Published in final edited form as:

Gene. 2010 January 1; 449(1-2): 50–58. doi:10.1016/j.gene.2009.09.004.

Mutational Analysis of *Drosophila Basigin* Function in the Visual System

Michelle Munro, Yazan Akkam, and Kathryn D. Curtin*

Department of Biological Sciences, 601 SCEN, Fulbright College of Arts and Sciences, University of Arkansas, Fayetteville, AR 72701 USA

Abstract

Drosophila basigin is a cell-surface glycoprotein of the Ig superfamily and a member of a protein family that includes mammalian EMMPRIN/CD147/basigin, neuropilin, and embigin. Our previous work on *Drosophila basigin* has shown that it is required for normal photoreceptor cell structure and normal neuron-glia interaction in the fly visual system. Specifically, the photoreceptor neurons of mosaic animals that are mutant in the eye for *basigin* show altered cell structure with nuclei, mitochondria and rER misplaced and variable axon diameter compared to wild-type. In addition, glia cells in the optic lamina that contact photoreceptor axons are misplaced and show altered structure. All these defects are rescued by expression of either transgenic fly *basigin* or transgenic mouse *basigin* in the photoreceptors demonstrating that mouse *basigin* can functionally replace fly *basigin*. To determine what regions of the basigin protein are required for each of these functions, we have created mutant *basigin* transgenes coding for proteins that are altered in conserved residues, introduced these into the fly genome, and tested them for their ability to rescue both photoreceptor cell structure defects and neuron-glia interaction defects of *basigin*. The results suggest that the highly conserved transmembrane domain and the extracellular domains are crucial for basigin function in the visual system while the short intracellular tail may not play a role in these functions.

Keywords

mutation; basigin; cell structure; glia; *Drosophila* visual system; Ig family glycoprotein

1. Introduction

Basigin/EMMPRIN/CD147 is a transmembrane glycoprotein of the Ig superfamily. Basigin homologs have been found in many animals and have been given various designations including basigin in mouse (Altruda *et al.*, 1989), CD147 or EMMPRIN in humans (Biswas *et al.*, 1995), OX47/gp55 in rats (Fossum *et al.*, 1991), 5A11 or HT7 in chicken (Fadool and Linser, 1993, Seuberger *et al.*, 1990). Basigin/EMMPRIN/CD147 is one member of a three member protein family that also includes embigin and neuropilin (SDR1, gp55/gp65).

© 2009 Elsevier B.V. All rights reserved.

*Author for correspondence (kcurtin@uark.edu), Phone: 479-575-4886, Cell: 479-236-2171, Address: 601 SCEN, Biological Sciences, University of Arkansas, Fayetteville, AR 72701.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Human basigin/CD147/EMMPRIN (extracellular **m**atrix **m**etallo**p**roteinase **i**nducer), is found on a variety of tumors (Biswas *et al.*, 1995; Davidson *et al.*, 2003; Reimers *et al.*, 2004) and is believed to play a role in tumor invasion and metastasis (Kanekura *et al.*, 2002; Zucker *et al.*, 2001) by stimulating matrix metalloproteinases (MMPs) secretion from fibroblasts (Biswas *et al.*, 1995; Guo *et al.* 1997; Kataoka *et al.* 1993; Nabeshima *et al.*, 2002). MMP secretion leads to a remodeling of the extracellular matrix allowing tumor cell migration through the matrix (Egebal and Werb, 2002). In addition to its role in stimulating MMPs, basigin/EMMPRIN/CD147 has been shown to promote adhesion-independent growth of cells, a hallmark of cancerous cells (Marieb *et al.*, 2004).

Basigin homologs are widely expressed in normal tissues in the nervous, reproductive, and immune systems. Mice and chicken express basigin in the developing retina (Fadool and Linser, 1993; Ochrietor *et al.*, 2003) the blood-brain barrier (Risau *et al.*, 1986, Schlosshauer and Herzog, 1990), the CNS (Fan *et al.*, 1998), epithelial tissues (Fadool and Linser, 1994) and on activated T and B cells (Fossum *et al.*, 1991). In *Drosophila*, basigin is expressed in the larval nerve cord and muscles (Besse *et al.* 2007) and in the adult in photoreceptor neurons (Curtin *et al.*, 2005; Curtin *et al.*, 2007), in a subset of glia and muscle cells in the head, and in the antennae (Curtin, unpublished).

Consistent with its wide range of expression, basigin/EMMPRIN/CD147 affects development and function of a wide range of tissues in the animal and *basigin* mutant animals show a wide variety of phenotypes. Basigin/CD147 plays roles in cell aggregation and leukocyte activation during immune response (Cho *et al.*, 2001; Fossum *et al.*, 1991; Kasinrerker *et al.*, 1992). In the mouse reproductive system, *basigin* is required for spermatogenesis (Igakura *et al.*, 1998; Toyama *et al.*, 1999) and for embryo implantation (Igakura *et al.*, 1998). *Drosophila basigin* is also required for male fertility (Castrillon, 1993). Avian, mouse and *Drosophila basigin* are all required for normal retinal development and function (Fadool and Linser, 1993; Ochrietor and Linser, 2004; Curtin *et al.*, 2005; Curtin *et al.*, 2007). *Basigin* plays a role in odor response in mouse (Igakura *et al.*, 1996) as well as in fruit flies (Anholt *et al.* 2003). *Basigin* is required for normal sensory and memory function in mice (Naruhashi *et al.*, 1997). *Basigin* is required for neuromuscular junction (NMJ) formation in fruit flies (Besse *et al.*, 2007).

Given the wide range of phenotypes associated with loss of *basigin* function, the finding that *basigin* plays fundamental roles in basic cell physiology and structure is not surprising. *Basigin* is required for normal cell structure (Curtin *et al.*, 2005). Genetic mosaic *Drosophila* in which *basigin* is mutant only in the eye show altered photoreceptor architecture with nuclei, rER and mitochondria all being misplaced within the photoreceptors (Curtin *et al.*, 2005). In addition, *basigin* mutant photoreceptors show abnormal variation in the thickness of axon terminals in the post-synaptic optic lamina (Curtin *et al.*, 2005). Expression of *Drosophila basigin* in non-adherent High Five insect cells leads to elaboration of microfilaments and microtubules and the consequent formation of lamellipodia and basigin partially colocalizes with actin in these cells (Curtin *et al.*, 2005). Besse *et al.* (2007) have more recently found that the actin cytoskeleton is altered in the pre-synaptic side of the NMJ in *basigin* hypomorphic mutants. All these data suggest that basigin plays a role in cytoskeleton organization. Interestingly, basigin has been found to partially co-localize with integrins in mammalian cells and to interact genetically with integrins in *Drosophila* (Reed *et al.*, 2004; Curtin *et al.*, 2005).

