pgant5* is shown. Exons are represented as *boxes* and introns are represented as *lines*. *Blue boxes* are coding regions and *white boxes* are noncoding regions of *pgant5*. Flanking genes are also shown. Regions used to generate PCR primers are represented as *triangles*. *B,* real-time PCR analysis of *pgant5* transcript levels using the primer pairs shown in *A* reveals a significant decrease in *pgant5* gene expression in *pgant5*$^{c03193}$/*Df(2L)BSC109* relative to *pgant5*$^{c03193}$/+ heterozygotes, *Df(2L)BSC109*/+ heterozygotes, *pgant5*$^{c03193(excision)}$/*Df(2L)BSC109*, or wild type. RNA was normalized to 18S rRNA. *C,* loss of *pgant5* results in lethality.

*CG30463* in the respiratory system also affected viability. *CG30463* was also found to be required in the amnioserosa, an embryonic tissue involved in developmentally regulated cell migration events. Finally, 3 of the 5 essential *pgants* were required in the mesoderm. The results presented here will form the basis for future studies investigating the mechanistic role of these essential *pgant*s in diverse tissues and developmental stages with the hope of gaining insight into the role of their mammalian orthologues.

To begin to address the specific role of one of the novel essential genes identified in this screen, we further characterized the phenotypes associated with the loss of *pgant5*. Interestingly, loss of *pgant5* resulted in the disruption of the proper structure and function of specialized cells of the digestive tract responsible for gut acidification. Our studies revealed that the loss of *pgant5* caused loss of *O*-glycoproteins along the apical and luminal surfaces of copper cells of the midgut, along with an irregular apical actin-based microvillar structure in *pgant5*$^{c03193}$/*Df(2L)BSC109.* Restoration of *pgant5* expression resulted in restoration of *O*-glycosylation, actin-based microvilli, and proper gut acidification. Western blots revealed that the loss of *pgant5* affected the glycosylation of many proteins, making it difficult to discern what the primary target(s) might be. It remains possible that PGANT5 could be glycosylating subunits of the ion transporters responsible for gut acidification. Alternatively, PGANT5 could be glycosylating other proteins involved in the localization of ion transporters or the establishment of proper apical polarity within the copper cells. In support of this, previous work on PGANT35A in the respiratory tract demonstrated that it also glycosylates apical and luminal proteins and affects apical-basal polarity when mutated (29). As *O*-glycoproteins are abundant along the apical regions of many developing tissues (11), it is possible that they are performing functions related to maintenance of the unique characteristics of apical surfaces.

Previous work on the mammalian orthologue of *pgant5* (*Galnt1*) did not examine the digestive system but did find lymphocyte homing defects (due to decreased presence of selectin ligands on lymphocytes and endothelial cells) and bleeding disorders (due to decreased plasma levels of blood coagulation factors) in *Galnt1*$^{-/-}$ mice (32). However, the specific substrates involved and the mechanism(s) by which these phenotypes occur remain unknown (32). Nonetheless, the results presented here highlight a previously unknown role for *pgant5* in digestive tract function and provide a basis for examining the

