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A new member of the GM130 golgin subfamily is expressed in the optic lobe anlagen of the metamorphosing brain of *Manduca sexta*

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Abstract

During metamorphosis of the insect brain, the optic lobe anlagen generate the proliferation centers for the visual cortices. We show here that, in the moth *Manduca sexta*, an 80 kDa Golgi complex protein (Ms-golgin80) is abundantly expressed in the cytoplasm of neuroblasts and ganglion mother cells in the optic lobe anlagen and proliferation centers. The predicted amino acid sequence for Ms-golgin80 is similar to that of several members of the GM130 subfamily of Golgi-associated proteins, including rat GM130 and human golgin-95. Homologs of Ms-golgin80 from *Drosophila melanogaster, Caenorhabditis elegans, and Brugia malayi* were identified through homology sequence search. Sequence similarities are present in three regions: the N-terminus, an internal domain of 89 amino acids, and another domain of 89 amino acids near the C-terminus. Structural similarities further suggest that these molecules play the same cellular role as GM130. GM130 is involved in the docking and fusion of coatomer (COP I) coated vesicles to the Golgi membranes; it also regulates the fragmentation and subsequent reassembly of the Golgi complex during mitosis. Abundant expression of Ms-golgin80 in neuroblasts and ganglion mother cells and its reduced expression in the neuronal progeny of these cells suggest that this protein may be involved in the maintenance of the proliferative state.

Keywords: Golgi complex proteins; mitosis; Ms-golgin80; neuroblasts; tobacco hornworm

Abbreviation:

BrdU	5-bromo-2'-deoxyuridine
COP I, II	coatomer proteins that coat vesicles and direct protein and membrane trafficking between early compartments of the secretory pathway in eukaryotic cells
GM130	Peripheral membrane proteins associated with the Golgi bodies.
GMC	ganglion mother cell
Ms-golgin80	The *Manduca sexta* homolog of the GM130 protein. Other homologs include Rn-GM130 (*Rattus norvegicus)*; Hs-GM130 (*Homo sapiens*), Dm-golgin90 (*Drosophila melanogaster*), Ce-golgin107 (*Caenorhabditis elegans*) and Bm-golgin (*Brugia malayi*)
PBS	phosphate buffered saline
PBST	phosphate buffered saline containing 0.05% Tween-20
TBST	Tris buffered saline containing 0.05% Tween-20

Introduction

In insects with complete metamorphosis, the compound eyes are present only in adults. Correspondingly, during metamorphosis, the optic lobes of the brain, which receive inputs from the new compound eyes, must increase dramatically in size. In Lepidoptera, the growth of each optic lobe in its horizontal dimension during metamorphosis is about twenty-fold: the width of the remainder of the protocerebrum increases only eight-fold during this time (Nordlander and Edwards, 1969a). This growth reflects intense mitotic activity in a population of neuronal precursor cells referred to as the optic lobe anlagen.

The adult optic lobe is composed of three major cortices and their associated neuropils, the lamina (outermost), the medulla, and the lobula (innermost). Neurons of these three optic lobe cortices are generated by proliferation centers of ganglion mother cells (GMCs). These centers are in turn derived from the optic lobe anlagen, which are aggregations of stem cells (neuroblasts). The optic lobe anlagen are divided into distinct outer and inner optic lobe anlagen during development. The outer optic lobe anlagen gives

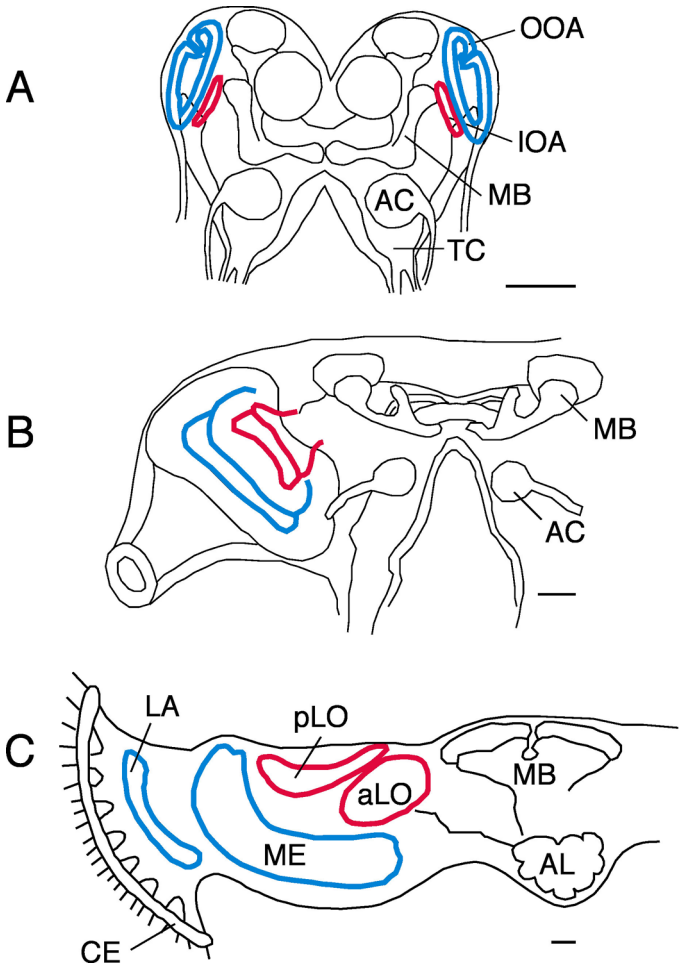

Figure 1. Overview of metamorphosis of the optic lobes of *Manduca sexta*. Schematic diagrams based on our sectioned material and on descriptions given in Nordlander and Edwards (1968). A. Overview of larval brain showing outer optic anlagen highlighted in blue and inner optic anlagen in red. The anlagen are shown in their entirety as they would be seen in wholemount rather than as they would appear in a single section. B. Representative section through a pupal brain showing the outer optic anlagen in blue and the inner optic anlagen in red. C. Top view of the adult brain, showing the distinct lamina, medulla, and lobula of the mature optic lobe. The lamina and medulla are outlined in blue to indicate their origin in the outer optic anlagen. The anterior and posterior portions of the lobula are outlined in red to reflect their origin in the inner optic anlagen. Abbreviations: AC, antennal center of the larval and pupal brain; AL, antennal lobe of the adult brain; aLO, anterior portion of the lobula; CE, compound eye; IOA, inner optic anlagen; LA, lamina; MB, neuropils associated with the mushroom bodies; ME, medulla; OOA, outer optic anlagen; pLO, posterior portion of the lobula; TC, tritocerebrum. Scale bar = 100 μm.

rise to the lamina and medulla; the inner optic lobe anlagen produces the lobula (Meinertzhagen 1973; Meinertzhagen and Hanson 1993). Fig. 1 provides a schematic overview of these general brain regions during metamorphosis in Lepidoptera. Both symmetric and asymmetric patterns of division have been demonstrated for these populations of neuroblasts (Panov, 1960; Monsma and Booker, 1996a). Symmetric divisions of neuroblasts give rise to a new pair of neuroblasts, and contribute to the expansion and maintenance of the stem cell population itself. Asymmetric divisions of neuroblasts, which typically occur at the edges of the optic lobe anlagen, yield a neuroblast and a GMC. The GMCs then undergo a final round of symmetric division, and their progeny are the neurons of the optic lobes (Nordlander and Edwards, 1969b; Monsma and Booker, 1996a).

Mitosis of optic lobe anlagen cells in *Manduca sexta* is responsive to the titer of ecdysone, with higher rates of mitosis associated with the presence of the steroid. The mechanisms of ecdysone control are not known, although interactions with nitric oxide signaling pathways appear to be involved (Monsma and Booker, 1996b; Champlin and Truman, 1998; Champlin and Truman, 2000). Several characterized *Drosophila melanogaster* genes are expressed in the optic lobe anlagen during development. *Asense*, and *lethal (1) optomotor-blind* are nuclear transcription factors (Gonzalez *et al.*, 1989; Pflugfelder *et al.*, 1992). *Giant lens* is a secretory protein with an EGF domain (Kretzschmar *et al.*, 1992). *Neuralized* is a ubiquitin ligase that functions to down-regulate the Notch ligand Delta (Boulianne *et al.*, 1991; Lai *et al.*, 2001; Deblandre *et al.*, 2001; Pavlopoulos *et al.*, 2001). Mutants for these genes show defects in neuronal pathfinding and morphological anomalies in the optic lobes. Many of these genes are apparently upstream neurogenic determinants. In contrast to our growing knowledge of the mechanisms of neuronal specification, the genes that maintain the mitotic properties of the neuroblasts in the optic lobe anlagen are unknown.

In a search for candidate genes that assist in maintenance of the proliferative status of cells in optic lobe anlagen, the Golgi complex attracts interest. The unitary Golgi complex must be partitioned between daughter cells during mitosis (Lowe *et al.*, 1998a). Although the Golgi complex has been traditionally viewed as having only an indirect involvement in mitosis (Farquhar and Palade, 1998), there is recent evidence that fragmentation of the Golgi is necessary condition for entry into the mitotic phase in mammalian cells (Sutterlin *et al.,* 2002). The structure of the Golgi complex varies among cell types, with differences often most clearly seen when proliferating and differentiated cell populations are compared. For example, the dispersed Golgi stacks double just prior to mitosis in the developing root tips of plants (Staehelin *et al.*, 1990; Mollenhauer and Morre, 1991; Lynch and Staehelin, 1992), and variations in Golgi morphology within the plant root tip are believed to reflect different functional demands upon the Golgi complex (Driouich and Staehelin, 1997; Nebenfuhr and Staehelin, 2001). These observations on the architecture and functional organization of the Golgi stacks suggest that regulation of the Golgi complex plays a role in maintenance of the intense proliferative state that characterizes postembryonic neurogenesis in the insect brain.