In addition to affecting cell structure, there are several lines of evidence that *basigin* mediates cell-cell interactions. *Basigin* is required for neuron-glia interactions as determined *in vitro* by experiments on dissociated chick retina (Fadool and Linser, 1993) and *in vivo* in the fly visual system (Curtin *et al.*, 2007). In *Drosophila*, epithelial glia in the post-synaptic lamina are misplaced in mosaic animals that have *basigin* mutant eyes (Curtin *et al.*, 2007). In addition,

when *basigin* is mutant in photoreceptors, specialized invaginations of these glia cells into photoreceptor terminals, called capitate projection, are largely absent (Curtin *et al.*, 2007). *Drosophila basigin* promotes cell aggregation in non-adherent *Drosophila* S2 cells (Besse *et al.*, 2007). *Drosophila basigin* is required for development of nerve-muscle contacts at the NMJ (Besse *et al.*, 2007) with *basigin* mutant larvae exhibiting larger and fewer synaptic boutons than wild-type. (Besse *et al.*, 2007). Lastly, *basigin* plays a role in dorsal closure in *Drosophila* suggesting an involvement in extraembryonic membrane apposition (Reed *et al.*, 2004).

The molecular mechanism by which *basigin* protein promotes normal cell structure or cell-cell interactions is unknown. As a first step in better understanding the molecular function of *basigin*, we were interested in determining what regions of the *basigin* protein are important for function. To do this, we generated and tested mutant *basigin* transgenes for their ability to replace endogenous *basigin* function in the fly visual system, specifically for their ability to rescue photoreceptor cell structural defects and neuron-glia defects of *basigin*. Our lab has previously shown that expression of mouse *basigin* (specifically *basigin 2*, NCBI ID: NP_001070652.1) can rescue photoreceptor structure defects (Curtin *et al.*, 2005) as well as neuron-glia interaction defects (Curtin *et al.*, 2007) in mosaic flies in which endogenous *basigin* is mutant in the eyes. This shows that mouse *basigin* protein can make the same molecular contacts as fly *basigin* in the fly visual system. For this reason, we focused on generating mutations in codons for conserved residues in each of the putative subdomains of the *basigin* protein including the putative extracellular, transmembrane and intracellular domains. The results of this mutagenic analysis will help us identify residues and regions of the *basigin* protein that are important for each of the cellular functions of this multifunctional protein.

2. Materials and Methods

2.1 Generating transgenic flies containing mutant *basigin* transgenes

Mutant *basigin* transgenes were created using site-directed mutagenesis with oligonucleotides designed with the appropriate codon changes. These were used with the Quickchange Kit (cat# 200523) from Stratagene (La Jolla, CA) following the manufacturer's instructions. Mutant transgenes were confirmed by DNA sequencing, cloned into the pUAST vector (Brand and Perrimon, 1993), and introduced into flies by P-element mediated mutagenesis by Best Gene, Inc. (Chino Hills, CA). All the cloned *basigin* genes were tagged with a V5 antibody tag at the C-terminus of the protein.

2.2 Flies and mosaics

Stocks of *Drosophila melanogaster* were maintained at 25°C. The *bsg*^{δ265} excision allele was previously described (Curtin *et al.*, 2005). Eye specific genetic mosaics of the *bsg*²⁶⁵ mutant were prepared according to the method reported by Stowers and Schwarz (1999). EGUF/hid lines for FRT40A were obtained from the Bloomington Fly Stock Center, Bloomington, IL. The ey-Gal4 construct in the EGUF/hid line was used to drive expression of the *basigin* mutant transgenes that were expressed from a UAS promoter.

2.3 Antibodies

A polyclonal antibody against *Drosophila basigin* was generated in rabbit, to a fusion protein containing the entire extracellular domain as previously described (Curtin *et al.*, 2007). The ability of this antibody to recognize *basigin* has been demonstrated on frozen sections by immuno-histochemistry. Flies with *basigin* mutant eyes show no labeling with this antibody on photoreceptor axons in the lamina, while wild-type animals show labeling (Curtin *et al.*, 2007 and unpublished). HRP tagged anti-V5 antibody (cat # R961-25) was obtained from

Invitrogen Corp, Carlsbad, CA, USA). Anti-repo (8D12) and anti-elav were obtained from the Developmental Studies Hybridoma Bank, University of Iowa, USA). 8D12, raised against amino acids 218–612 of *Drosophila* repo, was used at 1/20 dilution; 8D12 specificity has been confirmed by failure to immuno-label *repo* null mutant embryos (Halter *et al.*, 2005). Secondary antibodies included biotinylated goat anti-rabbit antibodies (Vector Labs, Burlingame, CA, USA, cat. No. BA-1000) and Alexa-568 conjugated goat anti-rabbit (cat. no. A11011 from Invitrogen/Molecular Probes, Carlsbad, CA, USA).

2.4 Immunohistochemistry

Partially dissected fly heads were fixed in ice-cold 3% para-formaldehyde in a phosphate buffer (13.5mM KCl, 50mM Na₂HPO₄, 10mM KH₂PO₄), for 5 h, washed 3 × 5 min in PBS, incubated overnight in PBS plus 20% sucrose, and subsequently mounted in TissueTek (Fisher Scientific, Pittsburgh, PA, USA) and quick frozen in liquid nitrogen. Sections 10–15µm thick were collected onto slides pre-treated with poly-L-lysine (Sigma, St. Louis, MO, USA). Sections to be labeled subsequently with rabbit anti-basigin were frozen and sectioned at 10 µm, and then subsequently fixed for 5 min in 3% formaldehyde. To block non-specific binding, samples were put for 1 h in PBS plus 1% TritonX-100 and either 2% BSA (Sigma Chemical Co.) or 10% normal goat serum (Vector Labs, cat. No. S-1000), and then incubated overnight in primary antibody in blocking solution. Slides were washed 3 × 10 minutes in PBS containing 0.5% Tween20. Alexa-568 conjugated goat anti-rabbit was applied for 1 h at 1/1000 dilution in block. Slides were mounted using glycerol (Sigma) with 1mg/ml *p*-phenylene diamine (Sigma). Images were photographed on a Zeiss Axiophot and assembled with Adobe Photoshop.