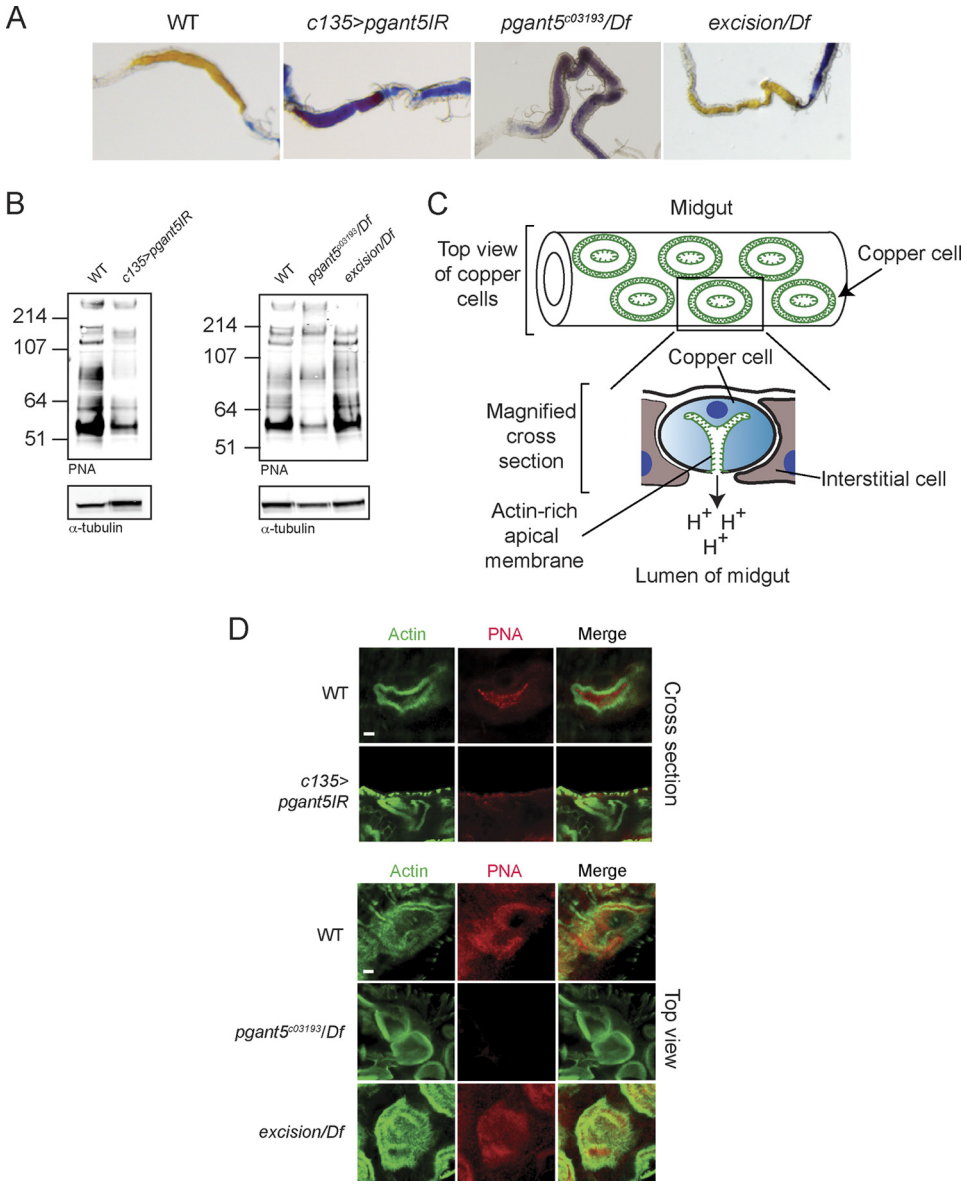



FIGURE 4. **Loss of *pgant5* results in loss of gut acidification and disruption of *O*-glycosylation in copper cells of the digestive tract.** *A*, larval gut acidification as detected with bromophenol blue shows acidified midguts (*yellow*) in wild type and *pgant5* transposon excision (*excision/Df*), but basic midguts (*blue*) in *pgant5* mutants (*pgant5*$^{c03193}$*/Df*) or larvae expressing dsRNA to *pgant5* in the digestive system (*c135>pgant5IR*). *B*, Western blots of proteins from wild type, *c135>pgant5IR*, *pgant5*$^{c03193}$*/Df*, and *excision/Df* third instar larval midguts probed with the lectin PNA to detect *O*-glycosylated proteins or with α-tubulin as a loading control. *C*, diagram of midgut and copper cell morphology. Shown is a diagram of a section of the midgut where copper cells stain for actin (*green*) in *concentric circles*. Below is a magnified cross-section of a copper cell, highlighting the unique invaginated actin-rich apical surface (*green*). *D*, copper cells from wild type, *c135>pgant5IR*, *pgant5*$^{c03193}$*/Df*, and *excision/Df* third instar larval midguts were stained with PNA (*red*) and phalloidin/actin (*green*). In wild type copper cells, phalloidin staining labels actin-based microvilli unique to the apical region of copper cells. PNA-reactive *O*-glycoproteins are found along the apical and luminal regions of wild type copper cells (shown in *upper panel cross-sections*). All apical and luminal PNA staining is lost from *c135>pgant5IR* (*upper panel*) and *pgant5*$^{c03193}$*/Df* copper cells (*lower panel*). Additionally, apical actin staining is irregular in size and shape in *pgant5*$^{c03193}$*/Df* copper cells. PNA staining and gut acidification are restored upon excision of the transposon (*excision/Df*). *Scale bars* = 4 μm.