Although it has been known for some time that inhibition of cellular membrane traffic is associated with the fragmentation of the Golgi complex during mitosis in animal cells, molecular evidence for a mechanism of mitosis-associated disassembly of the Golgi complex came more recently from studies of the Golgi complex structural protein, GM130 (Warren, 1993; Lowe *et al.*, 1998a). GM130, a peripheral membrane protein of the Golgi complex, is associated with the tethering machinery for vesicles coated with coatomer proteins COP I and II (Nakamura ***et*** *al.*, 1995; Warren and Malhotra, 1998; Lowe *et al.*, 1998a; Allan *et al.*, 2000).

Phosphorylation of GM130 during mitosis blocked traffic of COP I-coated vesicles, and resulted in the disassembly of the Golgi (Nakamura *et al.*, 1997; Lowe *et al.*, 1998b).

GM130 belongs to a recently described family of human autoantigens associated with the Golgi complex (Fritzler *et al.,* 1993). This family has been designated the golgins. These proteins, originally identified in antisera from patients with autoimmune diseases, all possess extensive coiled-coil motifs. The family includes GM130/golgin-95, golgin-67, golgin-160/mea-2/GCP170, golgin-97, golgin-230/245/256, and giantin/macrogolgin (Warren and Malhotra, 1998; Eystathioy *et al.*, 2000). Some of these Golgi matrix proteins were recently found to be required for maintenance of the Golgi architecture in animal cells (Seemann *et al.*, 2000). There is evidence that the Golgi structural proteins are the key partitioning units during mitosis, with the Golgi-associated enzymes distributed by some other mechanism (Seemann *et al*., 2002). Other studies have found that golgins have been tissue-specific patterns of distribution (e.g. Kondo and Sutou, 1997; Nagase *et al.*, 1998).

In the course of studies of postembryonic neurogenesis in the brain of the tobacco hornworm, *Manduca sexta*, we discovered an insect homolog of GM130, Ms-golgin80. After a full-length cDNA of Ms-golgin80 was cloned and sequenced, putative *D. melanogaster*, *Caenorhabditis elegans*, and *B. malayi* homologs were identified using the *M. sexta* sequence as a basis for database searches. Together with GM130 and Ms-golgin80, these proteins form a novel GM130 subfamily that is conserved in vertebrates and invertebrates. Ms-golgin80 is abundantly expressed in the neuroblasts of the optic lobe anlagen and the GMCs of the optic lobe proliferation centers, but is largely reduced or absent in the differentiated progeny of these cells. The subcellular localization of Ms-golgin80, to date studied only at the light microscopic level, suggests that it may be associated with the Golgi complex and possibly with cytoplasmic vesicles. We propose that elevated expression of Ms-golgin80 in the cells of the optic lobe anlagen is needed to ensure an appropriately high supply of the cellular components required by the prolonged mitotic status of these cells, and thus directly contributes to the maintenance of the proliferative state necessary for development.

Materials and Methods

Animals

Larvae of *M. sexta* were reared in the laboratory (Bell and Joachim, 1976). Molts were used as reference points for staging. There are five larval instars. Roman numerals designate successive larval instars and Arabic numerals successive days within an instar, with 0 denoting the day of the molt. The onset of wandering behavior during the final larval instar is denoted as W0, with subsequent days denoted as W1, W2 etc. (Booker and Truman, 1987a, b). Wandering denotes the initiation of a nonfeeding pre-pupal phase prior to the pupal molt. Pupae (P0 through P18) were staged using cuticle markers, following Schwartz and Truman (1983).

Generation of monoclonal antibody (mAb) P1G11

To prepare antigen from *M. sexta*, abdominal ganglia of 50 newly eclosed adults were collected and immediately fixed in 2% paraformaldehyde at room temperature for 1 hour. Ganglia were rinsed in phosphate buffered saline (PBS; 0.01 M phosphate, 140 mM NaCl, pH 7.4) and frozen at -80° C. At the time of injection, the ganglia were homogenized and then spun at 1000 rpm for 3 min to remove cellular debris. A BALB/c mouse was immunized with antigen mixed with an equal volume of the adjuvant RIBI (monophosphoryl lipid A and trehalose dimycolate emulsion; Corixa Corp. www.corixa.com). On the 21st day after the first injection, the mouse was immunized again. On the 37th day, the mouse was boosted with the same antigens. Three days later, spleen cells were fused with SP2/o-Ag myeloma cells using polyethylene glycol and then plated in a HAT selection medium from which HAT-resistant clones were selected (Kennett *et al.*, 1982). 192 hybridoma supernatants were screened using whole mount immunocytochemistry, as previously described (Ewer *et al.*, 1998). MAb P1G11 was further cloned, and ascites fluid was generated using standard procedures to prepare sufficient mAb P1G11 for further experiments.

Immunocytochemistry for Mab P1G11

Brains were dissected free of the head capsule and fixed in alcoholic Bouin's overnight at room temperature. They were dehydrated in ethanol, cleared in xylene, and embedded in Paraplast (Fisher, www1.fishersci.com). Each brain was sectioned at a fixed section thickness (6-10 μm) chosen according to developmental stage. Sections were mounted on poly-L-lysine-coated slides. After deparaffinizing, brain sections were rehydrated and then rinsed in PBS. Sections were quenched with 2% hydrogen peroxide in PBS for 5 min at room temperature to reduce background staining. After washing in PBS, sections were incubated with mAb P1G11 ascites diluted (1:100) in blocking buffer, 3% normal goat serum (NGS; Sigma, www.sigmaaldrich.com) in PBS-TX (PBS plus 0.3% Triton X-100), at 4° C overnight. The next day, sections were washed with PBS, and then incubated with goat anti-mouse horseradish peroxidase conjugates diluted 1:100 in 3% NGS in PBS-TX (Sigma A4416) for 2 h at room temperature. After washing with PBS, the conjugated complex was visualized using 0.5 mg/ml diaminobenzidine (Sigma) containing 0.01% hydrogen peroxide. Color development was stopped by rinsing sections with distilled water. Sections were dehydrated, cleared, and mounted with CytoSeal (Stephens Scientific, www1.fishersci.com). To confirm previously published cell division patterns within the optic lobe anlagen, larval brains were incubated in Grace's insect culture medium (GIBCO, www.lifetech.com) containing 1.5 mg/ml 5-bromo-2'-deoxyuridine (BrdU) at room temperature for 5-6 hours, with gentle shaking and then processed for immunocytochemistry with anti-BrdU monoclonal antibody (gift of Dr. S. Kaufman, Department of Cell and Structural Biology, UIUC) as previously described (Farris *et al.,* 1999)

cDNA Library Screens

To obtain the cDNA clones corresponding to the antigen P1G11, a *M. sexta* cDNA library derived from the ventral nerve cord (Chen *et al.* 1997) was immunoscreened using mAb P1G11. This cDNA library was constructed using the Uni-ZAP XR vector (Stratagene, www.stratagene.com). Approximately 5 x 10^4 phage formation units from the cDNA library were grown on XL1-Blue MRF cell lawns on the agar plates at 42° C until small plaques

became visible. Air-dried nitrocellulose filters (Schleicher & Schuell), pretreated with 10 mM isopropyl-b-D-thio-galactopyranoside (IPTG, Stratagene), were applied to the top of the agar plate. The expressed fusion proteins were then blotted to the filter by incubation at 37° C for 3.5 hours. The filter was carefully picked, rinsed with 20 mM Tris (pH 7.5) containing 150 mM NaCl (TBS), and blocked with blocking solution (3% bovine serum albumin in TBS) for 5 h at room temperature. The expressed fusion proteins were probed with mAb P1G11 using a 1:1000 dilution of ascites fluid with 2.5% dry milk in TBS containing 0.05% Tween-20 (TBST) at 4° C overnight. The filters were washed with TBST and then incubated with anti-mouse alkaline phosphatase conjugates diluted 1:7500 in blocking buffer (Promega, www.promega.com) for 2 h at room temperature. Each filter was washed with TBST three times, and the positive clones were visualized by incubation with 350 µg/ml nitroblue tetrazolium (NBT) and 175 µg/ml 5-bromo-4-chloro-3-indolyl phosphate (BCIP; Promega) in color development buffer. The filters were rinsed with TBS and air dried. A secondary screen was used to recover pure clones. The pBluescript phagemids from the positive clones were rescued in SOLR cells according to the manufacturer's protocols (Stratagene). Each single rescued phagemid clone was amplified using an alkaline lysis miniprep procedure (Feliciello and Chinali, 1993). The cDNA library immunoscreen did not recover any full-length cDNA clones. Another cDNA library screen (2 x 10^5 pfu) was carried out using a 900 bp DIG-labeled probe spanning the region near the 3' end of the clone 1-1-A1-a. A polymerase chain reaction mixture of the following: 1X reaction buffer (Promega), 2.5 mM $MgCl_2$, 100 µM dNTPs, 2 units/50 µl of Taq Polymerase (BRL), 400 nM P1G11-9F primer, 400 nM P1G11-2R primer, 1 ng/µl of 2.2 Kb purified insert (66 ng), and 20 µM DIG-11-dUTP was used to generate about 50 µl DIG-labeled PCR product. The sequences for primers P1G11-9F and P1G11-2R are 5'AATGAGCAACTTAAGTCA3' and 5'AAATCTGCTACTTCCTCCAT3' respectively. This library screen protocol has been previously described in detail (Chen *et al.*, 1997). The cDNA sequences were determined in both directions by the dideoxy-chain termination method (Sanger *et al.*, 1977) using Sequenase V2.0 (U. S. Biochemicals, www.usbweb.com).