2.5 Western Blot

Mosaic animals that were mutant in the eye for *bsg*^{δ265} but were expressing V5 tagged basigin proteins from mutant *basigin* transgenes in the eye were generated using the general method of Stowers and Schwarz (1999). Head extracts from such animals were prepared by grinding 50 heads in 100µL of SDS PAGE sample buffer, 30µl of this extract was run on an 8% denaturing polyacrylamide gel and the gel blotted to nitrocellulose using standard techniques. The nitrocellulose blot was incubated in block buffer (25mM Tris-HCl, pH 8, 125mM NaCl, 0.1% Tween 20, 5% Carnation Dry Milk) for an hour and subsequently incubated overnight in HRP tagged anti-V5 antibody (cat # R961-25, Invitrogen Corp, Carlsbad, CA, USA) diluted 1:1000 in block buffer. The transgenic basigin proteins were all tagged at the C-terminus with a V5 tag. The blot was subsequently washed 3 × 15 minutes in wash buffer (same as blocking buffer minus the dried milk) and the HRP subsequently detected using SuperSignal West Pico Chemiluminescent Reagent (cat # 34077) from Thermo Scientific (part of Fisher Scientific, Pittsburgh, PA, USA). The results were photographed using a Fluorchem 8900 (Alpha Innotech Corporation, San Leandro, CA, USA).

3. Results

3.1 Structure of *Drosophila* basigin

Although mammals contain three *basigin* related genes, *basigin*, *neuropilin*, and *embigin*, *Drosophila* contains only one *basigin* related gene. This gene, located on chromosome 2L at cytological map position 28E3–28E5, spans 25 kB and encodes nine transcripts coding for two distinct proteins, one with 265 amino acids and one with 298 amino acids (*Drosophila* genome project— <http://flybase.bio.indiana.edu/>). Both proteins are putative transmembrane proteins with a short intracellular tail and two putative extracellular Ig domains and they differ only in the N and C terminal residues. Though the fly gene has been dubbed *basigin*, the coded protein has similar degrees of homology to both mammalian basigin and mammalian neuropilin. Fly basigin has 26% identity and 34% chemical similarity to mouse basigin and 26% identity and

30% similarity to neuroplastin (Curtin *et al.*, 2005). The homology between fly and mouse basigin family members is not equally distributed along the length of the molecule. Fig. 1 shows a sequence alignment of several basigin related proteins from different species. Comparison of mouse and fly basigin extracellular domains, reveals 20% identity and 28% chemical similarity (Curtin *et al.*, 2005). The highest homology between basigin related proteins is in the TM domain (Curtin *et al.*, 2005) where mouse and fly basigin are 80% identical. The short cytoplasmic tail shows homology between mouse and fly basigin in a short sequence of five amino acids found immediately after the putative TM domain (YEKRR), as well as homology at L256 and E258 of *Drosophila basigin*.

3.2 Testing engineered basigin mutant genes for their ability to affect cell structure

Drosophila basigin has previously been shown to be required for normal cell architecture. Specifically, *basigin* has been shown to be required for proper localization of nuclei, endoplasmic reticulum, and mitochondria in photoreceptors, as well as normal axon diameter in photoreceptor neurons in the eye (Curtin *et al.*, 2005). To illustrate the effect of basigin on photoreceptor cell structure we created mosaic animals using the method of Stowers and Schwarz (1999) in which the entire eye and only the eye is homozygous mutant for *bsg*²⁶⁵, an embryonic lethal allele of *basigin* (Curtin *et al.*, 2005). Frozen head sections were labeled with antibody to elav, a neural nuclear protein to visualize photoreceptor nuclear placement (Fig. 2). Normal photoreceptor nuclei were arranged in orderly rows within the retina. The nuclei of photoreceptors R1–R6 were located in tight rows at the apical surface of the eye while R7 nuclei were just proximal to these. R8 nuclei were aligned near the basement membrane of the retina (e.g. Fig 2A). In *bsg*²⁶⁵ mutant eyes, photoreceptor nuclei were scattered throughout the retina (Fig. 2B). These results were similar to those previously published (Curtin *et al.*, 2005).

Mouse *basigin* can functionally replace fly *basigin* in the fly visual system, completely rescuing the photoreceptor nuclear placement defects (Curtin *et al.*, 2005). The ability of mouse *basigin* to replace fly *basigin* suggests that conserved residues between the fly and mouse protein are important for basigin's function in normal cell structure. To specifically identify residues in the protein that are required for basigin's role in promoting normal cell structure, we mutated codons for highly conserved amino acids within the basigin protein. We introduced these mutant *basigin* transgenes into *Drosophila* and we tested each mutant transgene for its ability to rescue the photoreceptor nuclear placement defect. We chose to alter amino acids of the basigin protein that were shared among all family members shown (Fig. 1). Some of our engineered mutant *basigin* transgenes have alterations of one aa codon, others were altered in more than one aa codon. The aa residues that were altered are denoted with asterisks in Fig 1. Residues with asterisks with a bar above them indicate several adjacent aa residues that were altered at once. Table 1 gives a list of the specific mutant basigin proteins encoded by engineered transgenes. These mutant proteins are named according to the following example: W151G denotes change of amino acid residue 151 from W to G.