role of *Galnt1* in mammalian digestive system function. There is precedent for mucins and mucin-type *O*-glycosylation having functional roles in the digestive system as the loss of a major *O*-glycosylated mucin (Muc2) or the disruption of certain *O*-glycan structures results in digestive tract abnormalities, including increased rates of infection, decreased barrier function, and increased susceptibility to colitis and colon cancer (26, 46–48). Future studies on PGANT5 in the *Drosophila* gut will identify direct *in vivo* targets of this enzyme and define how *O*-glycosylation regulates their function.

As mentioned above, the putative glycosyltransferase *CG30463* was found to be essential for viability and required in multiple developing tissues. Based on sequence conservation, *CG30463* is assumed to be a member of the *pgant* family. However, *in vitro* biochemical assays have failed to detect glycosyltransferase activity for the purified recombinant CG30463 protein (data not shown), thus its biological relevance has been unclear. The data shown here, indicating that knockdown of *CG30463* in specific tissues results in lethality, demonstrates a crucial *in vivo* role for this gene. It is possible that the CG30463 protein has very specific

**TABLE 4**
**Wing blistering in flies expressing dsRNA to *pgant* genes in the developing wing**

| Gene knocked down | Blistered[a] | n[b] |
|---|---|---|
| | % | |
| *pgant2* | 0 | 253 |
| *pgant3* | 18 | 200 |
| *pgant4* | 0 | 114 |
| *pgant5* | 0 | 120 |
| *pgant6* | 0 | 178 |
| *pgant7* | 0 | 160 |
| *pgant35A* | 0 | 172 |
| *CG30463* | 0 | 178 |
| *CG10000* | 0 | 235 |
| *CG31776* | 0 | 210 |

[a] Represents the (number of flies displaying blistered wings/total number of flies of denoted genotype) × 100.
[b] *n,* total number of flies of denoted genotype scored.

substrate preferences and thus its *in vitro* enzymatic activity may not have been detectable with the panel of substrate peptides used. Future experiments will use this RNAi system to investigate *in vivo* changes in *O*-glycosylation upon *CG30463* knockdown.

Finally, we used this *in vivo* RNAi system to address the role of each family member in a specific developmental process, integrin-mediated cell adhesion. Here we demonstrate that *pgant3* has a unique role in integrin-mediated wing blade adhesion, as RNAi to other family members had no effect on wing blistering. Our results, demonstrating a unique function for *pgant3* in the wing, are also supported by genetic data indicating that expression of another family member in the wing could not compensate for loss of *pgant3* (31). These studies indicate that PGANT3 may be uniquely responsible for glycosylating the extracellular matrix protein Tiggrin in the wing disc, further supporting the notion of unique substrate preferences *in vivo*. The role for other *pgant*s in specific cell adhesion events in other tissues will be investigated using this methodology.

In summary, we have identified 4 additional members of this glycosyltransferase family that are essential for viability in *Drosophila*, highlighting the importance of this protein modification and unique requirements for individual family members. Future work will define the specific functions and substrates of each member in *Drosophila* to gain insight into the roles of *O*-glycosylation in development and disease.