DNA sequence analysis

The cDNA and putative protein sequences of Ms-golgin80 and other golgins were analyzed using Phylogenetic Analysis Using Parsimony (PAUP 3.1.1; Swofford, 1993). The conserved domains of the golgins were aligned by FASTA (Altschul *et al.* 1990). Analysis for putative leucine zipper domains and phosphorylation sites was carried out with the GCG software package (GCG Inc.). Putative coiled-coil domains were predicted by the method of Lupas *et al.* (1991). Theoretical molecular weights and PIs of the putative GM130 subfamily proteins were obtained using Compute MW/PI, from ExPASy server, Swiss Institute of Bioinformatics. The hydropathy plot was performed according to the method of Kyte and Doolittle (1982).

Expression of the Ms-golgin80 fusion protein in the Escherichia coli BL-21 and the Drosophila melanogaster Schneider 2 (S_2) cell lines

The expression vector Uni-Zap was used to express fusion proteins with β-galactosidase (15 kilodaltons). Plasmids were transformed into *E. coli* strain BL-21 strains (Pharmacia, www.pharmacia.com) using the 3-1-b1 plasmid (Uni-Zap, http://www.stratagene.com). The recombinant cells were cultured in 3 ml LB medium to OD_{600} = 0.6 at 37° C and then incubated with IPTG to a final concentration of 1 mM overnight. The cells were harvested at 1500 rpm, 10 min, and resuspended with an equal volume of water. Cells were lysed using SDS-PAGE sample buffer (Laemmli, 1970). Lysate was stored at -70° C. The *D. melanogaster* S2 cell line, was transfected with P-Met-3-1-b1 plasmid as follows. A KpnI-EcoRI cDNA fragment containing full length 3-1-b1 was cloned into P-Met vector (Nose *et al.* 1992). The P-Met vector can express cloned sequences off the *D. melanogaster* metallothionine promoter by induction with $CuSO_4$. Because there are three EcoRI sites in the encoding region, this fragment was obtained by EcoRI partial digestion and KpnI complete digestion. The protocol for transfection has been previously described (Nose *et al.*, 1992). Two days after incubation with 0.7 mM $CuSO_4$, cells were harvested and lysed using SDS-PAGE sample buffer.

Tissue lysate preparation and immunoblot analysis

Thirty brains and ventral nerve cords of 5th instar larvae were collected on dry ice and then homogenized on ice in 50 µl PBS containing 1% SDS and protease inhibitors (5 mM EDTA, 20 µg/ml phenylmethylsulfonyl, 1 µg/ml pepstatin), and 80 µM phenylthiourea. The homogenate was centrifuged at 14,000 rpm for 10 min at 4° C. Supernatant was diluted with SDS-PAGE sample buffer prepared without dye and dithiothreitol, and stored at -70° C. Samples were separated on 1 mm thick, 10% SDS-polyacrylamide gels using the Bio-Rad Mini-Protean II electrophoresis system (www.bio-rad.com). Samples were heated at 70° C for 3 min. Approximately 10-20 µg of protein were loaded into each well. Gels were run at 80 V for 3 h in Laemmli running buffer (Laemmli, 1970). Proteins were then electroblotted onto nitrocellulose filter using a Bio-Rad Minigel Transblot apparatus. The filter was washed with TBST (20 mM Tris-HCl pH 7.8, 150 mM NaCl and 0.05% Tween-20) for 10 min and then incubated with blocking reagent (Pierce, www.piercenet.com) at 4° C, overnight. MAb P1G11 ascites 1: 800 diluted in 5% nonfat dry milk dissolved in PBST (PBS containing 0.05% Tween-20) was used to incubate the filter for 1 h at room temperature. The filter was washed with TBST six times (10 min each time) and then incubated with 1: 2000 dilution of secondary antibody (SuperSignal substrate Western blotting kit, Pierce) in 5% nonfat dry milk, PBST at room temperature for 1 h. The filter was then washed with TBST 6 times (15 min each time) and processed with chemiluminescence reagents according to the manufacturer's instructions (Pierce) and exposed to X-ray film.

In situ hybridization

The expression pattern of the gene coding for the P1G11 protein detected in the brain was examined by *in situ* hybridization. DIG-labeled antisense and sense Ms-golgin80 probes were prepared by standard methods (Tear *et al.*, 1996). The full-length 3-1-b1 cDNA clone served as the template for the labeling. Whole brains were hybridized using the method of Riddiford *et al.* (1994). Brains were hybridized with a 1: 100 dilution of probe at 55° C for 24-48 h. For the anti-DIG-alkaline phosphatase reaction, brains were washed with

50% formamide, 2X SSC, 0.1% Tween 20, three times, 1 h each time at room temperature; washed with PBST at room temperature, two times, 1 h each time; incubated in 5% NGS in PBST for 2 h at room temperature; and incubated in anti-DIG-AP antibody (Roche, www.roche.com) diluted 1:1000 in PBST, 5% NGS at 4° C , 24-48 h; washed with PBST, 3 times, 30 min each time. Color was developed in APB buffer (100 mM Tris, pH 9.5, 100 mM NaCl, 50 mM $MgCl_2$, 0.1% Tween-20 containing 675 µg/ml NBT and 350 µg /ml BCIP). Tissue was dehydrated in ethanol, cleared in methyl salicylate, and mounted with Permount (Fisher).

Results

Generation of MAb P1G11

Monoclonal antibody (mAb) P1G11 was isolated in a screen for antibodies against neuron-specific antigens in the central nervous system of *M. sexta*. MAbs with specific staining patterns restricted to subsets of neurons were chosen for further analysis from a total of 192 screened antibodies. P1G11 produced weak immunostaining in a majority of neurons in the segmental ganglia, but showed a dynamic and intense immunostaining pattern in the metamorphosing brain. The most intense expression of P1G11 antigen was found in the optic lobe anlagen, but also appeared transiently in other proliferation cell populations in the brain, for example, in the developing mushroom bodies. Because of its association with mitosis, we used mAb P1G11 to study the growth of the optic lobes during metamorphosis and to clone and sequence the gene encoding the P1G11 antigen.

Expression of P1G11 antigen during optic lobe development

Previous studies have shown that the neuroblasts in the lamina and medulla centers are gradually recruited from the optic lobe anlagen: that is, individual neuroblasts undergo a series of symmetric divisions, then switch to asymmetric divisions (Edwards, 1969; Monsma and Booker, 1996a; Champlin and Truman, 1998). This results in a switch from the production of additional neuroblasts to the production of the direct neuronal precursors, the GMCs. These changes could be generally tracked in our tissue sections because the nuclear diameters of GMCs are about 4 µm, while the nuclei of neuroblasts are 6-9 µm in diameter. We also confirmed this previously reported pattern of neuroblast proliferation by means of immunostaining for incorporated BrdU (data not shown).

Use of the P1G11 antibody as a marker allowed us to track the dynamic pattern of optic lobe formation previously inferred on the basis of cytology (Fig. 2). This was because the P1G11 antibody immunostained neuroblasts and GMCs but not the neuronal progeny of the GMCs. Overall, our results were highly consistent with earlier studies of lepidopteran neurometamorphosis (Nordlander and Edwards, 1969b; Monsma and Booker, 1996a). Specifically, from the first day of the second larval instar on, labeling by the mAb P1G11 within the optic lobes was confined to neuroblasts. This pattern did not change until late in the penultimate (IVth) larval instar, when asymmetric divisions of the neuroblasts can first be detected (Monsma and Booker, 1996a). At this time, GMCs were also immunopositive for P1G11.