To determine if our mutant *basigin* transgenes could promote normal cell structure we assayed the ability of these transgenes to promote normal placement of photoreceptor nuclei. Specifically, we expressed each engineered mutant *basigin* gene in the eyes of mosaic animals that were homozygous mutant in the eye for *bsg*²⁶⁵. Sixteen different mutant lines of animals were tested for rescue. Of these, frozen sections are shown for 12 (Fig. 2) while tabulated data is presented for all 16 (Table 1). Mosaic animals with *bsg*²⁶⁵ mutant eyes showed misplaced nuclei (Fig 2B) while normal placement of photoreceptor nuclei was seen when a wild-type *basigin* transgene was used to rescue (Fig. 2A). *Bsg*²⁶⁵-mutant-eye animals that expressed the basigin proteins YE246SG (Fig. 2C) and PFL228LFT (Fig. 2D) from transgenes showed normal nuclear placement, indistinguishable from the results seen with wild-type *basigin*,

indicating complete rescue. *Bsg*²⁶⁵ mosaic animals expressing the T128A basigin (Fig. 2E), RVK218LVT (Fig. 2F), EG132GV (Fig. 2G), WKK64LKT (Fig. 2H) and Y103S (Fig. 2I) all showed fewer misplaced nuclei than the *bsg*²⁶⁵ mutant eye animals, but failed to show normal nuclear placement, indicating partial rescue. Basigin mutant protein E235G (Fig. 2J), W151G (Fig. 2K) and DRGEY188AVRES (Fig. 2L) by contrast showed nuclear placement that was similar to *bsg*²⁶⁵ mutant eye animals with no rescue construct (Fig. 2B) indicating a complete failure of these constructs to rescue.

To more accurately evaluate the ability of each engineered mutant *basigin* transgenes to rescue the misplaced photoreceptor nuclei defect of *bsg*²⁶⁵ mosaics, we quantified the results for each of the 16 transgenic lines (Table 1). Nuclei that were clearly located between the normal position of the R7 and R8 nuclei were counted as misplaced and misplaced nuclei were averaged *per* eye for each line. Flies that are mutant in the eye for *bsg*²⁶⁵ show 18% $\pm$ 0.67% (S.E.) misplaced photoreceptor nuclei. This is consistent with previous report of 22% nuclei misplaced in *bsg*²⁶⁵ mutant eyes (Curtin *et al.*, 2005). Mutant *basigin* genes were classified as showing complete rescue if 3% or fewer nuclei were misplaced, partial rescue if 7%–13% of nuclei were misplaced and no rescue if 17%–19% of nuclei were misplaced (Table 1). Most basigin proteins that failed to rescue were altered in the TM or second Ig domain. In addition, a truncated version of basigin (Trunc V5) that terminates just prior to the transmembrane domain and was secreted when expressed in insect High Five cells (not shown) failed to rescue. Altered proteins that showed partial rescue were scattered throughout the extracellular portion of the molecule. Mutant proteins that showed complete rescue were altered in the intracellular tail of basigin (Table 1).

Average values for misplaced nuclei for each mutant were compared to both the positive and negative controls using Dunnett's Method (Table 1). P-values over 0.05 in such a comparison indicate significant similarity. Mutant basigin proteins that we designate as rescuing all had p-values greater than 0.8 when compared to the positive control in which wild-type basigin protein was affecting rescue. Mutants that failed to rescue had p-values which were greater than 0.9 when compared to the negative control, *bsg*²⁶⁵ mutant eye animals with no rescue construct. Those mutants which we labeled as partial rescue had p-values <0.0001 when compared to either the positive or negative controls, indicating that they were very different than either control.

3.3 Examining mutant basigin protein stability and expression

Mutant basigin proteins that failed to rescue may be unstable or not made in sufficient amounts due to position affects. To assess protein levels, we made head extracts from animals that are mutant in the eye for *bsg*²⁶⁵ but were expressing a basigin mutant transgene. All of our *basigin* transgenes encoded proteins with a V5 antibody tag at the C-terminus for detection on Westerns. The results are shown in Fig. 3. Mutant basigin proteins that failed to rescue such as W151G and E235G were detected at levels similar to wild-type basigin. We also examined the mutant basigin proteins that showed partial rescue phenotypes and these also expressed stable proteins (Fig. 3 and data not shown). TruncV5, a shortened version of basigin, appeared smaller on the blot, as expected. The predicted difference in MW between full length basigin and truncated basigin is 4.8kD, roughly consistent with the MW difference observed here.

In addition to testing protein levels by immunoblotting, we also examined mutant transgenic basigin protein by immuno-histochemistry. To do this we created animals with *bsg*²⁶⁵ mutant eyes that are expressing altered basigin proteins in the eye. We examined frozen head sections of these animals using anti-basigin antibody. Results for two of the altered basigin proteins, W151G (Fig. 4A) and E235G (Fig. 4B) are shown. Expression of basigin from these transgenes was indistinguishable from wild-type (not shown but see Curtin *et al.*, 2007) with protein expressed along the lengths of the photoreceptor axons into the post-synaptic tissues.

Examination of all altered basigin proteins, except trunc-V5 which was not tested, showed similar results (now shown).

3.4 Testing mutant basigin transgenes for effects on neuron-glia cell interactions

In addition to being necessary for proper placement of photoreceptor nuclei, *basigin* is required for normal neuron-glia interactions in the optic lamina of *Drosophila* (Curtin *et al.*, 2007). Neurons and glia are mutually dependent on each other for the final localization in the nervous system (Perez and Stellar, 1996; Poeck *et al.*, 2002; Rangarajan *et al.*, 1999; Rangarajan *et al.*, 2001; Simpson *et al.*, 2000). Axons of photoreceptors R1–R6 terminate in the first optic neuropile or lamina, whereas axons of R7 and R8 grow through the lamina into the second neuropile, the medulla. Adult wild-type animals have six distinct categories of glia in the optic lamina which include from distal to proximal: fenestrated glia adjacent to the retinal basement membrane, pseudocartridge glia located between fenestrated glia and lamina cortex, two layers of satellite glia located in lamina cortex, epithelial glia which are in lamina neuropile proper and the marginal glia located between the lamina and medulla (Saint Marie and Carlson, 1983).

A previous study by Curtin *et al.* (2007) demonstrated several points. First, basigin is expressed on photoreceptor axons along their entire length from retina to lamina in the adult visual system. Second, proper placement of epithelial glia in the lamina is dependent on basigin expression on the photoreceptors. Third, basigin is necessary for the normal formation of capitate projections (CPs), finger-like projections of epithelial glia into photoreceptor terminals. CPs are proposed sites for neurotransmitter recycling (Fabian-Fine *et al.*, 2003). Fourth, expression of either fly or mouse basigin in the eye of *bsg*²⁶⁵ mosaic mutant-eye animals can rescue these glia cell defects (Curtin *et al.* 2007).