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## REFERENCES

1. Hart, G. W., and Copeland, R. J. (2010) Glycomics hits the big time. *Cell* **143,** 672–676
2. Liu, L., Xu, Y. X., and Hirschberg, C. B. (2010) The role of nucleotide sugar transporters in development of eukaryotes. *Semin. Cell Dev. Biol.* **21,** 600–608
3. Love, D. C., Krause, M. W., and Hanover, J. A. (2010) *O*-GlcNAc cycling. Emerging roles in development and epigenetics. *Semin. Cell Dev. Biol.* **21,** 646–654
4. Nakamura, N., Lyalin, D., and Panin, V. M. (2010) Protein *O*-mannosylation in animal development and physiology. From human disorders to *Drosophila* phenotypes. *Semin. Cell Dev. Biol.* **21,** 622–630
5. Schachter, H. (2010) Mgat1-dependent *N*-glycans are essential for the normal development of both vertebrate and invertebrate metazoans. *Semin. Cell Dev. Biol.* **21,** 609–615
6. Tabak, L. A. (2010) The role of mucin-type *O*-glycans in eukaryotic development. *Semin. Cell Dev. Biol.* **21,** 616–621
7. Takeuchi, H., and Haltiwanger, R. S. (2010) Role of glycosylation of Notch in development. *Semin. Cell Dev. Biol.* **21,** 638–645
8. Kramer, K. L. (2010) Specific sides to multifaceted glycosaminoglycans are observed in embryonic development. *Semin. Cell Dev. Biol.* **21,** 631–637
9. Kingsley, P. D., Hagen, K. G., Maltby, K. M., Zara, J., and Tabak, L. A. (2000) Diverse spatial expression patterns of UDP-GalNAc:polypeptide *N*-acetylgalactosaminyltransferase family member mRNAs during mouse development. *Glycobiology* **10,** 1317–1323
10. Tian, E., and Ten Hagen, K. G. (2006) Expression of the UDP-GalNAc:polypeptide *N*-acetylgalactosaminyltransferase family is spatially and temporally regulated during *Drosophila* development. *Glycobiology* **16,** 83–95
11. Tian, E., and Ten Hagen, K. G. (2007) *O*-Linked glycan expression during *Drosophila* development. *Glycobiology* **17,** 820–827
12. Tian, E., and Ten Hagen, K. G. (2009) Recent insights into the biological roles of mucin-type *O*-glycosylation. *Glycoconj. J.* **26,** 325–334
13. Young, W. W., Jr., Holcomb, D. R., Ten Hagen, K. G., and Tabak, L. A. (2003) Expression of UDP-GalNAc:polypeptide *N*-acetylgalactosaminyltransferase isoforms in murine tissues determined by real-time PCR. A new view of a large family. *Glycobiology* **13,** 549–557
14. Gerken, T. A., Ten Hagen, K. G., and Jamison, O. (2008) Conservation of peptide acceptor preferences between *Drosophila* and mammalian polypeptide-GalNAc-transferase ortholog pairs. *Glycobiology* **18,** 861–870
15. Ten Hagen, K. G., Fritz, T. A., and Tabak, L. A. (2003) All in the family. The UDP-GalNAc:polypeptide *N*-acetylgalactosaminyltransferases. *Glycobiology* **13,** 1R–16R