By P4, the outer and inner optic lobe anlagen were greatly reduced in size, with few neuroblasts remaining as the development of the optic lobes proceeded. However, at this time P1G11-immunoreactivity in the GMCs of the medulla and lamina proliferation centers was still visible. By P7, the mitotic activity of neuroblasts and GMCs ended concurrent with the fragmentation of

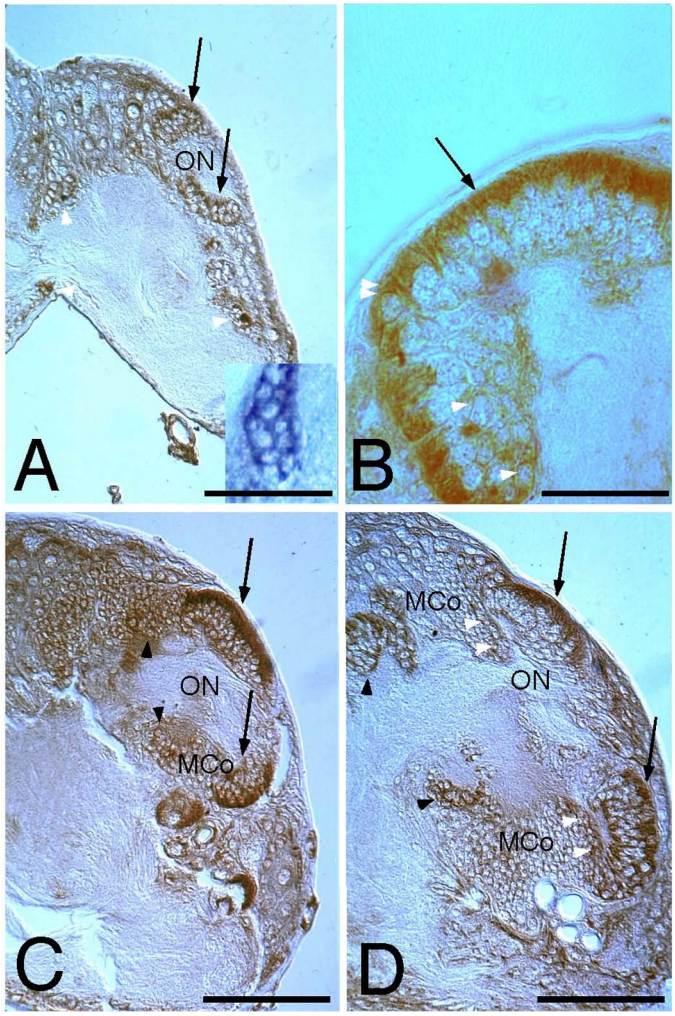

Figure 2. MAb P1G11 immunostaining in the developing optic lobes. A. At II2, labeling is present in the optic lobe anlagen, which contain populations of neuroblasts undergoing symmetrical divisions (black arrows). Inset shows a higher magnification view of the cytoplasmic staining observed in cells of the optic lobe anlagen. The olfactory proliferation center and some other scattered cells were also labeled (white arrowheads). The midline of the brain is at the left of the panel. B. A higher magnification view of the optic lobe anlagen at III2. Black arrow indicates the outer optic lobe anlagen. The labeling in the cortical cytoplasm of neuroblasts in the outer layer is intense, but diffusely distributed throughout the cytoplasm (double white arrowhead). Perinuclear staining is also present (single white arrowheads). C. At V0, the inner optic lobe anlagen is separated from the outer optic lobe anlagen by the newly formed medulla cortex (MCo), which displays only background levels of immunostaining. Black arrowheads indicate the neuroblasts of the inner optic lobe anlagen. The position of the unlabeled optic nerve (ON) is indicated. The midline of the brain (not shown) is at the left of the panel. D. At W0, the outer optic lobe anlagen (black arrow) and inner optic lobe anlagen (black arrowhead) are further separated from each other. Neuroblasts and GMCs are immunopositive for P1G11 in the proliferation centers (white arrowheads). A, C, and D are frontal sections; B is a horizontal section. Scale bars: (A, C, D) 50 µm (for inset in A: 20 µm); (B) 20 µm.

the optic lobe anlagen described by Monsma and Booker (1996a). Weak P1G11 immunoreactivity in the dwindling optic lobe anlagen and proliferation centers was still observed on P8, but had disappeared by P10.

Transient mAb P1G11 immunoreactivity was also found outside of the optic lobes. Immunostaining was located in the anterior part of about one-fourth of the medullar neuropil bundles at stages P2-10 and in the posterior part of about one-third of the anterior lobula neuropil bundles at stages P6-10 (not shown). Scattered elongated glial cells (Nordlander and Edwards, 1969b) within the lamina cortex showed strong but transient expression of P1G11 antigen at later stages (P6-10). Whether these glial cells are mitosis-potent at this time is not known.

Cytoplasmic staining with mAb P1G11 was also found, as our model predicts, in other brain formation centers and even in scattered individual neuroblasts (Fig. 2). By contrast, other brain regions showed much less P1G11-immunoreactivity during this period of postembryonic development than the optic lobe anlagen.

Characterization of cDNA clones recognized by MAb P1G11

A reverse genetic approach was undertaken to obtain P1G11 cDNAs. Immunoscreening was carried out using mAb P1G11. Two independent cDNA clones were recovered from approximately 3.5 X 10^6 plaques of cDNA expression library prepared from larval nerve cords of *M. sexta*. These two cDNA clones, designated as 1-1-A1a and 1-2-f2, are 2.1 kb and 1.2 kb respectively (Fig. 3). The sequences of the two clones share a 76 bp 5' untranslated region and a stretch of 1184 bp representing part of an open reading frame. Although the clone 1-1-A1-a provided an additional 0.9 kb of sequence, no termination signal sequence was found in either 1-1-A1a or 1-2-f2. To obtain the 3' end or full-length cDNAs, another screen using the same library was carried out using a 900 bp DIG-labeled DNA probe spanning the region between primers 9F and 2R.

A full-length 2.3 kb cDNA, designated 3-1-b1, was obtained using this method (Fig. 3). This clone overlapped completely with the other two cDNAs, and the sequence was submitted to GenBank under accession number AF244891. This clone comprised a complete open reading frame, a translation stop codon, a 95 bp 3' untranslated region with the polyadenylation recognition sequence, AATAAA, and a poly-(A) tail. The open reading frame predicts an 80 kilodalton protein of 708 amino acids. The complete sequence is provided as supplemental material.

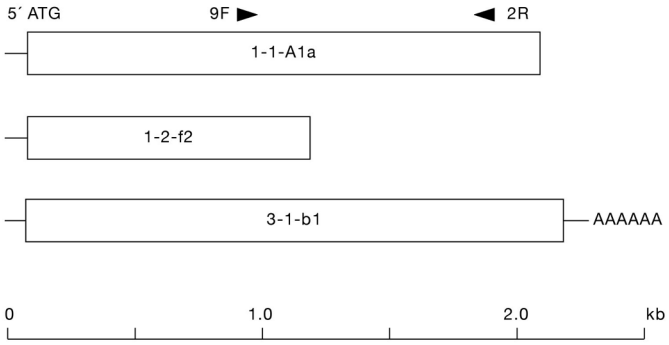

Figure 3. Schematic illustration of the three P1G11 cDNA clones isolated by immunoscreening of a cDNA expression library. The clones 1-1-A1a and 1-2-f2 are truncated versions, whereas the 3-1-b1 is full-length. The lines and boxes represent the untranslated and encoding regions, respectively. The primers, shown as arrowheads, were used to amplify a DIG-labeled probe for screening full-length cDNA clones. The sequence of the full-length cDNA clone 3-1-b1 and its putative amino acid sequence (Ms-golgin80) are provided as supplemental material.

Protein P1G11 belongs to a conserved Golgi complex protein subfamily of GM130-type proteins

Putative amino acid sequences of P1G11 were subjected to similar sequence BLAST searches (Altschul *et al.* 1990). This sequence similarity search revealed that this protein shares significant similarity with GM130-like proteins and several other alpha-helical coiled-coil structural proteins, Golgi complex proteins, such as the *D. melanogaster* CG11061 gene product (score: 216), GM130 (score: 116) from *Rattus norvegicus*, *Caenorhabditis elegans* F33G12.5 gene product (score: 108), golgin-95/human GM130 (score: 101), golgin-160 (score: 90) and giantin autoantigen (score: 89) (Adams *et al*., 2000; Fritzler *et al*., 1993; Kondo and Sutou 1997; Nakamura *et al*., 1995; Seelig *et al.*, 1994; Wilson *et al*., 1994). This protein is also similar to several cytoskeleton-related proteins, including interaptin (score: 116), myosin family proteins (myosin II heavy chain) (score: ~106), integrin homolog (score: 100) from yeast, and the yeast intracellular transport protein Uso1p (P115 homolog) (score: 100) (Goffeau *et al.*, 1996; Jacq et. al., 1997; Rivero *et al.*, 1998). Among these molecules, the cytoskeleton-related proteins, Uso1p, golgin-160 and giantin show no similarities other than coiled-coil to protein P1G11. However, four sequences not only gave high BLAST scores (216-106) but also share three conserved domains with protein P1G11: the putative CG11061 protein from *D. melanogaster*, two identified Golgi complex genes: GM130 and golgin-95/human GM130 and the hypothetical protein F33G12.5 from a *C. elegans* cosmid sequence.

Sequence comparison strongly suggested that the protein, recognized by mAb P1G11 in *M. sexta* as well as the *D. melnaogaster* CG11061 and nematode F33G12.5, are invertebrate homologs of rat GM130 and golgin-95/human GM130. Interestingly, three domains of protein P1G11 (amino acids 1-23, 369-451, and 560-648) are extremely conserved in the other four protein sequences as the second AUG of gene F33G12.5 is assumed to be its initiation code. The conserved N-termini identities range from 32-100% (Table 1). Notably, the positive-charged stretch KLAAARKK is almost identical among these five GM130-like sequences. This domain was mapped to be part of the N-terminal binding domain (amino acid 73) of GM130 to a vesicle docking protein, P115 (Nakamura *et al.* 1997). An internal span of 89 amino acids also shares high identities (33-85%) among these five sequences (Table 1). In addition, a domain corresponding to amino acids 560-648 of protein P1G11 (near the C-terminus) is also extremely conserved . EST sequence SW3ICA1433, which is derived from another species of nematode (*B. malayi*), also shows significant similarity in this domain after frame-shift adjustments for maximal alignment (Blaxter *et al.*, 1996). The identities of this domain among these six molecules range from 33% to 91% (Table 1).