In order to observe glia cell body placement, frozen head sections were labeled with antibody to the glia-specific nuclear homeodomain protein repo (Halter *et al.*, 1995; Xiong *et al.*, 1994). In mosaic flies with *bsg*²⁶⁵ mutant eyes, the nuclei of the subretinal (including the fenestrated and pseudocartridge glia) and satellite glia remained in their normal positions. However, the epithelial glia were displaced within the lamina neuropile (Fig. 5A) as previously described (Curtin *et al.*, 2007). This defect was rescued by transgene expression of wild-type *Drosophila basigin* (Fig 5B; and Curtin *et al.*, 2007). Likewise, expression of mouse *basigin* in the eyes of *bsg*²⁶⁵ mutant eye animals can rescue glia cell placement defects (Curtin *et al.*, 2007).

We examined each of our 16 mutant transgenes for their ability to rescue *basigin* mutant glia cell placement defects. Expression in the eye of the *basigin* transgene coding mutant protein YE246SG in the photoreceptors led to complete rescue of epithelial glia cell placement defects (Fig. 5C). Normal placement of the epithelial glia was also observed with mutant P147L basigin (Fig. 5D). No rescue of glia cell placement was observed in when basigin mutant proteins PFL228LFT (Fig. 5E) or EIE113GFG (Fig. 5F) were expressed. Expression of either E235G (Fig. 5G) or W151G (Fig. 5H) mutant basigin proteins in a *bsg*²⁶⁵ mutant-eye animal led to a novel epithelial glia cell body placement. Specifically, epithelial glia cell bodies were not found scattered throughout the lamina as in the *bsg*²⁶⁵ mutant eye animals, but instead were found at the proximal edge of the lamina near the termini of retinal cells R1–R6. Results for all 16 transgenes are shown in Table 1.

4. Discussion

Important cellular functions have been identified for *basigin* family genes through the study of *Drosophila basigin*. For example, *basigin* is required for normal cell structure in the animal (Curtin *et al.*, 2005). Though *basigin* is required for viability in *Drosophila*, it is possible to

create genetic mosaics in which *basigin* function is missing entirely from the eye and only the eye. Loss of *basigin* from the eye leads to altered photoreceptor architecture including misplaced nuclei, ER, and mitochondria and altered axon shape (Curtin *et al.*, 2005). In addition, expression of *Drosophila basigin* in non-adherent insect High Five cells leads to cell attachment to the culture dish and formation of filopodia and lamellopodia with elaboration of microfilaments and microtubules (Curtin *et al.*, 2005).

Drosophila basigin is also required for normal cell-cell interactions in *Drosophila*. Loss of *basigin* from the photoreceptors leads to misplaced epithelia glia and a failure of these glia to extend electron dense finger like projections (capitate projections) into photoreceptor terminals. Expression of *basigin* in *Drosophila* S2 cells leads to cell adherence (Besse *et al.*, 2007). *Basigin* mutants show altered neuromuscular junction formation (Besse *et al.*, 2007).

As a first step to better understand *basigin* protein function at a molecular level, we have altered conserved residues in *basigin* and tested these for their ability to promote normal photoreceptor cell structure and normal neuron-glia interactions in the visual system with the goal of identifying residues and/or regions of the protein that are essential for *basigin* function. Previous findings show that mouse *basigin* can functionally replace fly *basigin* in the visual system (Curtin *et al.*, 2005, 2007) indicating that residues that are conserved between mouse and fly *basigin* are crucial for *basigin* function. In this study, we mutated codons for amino acids that are conserved in all the *basigin*-related proteins shown in Fig. 1. These engineered mutant *basigin* transgenes were introduced into the animal and tested for their ability to rescue specific defects in the visual system of *basigin*-mutant-eye animals. *Basigin* is required for several aspects of normal photoreceptor cell structure in the visual system, including proper placement of nuclei, rER and mitochondria, as well as normal axon diameter. To assess the ability of mutant *basigin* proteins to promote normal cell structure, we examined their ability to promote proper placement of photoreceptor cell nuclei.

Mutant *basigin* proteins that promoted normal cell structure in the animal provide important information about the molecular function of *basigin* protein. For example, YEKRR are the only residues that are shared between all *basigin* family members in the putative intracellular tail of the protein, yet both YE246SG and KRR248MGG mutant *basigin* proteins functioned as well as wild-type *basigin* protein in rescuing photoreceptor structural defects. These mutant proteins functioned normally despite the dramatic chemical changes of the altered residues. In YE246SG, YE, a nonpolar aromatic and polar acidic respectively, were changed to SG, a polar uncharged and nonpolar residue respectively. In KRR248MGG, the positively charged KRR residues were changed to the neutral residues MGG. The fact that these residues, YEKRR, can be changed with no alteration in protein function suggests that *basigin* function may not depend on the intracellular portion of the protein. If this is true, it suggests that *basigin* protein is not affecting cell structure through direct intracellular signaling or direct attachment to cytoskeletal elements of the cell. In contrast to this result, Besse *et al.* (2007) found that changing KRR to NGG did affect *basigin* function at the NMJ. They found that expression of an altered *basigin* protein, KRR248NGG, failed to rescue *basigin* defects in NMJ synaptic bouton size and number. This suggests either that *basigin* protein functions differently at the NMJ or that NMJ development is more sensitive to disruptions in *basigin* function.

Flies which expressed the *basigin* transgene encoding mutant P147L, located in the putative first Ig domain, and PFL228LFT located in the putative TM region, also showed wild-type protein function. Proline can cause kinks or hinges in transmembrane alpha helices that can have profound effects on protein function (Ballesteros and Weinstein, 1992; Tieleman *et al.*, 2001). Generally speaking, proline is implicated in membrane protein folding (Lu *et al.*, 2001; Deber and Therien, 2002). The fact that both proline 147 in the putative first Ig domain and proline 228 in the putative TM domain could be changed without deleterious effects on

protein function shows that these prolines were not required for proper basigin conformation. Proline 228 is found in the TM helix, though proline 147 is not predicted to be in a helical region by the Eisenberg method of analysis. The negative D residue at 188 in the second putative Ig domain can be replaced by the positive N residue without deleterious effect indicating that this residue was also not crucial for function. Because DRGEY188AVRES fails to rescue, this indicates that it is the change of R189, G190 and/or Y192 that makes this mutant non-functional.