16. Perrine, C. L., Ganguli, A., Wu, P., Bertozzi, C. R., Fritz, T. A., Raman, J., Tabak, L. A., and Gerken, T. A. (2009) Glycopeptide-preferring polypeptide GalNAc-transferase 10 (ppGalNAc T10), involved in mucin-type *O*-glycosylation, has a unique GalNAc-*O*-Ser/Thr-binding site in its catalytic domain not found in ppGalNAc T1 or T2. *J. Biol. Chem.* **284,** 20387–20397
17. Ten Hagen, K. G., Tetaert, D., Hagen, F. K., Richet, C., Beres, T. M., Gagnon, J., Balys, M. M., VanWuyckhuyse, B., Bedi, G. S., Degand, P., and Tabak, L. A. (1999) Characterization of a UDP-GalNAc:polypeptide *N*-acetylgalactosaminyltransferase that displays glycopeptide *N*-acetylgalactosaminyltransferase activity. *J. Biol. Chem.* **274,** 27867–27874
18. Ten Hagen, K. G., Tran, D. T., Gerken, T. A., Stein, D. S., and Zhang, Z. (2003) Functional characterization and expression analysis of members of the UDP-GalNAc:polypeptide *N*-acetylgalactosaminyltransferase family from *Drosophila melanogaster*. *J. Biol. Chem.* **278,** 35039–35048
19. Ju, T., and Cummings, R. D. (2005) Protein glycosylation. Chaperone mutation in Tn syndrome. *Nature* **437,** 1252
20. Ichikawa, S., Guigonis, V., Imel, E. A., Courouble, M., Heissat, S., Henley, J. D., Sorenson, A. H., Petit, B., Lienhardt, A., and Econs, M. J. (2007) Novel GALNT3 mutations causing hyperostosis-hyperphosphatemia syndrome result in low intact fibroblast growth factor 23 concentrations. *J. Clin. Endocrinol. Metab.* **92,** 1943–1947
21. Topaz, O., Shurman, D. L., Bergman, R., Indelman, M., Ratajczak, P., Mizrachi, M., Khamaysi, Z., Behar, D., Petronius, D., Friedman, V., Zelikovic, I., Raimer, S., Metzker, A., Richard, G., and Sprecher, E. (2004) Mutations in GALNT3, encoding a protein involved in *O*-linked glycosylation, cause familial tumoral calcinosis. *Nat. Genet.* **36,** 579–581
22. Kathiresan, S., Manning, A. K., Demissie, S., D'Agostino, R. B., Surti, A., Guiducci, C., Gianniny, L., Burtt, N. P., Melander, O., Orho-Melander, M., Arnett, D. K., Peloso, G. M., Ordovas, J. M., and Cupples, L. A. (2007) A genome-wide association study for blood lipid phenotypes in the Framingham Heart Study. *BMC Med. Genet.* **8,** Suppl. 1, S17
23. Teslovich, T. M., Musunuru, K., Smith, A. V., Edmondson, A. C., Stylianou, I. M., Koseki, M., Pirruccello, J. P., Ripatti, S., Chasman, D. I., Willer, C. J., Johansen, C. T., Fouchier, S. W., Isaacs, A., Peloso, G. M., Barbalic, M., Ricketts, S. L., Bis, J. C., Aulchenko, Y. S., Thorleifsson, G., Feitosa, M. F., Chambers, J., Orho-Melander, M., Melander, O., Johnson, T., Li, X., Guo, X., Li, M., Shin Cho, Y., Jin Go, M., Jin Kim, Y., Lee, J. Y., Park, T., Kim, K., Sim, X., Twee-Hee Ong, R., Croteau-Chonka, D. C., Lange, L. A., Smith, J. D., Song, K.,