The *D. melanogaster* CG11061 protein shares the highest identity (21%) with protein P1G11 in overall sequence. A *D.*

Table 1. Comparisons of sequence identities, lengths, molecular weights, and PIs of the GM130 subfamily proteins

Protein	Identities to Rn-GM130 (%)				Length (a.a.)	Molecular Weight (kD)	PI
	Overall	28 a.a. at the N-terminus	89 a.a. domain in the middle	89 a.a. domain near the C-terminus			
Rn-GM130					986	130*	4.86
Hs-GM130	74	100	85	91	990	130*	4.97
Ms-golgin80	16	39	26	43	708	80*	5.23
Dm-golgin90	19	57	43	45	789	90#	5.19
Ce-golgin107	15	32	33	33	937	107#	5.27
Bm-golgin	ND	ND	ND	33	>81	ND	ND

Note: The identities of the three most conserved domains were compared among GM130 subfamily proteins. Amino acid number, molecular weight and PI point are also given.
Abbreviations: Bm: *Brugia malayi*; Ce: *Caenorhabditis elegans*; Dm: *Drosophila melanogaster*; Hs: *Homo sapiens*; Ms: *Manduca sexta*; Rn: *Rattus norvegicus*. The sequence of Bm-golgin is based on a partial EST. The nomenclature of these molecules is described in the Results section.
*: molecular weights based on estimation from Western blots; #: predictive molecular weights based on putative protein sequences; space left blank: 100% match; ND: no data available.

melanogaster EST sequence (clone GH01913) from a head library is identical to part of the putative *D. melanogaster* gene, strongly suggesting that the presumptive homolog is expressed in the fly brain. The overall identities of protein P1G11 and protein F33G12.5 of *C. elegans* to GM130 are 16% and 15%, respectively. Therefore, in addition to GM130 and golgin-95/human GM130, the insect and nematode genes together form a novel group of Golgi complex proteins we refer to as the GM130 subfamily. For convenience, P1G11 and the other GM130 homologs are hereafter referred to as Ms-golgin80 (*M. sexta*), Rn-GM130 (*R. norvegicus*), Hs-GM130 (*H. sapiens*), Dm-golgin90 (*D. melanogaster*), Ce-golgin107 (*C. elegans*) and Bm-golgin (*B. malayi*) according to the initials of their binomial names and predicted molecular weights.

Structural analysis of Ms-golgin80 and other invertebrate GM130 homologs

Ms-golgin80 as well as the fly and nematode GM130 homologs were subjected to further sequence and structure analysis. In addition to sequence similarity, their similar coiled-coil structures, similar hydropathy plots, and similar isoelectric points suggest that Ms-golgin80 and the other Rn-GM130-like proteins are invertebrate counterparts to Rn-GM130.

The extent of coiled-coil in these sequences was predicted by CoilScan (Lupas *et al.*, 1991). Ms-golgin80 possesses a high content of coiled-coils with several interruptions (Fig. 5). Extremely high contents of coiled-coils are also found in the *D. melanogaster* and *C. elegans* homologs. These predict a rod-like structure with several possible joints for these molecules, as seen in Rn-GM130 (Nakamura *et al.*, 1995). The lack of coiled-coils in the N- and C-termini (50-100 amino acids in length) of these molecules is also consistent with the structure-function pattern seen in Rn-GM130 in which the N- and C-termini are responsible for binding to the vesicle docking proteins P115 and GRASP65 respectively (Nakamura *et al.*, 1997; Barr *et al.*, 1998). The lack of coiled-coils at both ends may be important for the accessibility of GM130 subfamily molecules by their protein partners. The hydropathy plot showed that Ms-golgin80 is a hydrophilic molecule without any signal sequence and lacking transmembrane domains (Fig. 5). This protein therefore would not act as a secreted protein or transmembrane protein, consistent with the restricted cytoplasmic distribution revealed by immunostaining. The Ms-golgin80 protein sequence also revealed 12 potential protein kinase C phosphorylation sites, 17 casein kinase II phosphorylation sites, a tyrosine kinase phosphorylation site, 2 N-myristoylation sites (amino acids 229-234, GTVFNL and 423-428, GLEDAY) and 6 microbodies C-terminal targeting signals. The potential phosphorylation sites resemble those in Rn-GM130. The Rn-GM130 phosphorylation sites control the binding of Rn-GM130 to its protein partner P115: Rn-GM130 does not bind to P115 in mitotic cytosol, when it has undergone N-terminal phosphorylation, but does bind to P115 in interphase cytosol, at which time it is not phosphorylated (Nakamura *et al.*, 1997). These sites are also important for the functional regulation of Rn-GM130 during mitotic fragmentations and partitioning of the Golgi apparatus (Lowe *et al.*, 1998b). The isoelectric point of protein Ms-golgin80 is pH 5.23. This acidic property is also shared with other known GM130 subfamily members (Table 1).

Protein sequence analysis of Ms-golgin80 also revealed that several putative leucine zipper domains, characterized by at least four periodic repeats of leucine residues at every seventh position in a stretch of amino acids (Landschulz *et al.*, 1988). One such domain is present between amino acids 100-121 in the non-conserved region of the protein. Another domain near the C-terminus of protein P1G11 was found to be remarkably conserved across the Rn-GM130-like proteins (Fig. 4). This is the region that Rn-GM130 uses for dimerization (Nakamura *et al.* 1997). The alignment of the domains shows that a fragment corresponding to amino acids 568-589 of Ms-golgin80 could also be a conserved leucine-zipper-like domain. Rn-GM130 and these homologous molecules may potentially dimerize through parallel interdigitations using this domain. The sequence Q(3x)E(2x)TI(5x)LYQ(2x)R in this domain is conserved throughout the six homologs and indicates that this might be important for dimerization.

Taken together, those predicted invertebrate proteins, along with Rn-GM130 and Hs-GM130, emerge as a novel subfamily of Golgi complex proteins. Subfamily members show conservation in

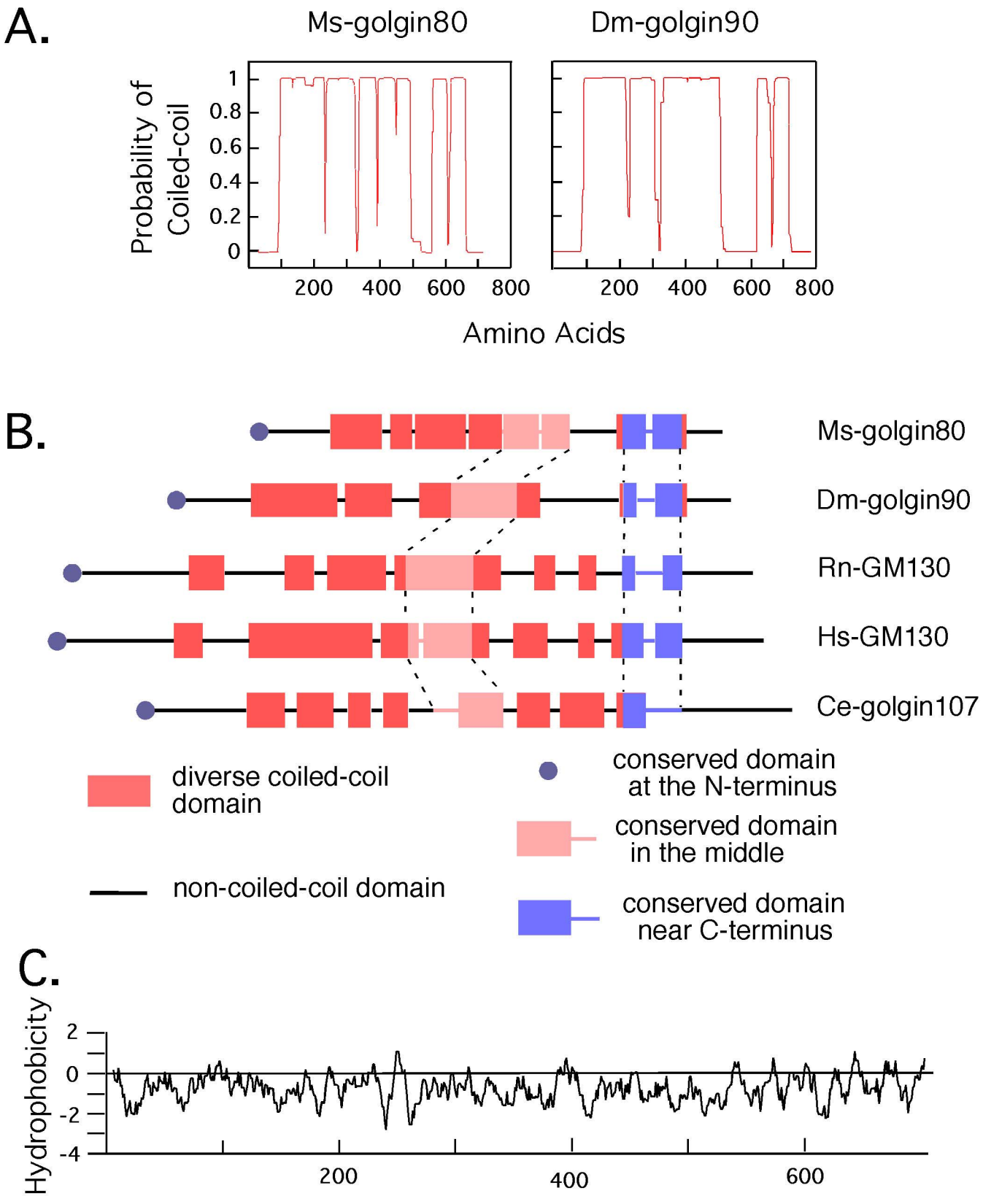

Figure 4. Sequence alignment of three conserved domains of the GM130 subfamily. Panel A shows the N-terminal, amino acids 1-23. Panel B shows amino acids 369-451; Panel C shows amino acids 560-648. Amino acid residues are highlighted with black blocks if at least 50% of sequence is identical. Dm-glogin90: *D. melanogaster* homolog derived from a *Drosophila* EST clone; GH01913 from a head library; genomic P1 clone DS04894; CG11061 putative protein. Ce-golgin107: *C. elegans* homolog from conceptual protein F33G12.5 of a hypothetical gene from the nematode genome project (Waterston and Sulston, 1995) and BM-golgin: *B. malayi* homolog from a 5' EST cDNA clone SW3ICA1433.