Mutant proteins that fail to rescue *basigin* function also shed light on basigin function in the animal. All of the following transgenic proteins failed to rescue photoreceptor nuclear placement defects: 1) E235G in the putative TM domain, 2) trunc-V5 which terminates before the putative TM domain and is found to be secreted when expressed in insect High Five cells (not shown), 3) W151G and 4) DRGEY188AVRES, both located in second putative Ig domain. These results suggest that the TM domains and the second putative Ig domain are both essential for basigin protein function.

The failure of trunc-V5 to rescue photoreceptor nuclear placement shows that basigin must be in the membrane to function. The failure of E235G to rescue coupled with the fact that the TM domain shows 80% identity between mouse and fly basigin argues for an essential role for the TM domain in basigin protein function. One hypothesis is that the TM domain is required for protein-protein interactions. Charged amino acid residues in TM domains, such as E235 in basigin, usually mediate protein oligomerization in the membrane by engaging in interhelical hydrogen bonding interactions with charged residues in the in other TM helices (Smith *et al.*, 1996; Zhou *et al.*, 2001). Consistent with this hypothesis, we have found basigin in large ca. 300kD MW complexes in membrane extracts from fly heads (Curtin, unpublished).

Basigin proteins altered in the second putative Ig domain fail to function. This region of the protein could be involved either in formation of basigin containing complexes within the membrane or in interaction of basigin with proteins on apposing cells or both. The first putative Ig domain and other extracellular regions of basigin also play some role in basigin function because proteins mutant in these regions show incomplete basigin protein activity. Examples of mutant basigin proteins that showed incomplete rescue include WKK64LKT, Y103S which are both in the first putative Ig domain, EIE113GFG, T128A and EG132GV which are all located between first and second putative Ig domain and RVK218LVT located between the second putative Ig domain and the TM domain.

In addition to its requirement for normal photoreceptor cell structure, basigin is also required for normal neuron-glia interactions in the visual system. We have shown that *basigin* expression in photoreceptors is required for normal placement of epithelia glia in the visual lamina as well as for the formation of CPs between these glia and the photoreceptor axon terminals (Curtin *et al.*, 2007). Given that basigin seems to have two distinct roles in the visual system, namely normal cell structure of the cells in which it is expressed, and proper placement and structure of cells that interact with basigin-expressing cells we were interested in testing each of our mutant transgenes for their effects on neuron-glia interaction. We were especially interested to see if there were mutant basigin proteins that would rescue one class of defects and not the other. We found that all transgenes that rescued photoreceptor structural defects of basigin also rescued the neuron-glia interaction defects of *basigin*. Likewise transgenes that failed to rescue, failed to rescue both classes of defects. This fact shows that there is considerable overlap in the molecular function of basigin for both classes of cellular functions, cell structure and cell-cell interactions. For example, the fact that the E235G basigin protein, which is altered in the TM domain fails to rescue the glia cell placement when expressed in photoreceptors suggests that basigin may have to form a functional complex within the membrane of photoreceptor cells to affect epithelial glia cell placement and structure.

Constructs that showed partial rescue of the cell structure defects failed to rescue the glia placement defects. This is probably due to the fact that the glia placement defects show higher penetrance in *bsg*²⁶⁵ mutants than the photoreceptor nuclear placement defect. For example, the range in the number of misplaced photoreceptor nuclei in animals with *bsg*²⁶⁵ eyes is 16%–50% (Curtin *et al.*, 2005) whereas glia misplacement is consistently over 80% (unpublished).

In summary, the results show that the TM and extracellular domains of basigin are essential for basigin's ability to promote normal photoreceptor cell structure and proper neuron-glia interactions, while the intracellular region of the protein may not be essential. These results are consistent with a model in which basigin acts either through protein interactions in the membrane, interactions with proteins on apposing cells, or both to affect cell structure.

How might one explain the fact that basigin seems to have two distinct roles, namely its involvement in both normal cell structure and normal cell-cell interaction? The most likely explanation is that basigin's primary function is to mediate cellular interactions and that its effects on photoreceptor cell structure are secondary. There is clear evidence that cellular interactions and cell structure are linked. For example, in mosaic animals that are mutant only in the eye for *bsg*²⁶⁵ epithelia glia fail to extend electron dense finger-like projections into *basigin* photoreceptor terminals (Curtin *et al.*, 2007). This defect is rescued by expression of basigin in photoreceptor cells. Thus failure of these glia to properly interact with photoreceptors causes changes in glia cell structure. In addition, in *basigin* larvae, cellular contacts are altered during NMJ formation, and this coincides with altered cytoskeletal arrangement in the muscle (Besse *et al.*, 2008).

There are several examples of proteins that both mediate cell adhesion and organize cell structure, including cadherins and integrins (reviewed in Aplin *et al.*, 1998). Indeed, for cellular structure to be normal the cytoskeleton of the cell must be anchored to something outside the cell, either to the extracellular matrix at focal adhesions or to the cytoskeleton of adjacent cells through adherens junctions. Thus it is not surprising to find proteins that affect both processes. However, because the portions of basigin needed for function are in the extracellular domains or in the TM domain, it seems likely that basigin's effect on cell structure is indirect while its role in cell-cell interactions may be direct. In all, the evidence we present suggests significant overlap in the molecular function of basigin in its role in cell structure and cell-cell interactions consistent with these two functions being tightly linked.

Acknowledgments

This work was supported by NIH Grant Number P20 RR15569 from the COBRE Program of the National Center for Research Resources to the University of Arkansas and by a grant from the Arkansas Biosciences Institute.