Hua Zhao, J., Yuan, X., Luan, J., Lamina, C., Ziegler, A., Zhang, W., Zee, R. Y., Wright, A. F., Witteman, J. C., Wilson, J. F., Willemsen, G., Wichmann, H. E., Whitfield, J. B., Waterworth, D. M., Wareham, N. J., Waeber, G., Vollenweider, P., Voight, B. F., Vitart, V., Uitterlinden, A. G., Uda, M., Tuomilehto, J., Thompson, J. R., Tanaka, T., Surakka, I., Stringham, H. M., Spector, T. D., Soranzo, N., Smit, J. H., Sinisalo, J., Silander, K., Sijbrands, E. J., Scuteri, A., Scott, J., Schlessinger, D., Sanna, S., Salomaa, V., Saharinen, J., Sabatti, C., Ruokonen, A., Rudan, I., Rose, L. M., Roberts, R., Rieder, M., Psaty, B. M., Pramstaller, P. P., Pichler, I., Perola, M., Penninx, B. W., Pedersen, N. L., Pattaro, C., Parker, A. N., Pare, G., Oostra, B. A., O'Donnell, C. J., Nieminen, M. S., Nickerson, D. A., Montgomery, G. W., Meitinger, T., McPherson, R., McCarthy, M. I., McArdle, W., Masson, D., Martin, N. G., Marroni, F., Mangino, M., Magnusson, P. K., Lucas, G., Luben, R., Loos, R. J., Lokki, M. L., Lettre, G., Langenberg, C., Launer, L. J., Lakatta, E. G., Laaksonen, R., Kyvik, K. O., Kronenberg, F., Konig, I. R., Khaw, K. T., Kaprio, J., Kaplan, L. M., Johansson, A., Jarvelin, M. R., Janssens, A. C., Ingelsson, E., Igl, W., Kees Hovingh, G., Hottenga, J. J., Hofman, A., Hicks, A. A., Hengstenberg, C., Heid, I. M., Hayward, C., Havulinna, A. S., Hastie, N. D., Harris, T. B., Haritunians, T., Hall, A. S., Gyllensten, U., Guiducci, C., Groop, L. C., Gonzalez, E., Gieger, C., Freimer, N. B., Ferrucci, L., Erdmann, J., Elliott, P., Ejebe, K. G., Doring, A., Dominiczak, A. F., Demissie, S., Deloukas, P., de Geus, E. J., de Faire, U., Crawford, G., Collins, F. S., Chen, Y. D., Caulfield, M. J., Campbell, H., Burtt, N. P., Bonnycastle, L. L., Boomsma, D. I., Boekholdt, S. M., Bergman, R. N., Barroso, I., Bandinelli, S., Ballantyne, C. M., Assimes, T. L., Quertermous, T., Altshuler, D., Seielstad, M., Wong, T. Y., Tai, E. S., Feranil, A. B., Kuzawa, C. W., Adair, L. S., Taylor, H. A., Jr., Borecki, I. B., Gabriel, S. B., Wilson, J. G., Holm, H., Thorsteinsdottir, U., Gudnason, V., Krauss, R. M., Mohlke, K. L., Ordovas, J. M., Munroe, P. B., Kooner, J. S., Tall, A. R., Hegele, R. A., Kastelein, J. J., Schadt, E. E., Rotter, J. I., Boerwinkle, E., Strachan, D. P., Mooser, V., Stefansson, K., Reilly, M. P., Samani, N. J., Schunkert, H., Cupples, L. A., Sandhu, M. S., Ridker, P. M., Rader, D. J., van Duijn, C. M., Peltonen, L., Abecasis, G. R., Boehnke, M., and Kathiresan, S. (2010) Biological, clinical and population relevance of 95 loci for blood lipids. *Nature* **466,** 707–713