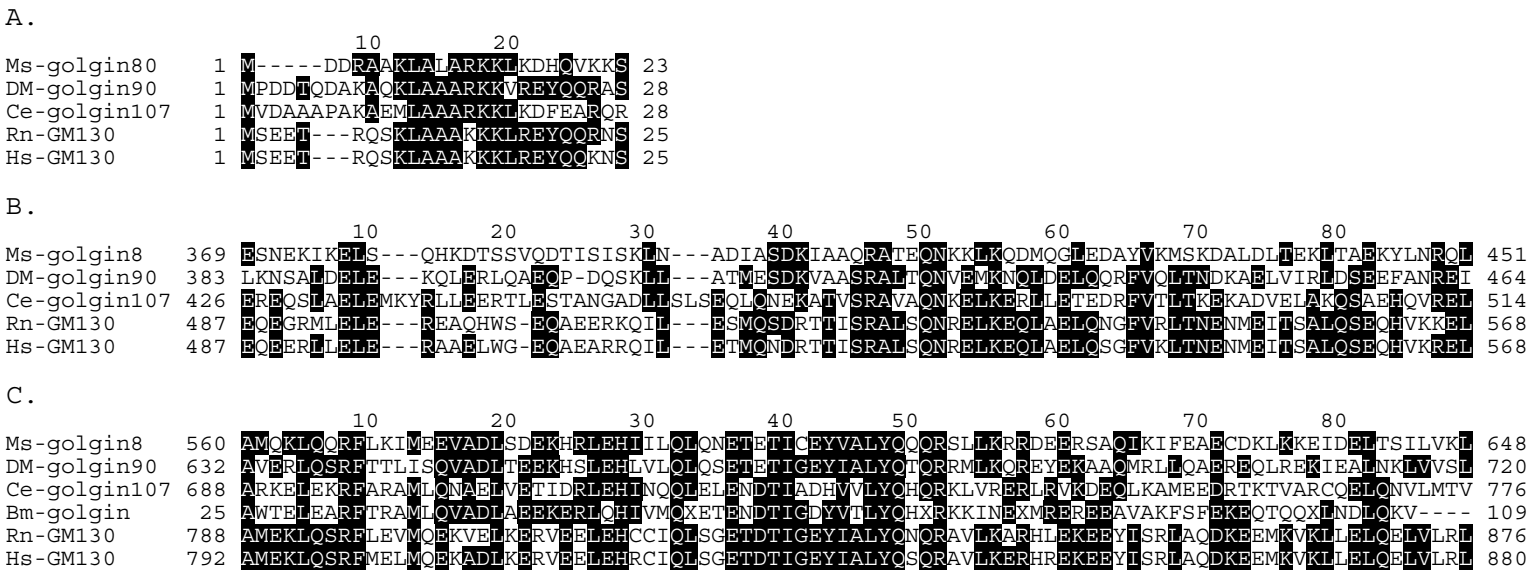

Figure 5. Sequence and structure analysis for the GM130 subfamily. A. Predicted coiled-coils in Ms-golgin80 and Dm-glogin90. B. Schematic illustration of the general molecular structure of the GM130 subfamily. C. Hydropathy plot of Ms-golgin80. There are no hydrophobic regions, indicating the absence of a signal sequence or a transmembrane domain.

primary translated sequences and might also share conserved functions common to vertebrates and invertebrates.

Recombinant Ms-golgin80 proteins and Western blotting

To verify that the protein encoded by clone 3-1-b1 is the same antigen immunoreactive to the mAb P1G11 in the brain, antigen extracted from the central nervous system was analyzed together with fusion proteins on Western blots. A single protein of 80 kilodaltons from the CNS of 5th instar larvae was detected (Fig. 6, lane 6) by immunoblots using mAb P1G11. As expected, normal S2 cells without or with $CuSO_4$ induction and transformed bacteria without IPTG induction did not generate any proteins immunoreactive to mAb P1G11 (Fig. 6, lanes 1, 2, 4). Under induction, the pBluescript plasmid and p-Met vector carrying a full length 3-1-b1 cDNA clone encoded a 95 kilodalton protein (with additional 15 kilodalton portion of ß-galactosidase) and an 80 kilodalton protein in transformed bacteria and S2 cells respectively (Fig. 6, lanes 5, 3). Therefore, the fusion protein sizes are consistent with the predicted molecular weight of approximately 80 kilodaltons.

To gain further information on the subcellular localization of the P1G11 antigen, we attempted to stain the S2 cells transfected with the P1G11 gene driven by the metallothionine promoter with the P1G11 mAb. We did not obtain a distinct staining pattern comparable to that seen *in vitro*, although as described above we were able to detect P1G11 in the same cells using Western blots. It is possible that the expression level of P1G11 in these cells is so low that it fell below the threshold for immunodetection. Another possibility is that MsGM80 is unable to interact with the GRASP65 of *D. melanogaster*, which would prevent localization to the Golgi of S2 cells.

Whole mount brain in situ hybridization

The authenticity of the 3-1-b1 cDNA clone was demonstrated by whole mount brain *in situ* hybridization. If the cDNA clone 3-1-b1 corresponds to the P1G11 gene, it should be expressed abundantly in the optic lobe anlagen. DIG-labeled antisense and sense (as a control) riboprobes generated from 3-1-b1 clone were used to detect the P1G11 mRNA in brains. In each hemisphere of the 5th larval brain, two adjacent ring-like structures corresponding to the optic lobe anlagen (see Fig. 1) were intensely labeled by the antisense probe (Fig. 7), whereas there was no specific staining seen when hybridization was conducted with the sense probe

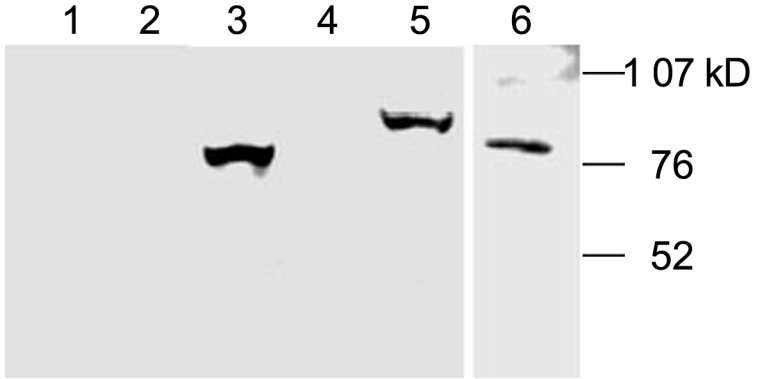

Figure 6. Immunoblots of Ms-golgin80 from tissue lysates and fusion proteins. A protein band of approximately 80 kilodaltons is present in lanes 3 and 6. There is a band of protein at 95 kilodaltons in lane 5. No protein bands are found in lane 1, 2, and 4. Lane 1: plain S2 cell; Lane 2: induced plain S2 cell; Lane 3: induced transfected S2 cell. Lane 4: non-induced transformed BL21 cell; Lane 5: induced transformed BL-21 cell; Lane 6: the larval CNS. BL-21 bacteria were transformed with 3-1-b1 plasmid (Uni-Zap), whereas the S2 cells were transfected with P-Met-3-1-b1 plasmid.