References

- Altruda F, Cervella P, Gaeta ML, et al. Cloning of cDNA for a novel mouse membrane glycoprotein (gp 42): shared identity to histocompatibility antigens, immunoglobulins and neural-cell adhesion molecules. *Gene* 1989;85:445–452. [PubMed: 2697642]
- Anholt RR, Dilda CL, Chang S, Fanara JJ, et al. The genetic architecture of odor-guided behavior in *Drosophila*: epistasis and the transcriptome. *Nature Genetics* 2003;35:180–184. [PubMed: 12958599]
- Aplin AE, Howe A, Alahari SK, et al. Signal transduction and signal modulation by cell adhesion receptors: the role of integrins, cadherins, immunoglobulin-cell adhesion molecules, and selectins. *Pharmacol. Rev* 1998;50:197–264. [PubMed: 9647866]
- Ballesteros JA, Weinstein H. The role of Pro/Hyp-kinks in determining the transmembrane helix length and gating mechanism of a Leu-zervamicin channel. *Biophys. J* 1992;62:110–111. [PubMed: 1376162]

- Berdichevski F, Chang S, Bodorova J, et al. Generation of monoclonal antibodies to integrin-associated proteins. Evidence that alpha3beta1 complexes with EMMPRIN/basigin/OX47/M6. *J. Biol. Chem* 1997;272:29174–29180. [PubMed: 9360995]
- Besse F, Mertel S, Kittel RJ, et al. The Ig cell adhesion molecule Basigin controls compartmentalization and vesicle release at *Drosophila melanogaster* synapses. *J. Cell Bio* 2007;177:843–855. [PubMed: 17548512]
- Biswas C, Zhang Y, DeCastro R, et al. The human tumor cell-derived collagenase stimulatory factor (renamed EMMPRIN) is a member of the immunoglobulin superfamily. *Cancer Res* 1995;55:434–439. [PubMed: 7812975]
- Brand AH, Perrimon N. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* 1993;118:401–415. [PubMed: 8223268]
- Castrillon DH, Gonczy P, Alexander S, et al. Toward a molecular genetic analysis of spermatogenesis in *Drosophila melanogaster*: characterization of male-sterile mutants generated by single P element mutagenesis. *Genetics* 1993;135:489–505. [PubMed: 8244010]
- Cho JY, Fox DA, Horejsi V, et al. The functional interactions between CD98, beta1-integrins, and CD147 in the induction of U937 homotypic aggregation. *Blood* 2001;98:374–382. [PubMed: 11435306]
- Curtin KD, Meinertzhagen IA, Wyman RJ. Basigin (EMMPRIN/CD147) interacts with integrin to affect cellular. *J. Cell Sci* 2005;118:2649–2660. [PubMed: 15928045]
- Curtin KD, Wyman RJ, Meinertzhagen IA. Basigin/EMMPRIN/CD147 mediates neuron-glia interactions in the optic lamina of *Drosophila*. *Glia* 2007;55:1542–1553. [PubMed: 17729283]
- Daosong X, Hemler M. Metabolic Activation-related CD147-CD98 Complex. *Molecular and Cellular Proteomics* 2005;4:1061–1071. [PubMed: 15901826]
- Davidson B, Goldberg I, Berner A, et al. EMMPRIN (extracellular matrix metalloproteinase inducer) is a novel marker of poor outcome in serous ovarian carcinoma. *Clin. Exp. Metastasis* 2003;20:161–169. [PubMed: 12705637]
- Deber CM, Therien AG. Putting the β -breaks on membrane protein misfolding. *Nature Struct. Biol* 2002;9:318–319. [PubMed: 11976722]
- Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. *Nat Rev Cancer* 2002;2:161–174. [PubMed: 11990853]
- Fabian-Fine R, Verstreken P, Hiesinger PR, et al. Endophilin Promotes a Late Step in Endocytosis at Glial Invaginations in *Drosophila* Photoreceptor Terminals. *J. Neurosci* 2003;23:10732–10744. [PubMed: 14627659]
- Fadool JM, Linser PJ. 5A11 antigen is a cell recognition molecule which is involved in neuronal-glia interactions in avian neural retina. *Develop. Dyn* 1993;196:252–262.
- Fadool JM, Linser PJ. Spatial and temporal expression of 5A11/HT7 antigen in the chick embryo. *Roux's Arch. Develop. Biol* 1994;203:3228–3339.
- Fadool JM, Linser PJ. Evidence for the formation of multimeric forms of the 5A11/HT7 antigen. *Biochem and Biophys. Res. Comm* 1996;229:280–286.
- Fan Q-W, Yuasa S, Kuno N, Senda, et al. Expression of basigin, a member of the immunoglobulin super family, in the mouse central nervous system. *Neurosci. Res* 1998;30:53–63. [PubMed: 9572580]
- Fossum S, Mallett S, Barclay AN. The MRC OX-47 antigen is a member of the immunoglobulin superfamily with an unusual transmembrane sequence. *Eur. J. Immunol* 1991;21:671–679. [PubMed: 2009910]
- Guo H, Zucker S, Gordon MK, et al. Stimulation of matrix metalloproteinase production by recombinant extracellular matrix metalloproteinase inducer from 483 transfected Chinese hamster ovary cells. *J Biol. Chem* 1997;272:24–27. [PubMed: 8995219]
- Halter DA, Uban J, Rickert C, et al. The homeobox gene *repo* is required for the differentiation and maintenance of glia function in the embryonic nervous system of *Drosophila melanogaster*. *Development* 1995;121:317–332. [PubMed: 7768175]
- Igakura T, Kadomatsu K, Kaname T, et al. A null mutation in basigin, an immunoglobulin superfamily member, indicates its important roles in peri-implantation development and spermatogenesis. *Dev. Biol* 1998;194:152–165. [PubMed: 9501026]