24. Willer, C. J., Sanna, S., Jackson, A. U., Scuteri, A., Bonnycastle, L. L., Clarke, R., Heath, S. C., Timpson, N. J., Najjar, S. S., Stringham, H. M., Strait, J., Duren, W. L., Maschio, A., Busonero, F., Mulas, A., Albai, G., Swift, A. J., Morken, M. A., Narisu, N., Bennett, D., Parish, S., Shen, H., Galan, P., Meneton, P., Hercberg, S., Zelenika, D., Chen, W. M., Li, Y., Scott, L. J., Scheet, P. A., Sundvall, J., Watanabe, R. M., Nagaraja, R., Ebrahim, S., Lawlor, D. A., Ben-Shlomo, Y., Davey-Smith, G., Shuldiner, A. R., Collins, R., Bergman, R. N., Uda, M., Tuomilehto, J., Cao, A., Collins, F. S., Lakatta, E., Lathrop, G. M., Boehnke, M., Schlessinger, D., Mohlke, K. L., and Abecasis, G. R. (2008) Newly identified loci that influence lipid concentrations and risk of coronary artery disease. *Nat. Genet.* **40,** 161–169
25. Fakhro, K. A., Choi, M., Ware, S. M., Belmont, J. W., Towbin, J. A., Lifton, R. P., Khokha, M. K., and Brueckner, M. (2011) Rare copy number variations in congenital heart disease patients identify unique genes in left-right patterning. *Proc. Natl. Acad. Sci. U.S.A.* **108,** 2915–2920
26. Guda, K., Moinova, H., He, J., Jamison, O., Ravi, L., Natale, L., Lutterbaugh, J., Lawrence, E., Lewis, S., Willson, J. K., Lowe, J. B., Wiesner, G. L., Parmigiani, G., Barnholtz-Sloan, J., Dawson, D. W., Velculescu, V. E., Kinzler, K. W., Papadopoulos, N., Vogelstein, B., Willis, J., Gerken, T. A., and Markowitz, S. D. (2009) Inactivating germ-line and somatic mutations in polypeptide *N*-acetylgalactosaminyltransferase 12 in human colon cancers. *Proc. Natl. Acad. Sci. U.S.A.* **106,** 12921–12925
27. Schwientek, T., Bennett, E. P., Flores, C., Thacker, J., Hollmann, M., Reis, C. A., Behrens, J., Mandel, U., Keck, B., Schäfer, M. A., Haselmann, K., Zubarev, R., Roepstorff, P., Burchell, J. M., Taylor-Papadimitriou, J., Hollingsworth, M. A., and Clausen, H. (2002) Functional conservation of subfamilies of putative UDP-*N*-acetylgalactosamine:polypeptide *N*-acetylgalactosaminyltransferases in *Drosophila*, *Caenorhabditis elegans*, and mammals. One subfamily composed of l(2)35Aa is essential in *Drosophila*. *J. Biol. Chem.* **277,** 22623–22638
28. Ten Hagen, K. G., and Tran, D. T. (2002) A UDP-GalNAc:polypeptide *N*-acetylgalactosaminyltransferase is essential for viability in *Drosophila melanogaster*. *J. Biol. Chem.* **277,** 22616–22622
29. Tian, E., and Ten Hagen, K. G. (2007) A UDP-GalNAc:polypeptide *N*-acetylgalactosaminyltransferase is required for epithelial tube formation. *J. Biol. Chem.* **282,** 606–614
30. Zhang, L., Tran, D. T., and Ten Hagen, K. G. (2010) An *O*-glycosyltransferase promotes cell adhesion during development by influencing secretion of an extracellular matrix integrin ligand. *J. Biol. Chem.* **285,** 19491–19501
31. Zhang, L., Zhang, Y., and Hagen, K. G. (2008) A mucin-type *O*-glycosyltransferase modulates cell adhesion during *Drosophila* development. *J. Biol. Chem.* **283,** 34076–34086
32. Tenno, M., Ohtsubo, K., Hagen, F. K., Ditto, D., Zarbock, A., Schaerli, P., von Andrian, U. H., Ley, K., Le, D., Tabak, L. A., and Marth, J. D. (2007) Initiation of protein *O*-glycosylation by the polypeptide GalNAcT-1 in vascular biology and humoral immunity. *Mol. Cell. Biol.* **27,** 8783–8796
33. Pastor-Pareja, J. C., and Xu, T. (2011) Shaping cells and organs in *Drosophila* by opposing roles of fat body-secreted collagen IV and perlecan. *Dev. Cell* **21,** 245–256
34. Neumüller, R. A., Richter, C., Fischer, A., Novatchkova, M., Neumüller, K. G., and Knoblich, J. A. (2011) Genome-wide analysis of self-renewal in *Drosophila* neural stem cells by transgenic RNAi. *Cell Stem Cell* **8,** 580–593