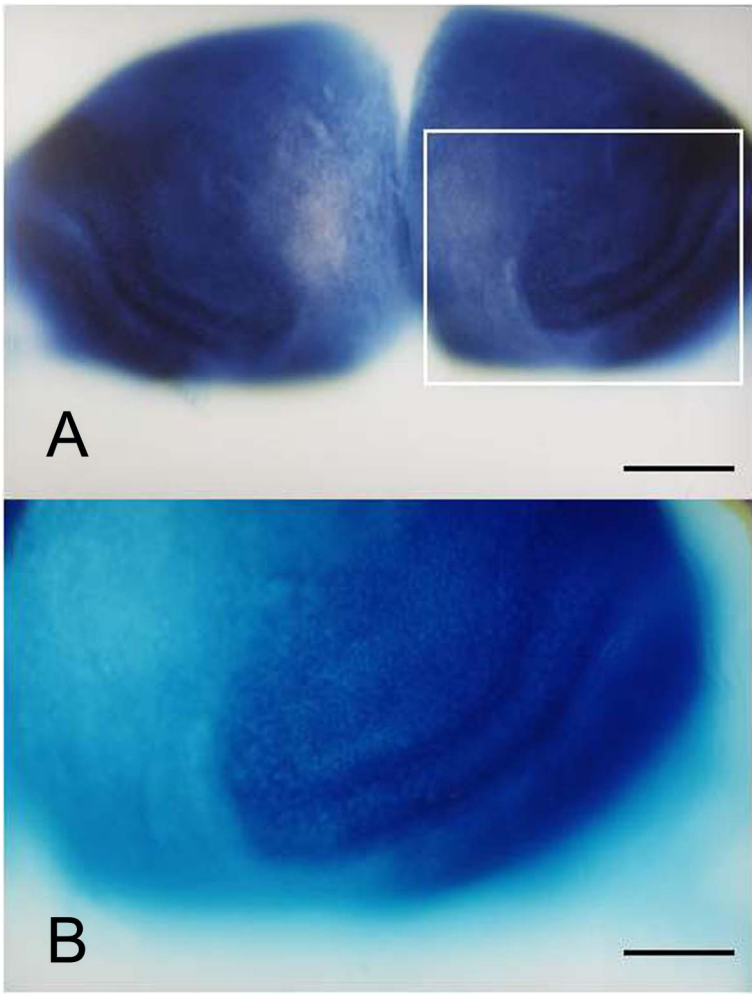

Figure 7. *In situ* hybridization on whole mount brain of *Manduca sexta* with antisense RNA probe templated by cDNA clone 3-1-b1. A. The crescent-shaped optic lobe anlagen are visible in the forming optic lobes of the Vth instar larva . Scale bar: 200 µm. B. Close-up of the optic lobe anlagen of A. Scale bar: 100 µm.

(not shown). This confirms that the 3-1-b1 cDNA encodes Ms-golgin80.

Discussion

An emerging subfamily of GM130-like proteins

The putative protein sequence and our structural analysis lead us to conclude that Ms-golgin80 is an insect homolog of Rn-GM130 (Nakamura *et al.*, 1995). Apparent homologs were also identified from *D. melanogaster, C. elegans, and B. malayi*. The subfamily is therefore conserved between vertebrates and the Ecdysozoa lineage of invertebrates (Aguinaldo *et al.*, 1997). The members of this subfamily share three conserved domains that distinguish them from other identified Golgi complex proteins (Barr, 1999; Munro and Nichols, 1999; Eystathioy *et al.*, 2000). Subfamily members also share a high content of coiled-coils, hydrophilicity, low (acidic) isoelectric point, and multiple phosphorylation sites. There are at least two conserved functional domains, a positive-charged N-terminus and a leucine zipper-like domain (Nakamura *et al.*, 1995; 1997; Barr *et al.*, 1998). The N-terminal 28 amino acid and the leucine zipper-like domain at amino acids.568-589 share identities up to 30% and 41%, respectively.

Whether these invertebrate homologs perform the same functions as their vertebrate counterparts remains to be investigated. The N-terminal cdk2 phosphorylation site and C-terminal GRASP65 binding sequence of Rn-GM130 and Hs-GM130 are apparently not present in their invertebrate counterparts. However, the C-terminal sequences for Dm-golgin90 and Ce-golgin107 are not yet completely defined because they are predicted from genomic DNA sequences. Full-length cDNA sequences for these two genes are required to address this question.

Ms-golgin80 is differentially expressed in proliferative cells in the developing optic lobes

Immunostaining using mAb P1G11 revealed that Ms-golgin80 is predominantly and consistently confined to the neuroblasts and the GMCs in the optic lobe anlagen and proliferation centers of the developing optic lobes from the second larval instar through Day 10 of pupal life. By contrast, differentiated optic lobe neurons derived from the GMCs do not express Ms-golgin80. We assume that the expression patterns of Ms-golgin80 reflect differences in level of abundance rather than all-or-none differences, as all eukaryotic cells must synthesize Golgi-associated proteins involved in COP I vesicle docking. The elevated expression of Ms-golgin80 protein in these tissues is apparently attributable to its high transcript level.

Subcellular distribution of Ms-golgin80 suggests an association with the Golgi apparatus in the optic anlagen of Manduca sexta

The subcellular distribution of Ms-golgin80 in the neuroblasts of the optic lobe anlagen and proliferation centers displayed several variants, including a punctate and tubular network, perinuclear staining, and occasionally, a diffuse distribution of reaction product throughout the cytoplasm. The first two patterns of distribution appeared in all neuroblasts and GMCs, while the diffuse pattern was found primarily in the most superficial layer of neuroblasts in the optic lobe anlagen. Overall, the punctate cytoplasmic immunoreactivity suggested that the P1G11 antigen is associated with the cytoskeleton or a specific organelle. The polarized perinuclear localization further suggested that P1G11 antigen might be associated with the Golgi apparatus because this pattern resembles the distribution of known Golgi complex proteins and enzymes in mammalian cells (Nakamura *et al.*, 1995; Roth and Berger, 1982). These possible associations remain to be confirmed in future ultrastructural studies.

A punctate Golgi complex has previously been described in several insects (Beams and Kessel, 1968), including in embryonic and adult cells of *D. melanogaster* (Ripoche *et al.*, 1994; Stanley *et al.*, 1997). Punctate Golgi complex has also been described in yeast, *Saccharomyces cerevisiae* (Duden and Schekman, 1997) and plants (Driouich and Staehelin, 1997). The perinuclear arrangement of Golgi stacks was discovered in animal (mammalian) cells (Rambourg and Clermont, 1990) and has also been reported for yeast (*Schizosaccharomyces pombe*; Chappell and Warren, 1989). The

proliferative cells from the insect optic lobes occasionally showed both of these patterns.

The diffuse cytoplasmic staining seen in some neuroblasts in the superficial layer of the optic lobe anlagen suggests that Ms-golgin80 might also be targeted to other, smaller, cytoplasmic components, such as vesicles. Two lines of evidence support this speculation. One comes from our observation that Ms-golgin80 immunoreactivity is present in both the medulla and anterior lobula neuropils, regions that do not contain neuronal somata. In addition, sequence analysis of Ms-golgin80 reveals 2 N-myristoylation sites (amino acids 229-234, GTVFNL and 423-428, GLEDAY) and 6 microbodies C-terminal targeting signal. These structural features suggest that Ms-golgin80 could be associated with transport vesicles.

Another possibility is that the diffuse staining reflects disassembly of the Golgi complex as seen in mitotic mammalian cells (Shima *et al.*, 1997). Two observations, however, argue against this possibility. First, mitotic fragmentations of the Golgi complex were previously observed to result in scattered punctate tubulo-vesicles, regardless of whether they originated from stacks or the larger punctate Golgi complex (Shima *et al.*, 1997; Stanley *et al.*, 1997). Second, the diffuse staining in the superficial layer of neuroblasts in the optic lobe anlagen persists across all the stages. We have not observed the diffuse staining disappearing or even being reduced in any of these neuroblasts, which would result from reassembly of smaller diffused fragments (as seen in HELA cells in interphase; Lowe *et al.* 1998a).

Functions of Ms-golgin80 and the GM130 subfamily

Rn-GM130 has been shown to be localized on the cytoplasmic side (periphery) of Golgi membranes, but does not appear to bind directly to the membrane itself (Nakamura *et al.*, 1995). Rn-GM130 has also been demonstrated to interact with P115 and GRASP65, which are part of the machinery for the docking of COP I vesicles at the Golgi complex (Nakamura *et al.*, 1997; Barr *et al.*, 1997). Rn-GM130, giantin, P115, and GRASP65 have been shown to form an array of rod-like molecules that function as a long arm to tether vesicles budding from cisternae to their target membranes. Phosphorylation of the serine25 at the N-terminus of Rn-GM130 disrupts its interaction with P115, and leads to disassembly of the Golgi complex during mitosis (Nakamura *et al.*, 1997). Therefore, Rn-GM130 is a key molecule in the maintenance of the Golgi complex in mammalian cells, both in interphase and mitosis. Because the N- and C-termini of Rn-GM130 are conserved in Ms-golgin80, we speculate that Ms-golgin80 plays a counterpart role in the docking of COP I vesicles and functions with P115- and GRASP65-like insect proteins to maintain the Golgi complex in *M. sexta*. Whether some of the multiple phosphorylation sites located at the N-terminus are the targets of Cdc2 kinase (and in turn whether their phosphorylation status would affect Ms-golgin80 binding activity) needs to be further investigated (Lowe *et al.*, 1998b). We speculate that the other invertebrate homologs described here also play an important role in Golgi vesicle docking. However, the conserved amino acid residues at C-termini are not identical to the sequence (xxNDxxIMVE) mapped in Rn-GM130 responsible for binding GRASP65. It is not yet known if the GRASP65-like molecules in other animals are correspondingly diverged from their vertebrate counterparts. It is interesting to note that Golgi fragmentation also occurs during apoptosis, at which time GRASP65 is a substrate for caspase-3 (Lane *et al.,* 2002).

Ms-golgin80, a GM130 homolog, is the first Golgi complex protein homolog demonstrated to show differential expression in the proliferative cells of the metamorphosing insect. High levels of expression of Ms-golgin80 in the proliferative neuroblasts and GMCs, but not in differentiated neurons, suggest a function of this protein associated with the highly active vesicle trafficking needed to meet the demands for cell components imposed by rapid cell division.

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Supplemental Material The full-length cDNA clone 3-1-b1 and its putative amino acid sequence (Ms-golgin80) of *Manduca sexta*. The cDNA clone 3-1-b1 is composed of 2298 bp with an 76 bp 5' untranslated region and a 3' untranslated region. An open reading frame starting from position 77 predicts a 708 amino acids protein. The conserved N-terminus, coiled-coil domains in the middle (amino acids 369-451), and near the C-terminus (amino acids 560-468) sequences are underlined with a dotted line, a solid line, and double lines respectively. The coiled-coil region near the C-terminus provides a putative dimerization site. Symbol * indicates the stop codon. GenBank accession no. AF244891

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   1                                GTTTTACTGAATTTTGCAAGTTCTGTATTGTCTAAACATATAGTGAATTTTCTCGGCAAACAATATTATTTTTATAATGGATGACAGAGCTGCTAAATTGGCTTTA
                                                                                                    M  D  D  R  A  A  K  L  A  L      10
 107   GCTAGAAAAAAGTTAAAAGATCACCAAGTGAAAAAATCATACACCCAACAAAGAGATGTAAATTGTCATAATATACCAGGGTTAAAAACTAATCCAGACAATGTTATGCCACATATAATA
       A  R  K  K  L  K  D  H  Q  V  K  K  S  Y  T  Q  Q  R  D  V  N  C  H  N  I  P  G  L  K  T  N  P  D  N  V  M  P  H  I  I      50
 227   AACGAGGAACAAGTTCAACCTATGTTTTCAACTGAAAGTCGTAATGATAATACTATACAAAATATCAATTCAGAAGTTGCTGACACAAAACAGACAATAGTAAATTCACCAACTTTAACA
       N  E  E  Q  V  Q  P  M  F  S  T  E  S  R  N  D  N  T  I  Q  N  I  N  S  E  V  A  D  T  K  Q  T  I  V  N  S  P  T  L  T      90
 347   GATAGCTTAAATGCTACAGAATTCCTAATATCAAGCAAGAGAAATCTTGAAATACAAGTTGATAATTTACAGTCAAAATTAGCAGATTTGCAAGATAAATACACATTAGCACTAACAAAC
       D  S  L  N  A  T  E  F  L  I  S  S  K  R  N  L  E  I  Q  V  D  N  L  Q  S  K  L  A  D  L  Q  D  K  Y  T  L  A  L  T  N     130
 467   GATAATGCATCTAAACAAATTATTCAAAATTTAGAAAGAGACCTCAGAAATATTGAAGATAAATATAACCAGATTGGTAAAGAAATTTTAGAGAAAAATGACACTATAAAGGAACTTCAC
       D  N  A  S  K  Q  I  I  Q  N  L  E  R  D  L  R  N  I  E  D  K  Y  N  Q  I  G  K  E  I  L  E  K  N  D  T  I  K  E  L  H     170
 587   ACAATGAAAACATTGTTGTCAGATGAAAATAGCAACTACCAAGAACAGTTGGAATTCACAAAGAGCATTTTGACCGCCAAAGAAGCAGAGAATAACTCTCTCCATAGCCAATTATTCAAT
       T  M  K  T  L  L  S  D  E  N  S  N  Y  Q  E  Q  L  E  F  T  K  S  I  L  T  A  K  E  A  E  N  N  S  L  H  S  Q  L  F  N     210
 707   CTTCAAAATCAATTAGAGGCAACACAGCTTCAACTGCAGCAATTGACGAATGGAACAGTCTTTAATTTATGCAAAAATAAAACTGATGAGACTCAACAAAACGAaGTTTTAATGCAAAAA
       L  Q  N  Q  L  E  A  T  Q  L  Q  L  Q  Q  L  T  N  G  T  V  F  N  L  C  K  N  K  T  D  E  T  Q  Q  N  E  V  L  M  Q  K     250
 827   ATATTAATATTAGAACAACAACTTAAAGACTCTCAAAAGGAGAGGAATCAAATCAGTGTACATTATGAACATTATGTCAGTGAATTGAATGAGCAACTTAAGTCAGTTTTGGAGAAAAAT
       I  L  I  L  E  Q  Q  L  K  D  S  Q  K  E  R  N  Q  I  S  V  H  Y  E  H  Y  V  S  E  L  N  E  Q  L  K  S  V  L  E  K  N     290
 947   GAAAATCTCAGTGCTGAACTTCAATACCTAGCAAACAGAGAAAATAGTTTGATTGAACAGATAAGTGAAATGGAAATTAGGTATCAAAATTATCATATCCAACCAAAAGCCACTGCTGAC
       E  N  L  S  A  E  L  Q  Y  L  A  N  R  E  N  S  L  I  E  Q  I  S  E  M  E  I  R  Y  Q  N  Y  H  I  Q  P  K  A  T  A  D     330
1067   GTCACACCTGGCAGCAATGATGAATTACAAGAGTTACAAACTAAATATGAAGAAATCCAAAACCATTTTCAAGCATTGAATATTAAATATAAAGAGCTTGAAAAGCAGTGTATAGAGAGT
       V  T  P  G  S  N  D  E  L  Q  E  L  Q  T  K  Y  E  E  I  Q  N  H  F  Q  A  L  N  I  K  Y  K  E  L  E  K  Q  C  I  E  S     370
1187   AATGAGAAAATCAAAGAATTAAGTCAACACAAGGATACCAGTAGTGTGCAAGACACCATCAGCATTTCAAAATTAAATGCAGATATAGCAAGTGACAAAATTGCAGCCCAAAGAGCAACC
       N  E  K  I  K  E  L  S  Q  H  K  D  T  S  S  V  Q  D  T  I  S  I  S  K  L  N  A  D  I  A  S  D  K  I  A  A  Q  R  A  T     410
1307   GAACAGAATAAAAAACTCAAGCAAGATATGCAAGGATTGGAAGATGCATATGTTAAAATGAGTAAAGATAAATTAGATTTAACGGAAAAGTTAACAGCCGAAAAATATTTAAACAGGCAA
       E  Q  N  K  K  L  K  Q  D  M  Q  G  L  E  D  A  Y  V  K  M  S  K  D  K  L  D  L  T  E  K  L  T  A  E  K  Y  L  N  R  Q     450
1427   CTCACTATAAAATTAGCTGAAATAGAAGAAAAAACTAAAGATATGCAAGTCAAATTAAAAGCTAAAGACGAAGAAATGATCAGATTACAAGCTAGTTACAGAAAATTAGAAAAGTATGAA
       L  T  I  K  L  A  E  I  E  E  K  T  K  D  M  Q  V  K  L  K  A  K  D  E  E  M  I  R  L  Q  A  S  Y  R  K  L  E  K  Y  E     490
1547   AGTTCTCAGACGGATAAACTCGTAGACAACTATACTGATAATGTTATCGAATATGAAGAACTGAAAGAGACACACTCTCACAACGAGGAATGTGTAGGTCATCAGTCAGATGGCAGCGAG
       S  S  Q  T  D  K  L  V  D  N  Y  T  D  N  V  I  E  Y  E  E  L  K  E  T  H  S  H  N  E  E  C  V  G  H  Q  S  D  G  S  E     530
1667   GAGAAAATGaCAACgtCTGACGTTATGACGCCAgAAATCTTGTGTAACTCCGGaTCAAGAcAAAGTCCAAATATTGCAAGAGAAGACGCAATGCAGAAACTTCAACAGCGCTTTTTAAAA
       E  K  M  T  T  S  D  V  M  T  P  E  I  L  C  N  S  G  S  R  Q  S  P  N  I  A  R  E  D  A  M  Q  K  L  Q  Q  R  F  L  K     570
1787   ATTATGGAGGAAGTAGCAGATTTATCGGATGAGAAACACAGACTAGAGCATATAATACTCCAGTTACAGAATGAAACTGAAACAATTTGTGAATATGTAGCTTTGTACCAACAACAGCGC
       I  M  E  E  V  A  D  L  S  D  E  K  H  R  L  E  H  I  I  L  Q  L  Q  N  E  T  E  T  I  C  E  Y  V  A  L  Y  Q  Q  Q  R     610
1907   AGTTTATTAAAGAGGAGAGATGAAGAAAGAAGTGCTCAAATAAAAATATTTGAAGCTGAATGTGATAAACTAAAAAAAGAAATAGATGAGTTGACTTCTATACTCGTAAAGTTATCGCAA
       S  L  L  K  R  R  D  E  E  R  S  A  Q  I  K  I  F  E  A  E  C  D  K  L  K  K  E  I  D  E  L  T  S  I  L  V  K  L  S  Q     650
2027   GATAAGGATTTATCGCACTTCTTAAAAACAGATTCCAAAAATATTGATATACAAAGAGTAATGAATTTGCTTTCAAATTTACAAAGCAATTCCTTAATTGACCCCCATAAGAAAAATGTA
       D  K  D  L  S  H  F  L  K  T  D  S  K  N  I  D  I  Q  R  V  M  N  L  L  S  N  L  Q  S  N  S  L  I  D  P  H  K  K  N  V     690
2147   GACTTTAAAAATTTTTACCCATGTAATTGTTGCTCTGGAAAACTTATCGAGGTATAAGAAATGTAAATGTAGGGCAGGAATTCAAGATACTAATATGTAGCATTAGTTTTATGGTTCTAA
       D  F  K  N  F  Y  P  C  N  C  C  S  G  K  L  I  E  V  *
2267   TAAATGTTTGTTACAAAAAAAAAAAAAAAAAA
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