35. Zacharogianni, M., Kondylis, V., Tang, Y., Farhan, H., Xanthakis, D., Fuchs, F., Boutros, M., and Rabouille, C. (2011) *EMBO J.* **30,** 3684–3700
36. Shiga, Y., Tanaka-Matakatsu, M., and Hayashi, S. (1996) A nuclear GFP/$\beta$-galactosidase fusion protein as a marker for morphogenesis in living *Drosophila*. *Dev. Growth Differ.* **38,** 99–106
37. Dietzl, G., Chen, D., Schnorrer, F., Su, K. C., Barinova, Y., Fellner, M., Gasser, B., Kinsey, K., Oppel, S., Scheiblauer, S., Couto, A., Marra, V., Keleman, K., and Dickson, B. J. (2007) A genome-wide transgenic RNAi library for conditional gene inactivation in *Drosophila*. *Nature* **448,** 151–156
38. Thibault, S. T., Singer, M. A., Miyazaki, W. Y., Milash, B., Dompe, N. A., Singh, C. M., Buchholz, R., Demsky, M., Fawcett, R., Francis-Lang, H. L., Ryner, L., Cheung, L. M., Chong, A., Erickson, C., Fisher, W. W., Greer, K., Hartouni, S. R., Howie, E., Jakkula, L., Joo, D., Killpack, K., Laufer, A., Mazzotta, J., Smith, R. D., Stevens, L. M., Stuber, C., Tan, L. R., Ventura, R., Woo, A., Zakrajsek, I., Zhao, L., Chen, F., Swimmer, C., Kopczynski, C., Duyk, G., Winberg, M. L., and Margolis, J. (2004) A complementary transposon tool kit for *Drosophila melanogaster* using P and piggyBac. *Nat. Genet.* **36,** 283–287
39. Lee, Y. S., and Carthew, R. W. (2003) Making a better RNAi vector for *Drosophila*. Use of intron spacers. *Methods* **30,** 322–329
40. Rubin, G. M., and Spradling, A. C. (1982) Genetic transformation of *Drosophila* with transposable element vectors. *Science* **218,** 348–353
41. Spradling, A. C., and Rubin, G. M. (1982) Transposition of cloned P elements into *Drosophila* germ line chromosomes. *Science* **218,** 341–347
42. Dubreuil, R. R. (2004) Copper cells and stomach acid secretion in the *Drosophila* midgut. *Int. J. Biochem. Cell Biol.* **36,** 745–752
43. Shanbhag, S., and Tripathi, S. (2009) Epithelial ultrastructure and cellular mechanisms of acid and base transport in the *Drosophila* midgut. *J. Exp. Biol.* **212,** 1731–1744
44. Ten Hagen, K. G., Bedi, G. S., Tetaert, D., Kingsley, P. D., Hagen, F. K., Balys, M. M., Beres, T. M., Degand, P., and Tabak, L. A. (2001) Cloning and characterization of a ninth member of the UDP-GalNAc:polypeptide *N*-acetylgalactosaminyltransferase family, ppGaNTase-T9. *J. Biol. Chem.* **276,** 17395–17404
45. Wandall, H. H., Hassan, H., Mirgorodskaya, E., Kristensen, A. K., Roepstorff, P., Bennett, E. P., Nielsen, P. A., Hollingsworth, M. A., Burchell, J., Taylor-Papadimitriou, J., and Clausen, H. (1997) Substrate specificities of three members of the human UDP-*N*-acetyl-$\alpha$-D-galactosamine:polypeptide *N*-acetylgalactosaminyltransferase family, GalNAc-T1, -T2, and -T3. *J. Biol. Chem.* **272,** 23503–23514
46. Van der Sluis, M., De Koning, B. A., De Bruijn, A. C., Velcich, A., Meijerink, J. P., Van Goudoever, J. B., Büller, H. A., Dekker, J., Van Seuningen, I., Renes, I. B., and Einerhand, A. W. (2006) Muc2-deficient mice spontaneously develop colitis, indicating that MUC2 is critical for colonic protection. *Gastroenterology* **131,** 117–129
47. Velcich, A., Yang, W., Heyer, J., Fragale, A., Nicholas, C., Viani, S., Kucherlapati, R., Lipkin, M., Yang, K., and Augenlicht, L. (2002) Colorectal cancer in mice genetically deficient in the mucin Muc2. *Science* **295,** 1726–1729
48. An, G., Wei, B., Xia, B., McDaniel, J. M., Ju, T., Cummings, R. D., Braun, J., and Xia, L. (2007) Increased susceptibility to colitis and colorectal tumors in mice lacking core 3-derived *O*-glycans. *J. Exp. Med.* **204,** 1417–1429