- Igakura T, Kadomatsu K, Taguchi O, et al. Roles of basigin, a member of the immunoglobulin superfamily, in behavior as to an irritating odor, lymphocyte response, and blood-brain barrier. *Biochem. Biophys. Res. Commun* 1996;224:33–36. [PubMed: 8694829]
- Kanekura T, Xiang C, Kanzaki T. Basigin (CD147) is expressed on melanoma cells and induces tumor cell invasion by stimulating production of matrix metalloproteinases by fibroblasts. *Int. J. Cancer* 2002;99:520–528. [PubMed: 11992541]
- Kasinrerk W, Fiebiger E, Stefanova I, et al. Human leukocyte activation antigen M6, a member of the Ig superfamily, is the species homologue of rat OX-47, mouse basigin, and chicken HT7 molecule. *J. Immunol* 1992;149:847–854. [PubMed: 1634773]
- Kataoka H, DeCastro R, Zucker S, et al. Tumor cell-derived collagenase-stimulatory factor increases expression of interstitial collagenase, stromelysin, and 72-kDa gelatinase. *Cancer Res* 1993;53:3154–3158. [PubMed: 8319225]
- Kirk P, Wilson MC, Heddle C, et al. CD147 is tightly associated with lactate transporters MCT1 and MCT4 and facilitates their cell surface expression. *EMBO J* 2000;19:3896–3904. [PubMed: 10921872]
- Lu H, Marti T, Booth PJ. Proline residues in transmembrane alpha helices affect the folding of bacteriorhodopsin. *J. Mol. Biol* 2001;308:437–446. [PubMed: 11327778]
- Marieb EA, Zoltan-Jones A, Rongsong L, et al. Emmprin promotes anchorage-independent growth in human mammary carcinoma cells by stimulating hyaluronan production. *Cancer Research* 2004;64:1229–1232. [PubMed: 14983875]
- Nabeshima K, Inoue T, Shimao Y, et al. Matrix metalloproteases in tumor invasion: role for cell migration. *Pathol. Int* 2002;52:255–264. [PubMed: 12031080]
- Narhashi K, Kadomatsu K, Igakura T, et al. Abnormalities of Sensory and Memory Functions in Mice Lacking *Bsg* Gene. *Biochem. Biophys. Res. Commun* 1997;236:733–737. [PubMed: 9245724]
- Ochrietor JD, Linser PJ. 5A11/Basigin Gene Products Are Necessary for Proper Maturation and Function of the Retina. *Dev. Neurosci* 2004;26:380–387. [PubMed: 15855767]
- Ochrietor JD, Moroz TP, van Ekeris L, et al. Retina-specific expression of 5A11/ Basigin-2, a member of the immunoglobulin gene superfamily. *Invest. Ophthalmol Vis. Sci* 2003;44:4086–4096. [PubMed: 12939332]
- Perez SE, Steller H. Migration of glial cells into retinal axon target field in *Drosophila melanogaster*. *J. Neurobiol* 1996;30:359–373. [PubMed: 8807529]
- Poeck B, Fischer S, Gunning D, et al. Glial cells mediate target layer selection of retinal axons in the developing visual system of *Drosophila*. *Neuron* 2002;1:99–113.
- Rangarajan R, Gong Q, Gaul U. Migration and function of glia in the developing *Drosophila* eye. *Development* 1999;126:3285–3292. [PubMed: 10393108]
- Rangarajan R, Courvoisier H, Gaul U. Dpp and Hedgehog mediate neuron-glia interactions in *Drosophila* eye development by promoting the proliferation and motility of subretinal glia. *Mech. Dev* 2001;108:93–103. [PubMed: 11578864]
- Reed BH, Wilk R, Schock F, et al. Integrin-dependent apposition of *Drosophila* extraembryonic membranes promotes morphogenesis and prevents anoikis. *Current Biol* 2004;14:372–380.
- Reimers N, Zafrakas K, Assmann V. Expression of extracellular matrix metalloproteases inducer on micrometastatic and primary mammary carcinoma cells. *Clin Cancer Res* 2004;15:3422–3428. [PubMed: 15161697]
- Risau W, Hallmann R, Albrecht U. Brain induces the expression of an early cell surface marker for blood-brain barrier-specific endothelium. *EMBO J* 1986;5:3179–3183. [PubMed: 3545814]
- Saint Marie RL, Carlson SD. The fine structure of neuroglia in the lamina ganglionaris of the housefly, *Musca domestica* L. *J Neurocytol* 1983;12:213–241. [PubMed: 6842275]
- Schlosshauer B, Herzog KH. Neurothelin. An inducible cell surface glycoprotein of blood-brain barrier-specific endothelial cells and distinct neurons. *J. Cell Biol* 1990;110:1261–1274. [PubMed: 2324198]
- Seulberger H, Lottspeich F, Risau W. The inducible blood-brain barrier specific molecule HT7 is a novel immunoglobulin-like cell surface glycoprotein. *EMBO J* 1990;9:2151–2158. [PubMed: 2357963]
- Simpson JH, Bland KS, Fetter RD, et al. Short-range and long-range guidance by slit and its robo receptors: A combinatorial code of robo receptors controls lateral position. *Cell* 2000;103:1019–1032. [PubMed: 11163179]

- Smith SO, Smith CS, Bormann BJ. Strong hydrogen bonding interactions involving a buried glutamic acid in the transmembrane domain of the neu/erbB-2 receptor. *Nature Struct. Biol* 1996;3:252–258. [PubMed: 8605627]
- Stowers RS, Schwarz TL. A genetic method for generating *Drosophila* eyes composed exclusively of mitotic clones of a single genotype. *Genetics* 1999;152:1631–1639. [PubMed: 10430588]
- Tang W, Chang SB, Hemler ME. Links between CD147 function, glycosylation, and caveolin-1. *Mol. Biol. Cell* 2004;15:4043–4050. [PubMed: 15201341]
- Tieleman DP, Shrivastava IH, Ulmschneider MB, et al. Proline-induced hinges in transmembrane helices: possible roles in ion channel gating. *Proteins. Struct. Funct. Genet* 2001;44:63–72.
- Toyama Y, Maekawa M, Kadomatsu K, et al. Histological characterization of defective spermatogenesis in mice lacking the basigin gene. *Anat Histol Embryol* 1999;28:205–213. [PubMed: 10458027]
- Xiong WC, Okano H, Patel NH, et al. *repo* encodes a glia-specific homeodomain protein required in the *Drosophila* nervous system. *Genes & Dev* 1994;8:981–994. [PubMed: 7926782]
- Yurchenko V, O'Connor M, Dai WW, et al. CD147 is a signaling receptor for cyclophilin B. *Biochem. Biophys. Res. Commun* 2001;288:786–788. [PubMed: 11688976]
- Yurchenko V, Zybarth G, O'Connor M, et al. Active site residues of cyclophilin A are crucial for its signaling activity via CD147. *J Biol. Chem* 2002;277:22959–22965. [PubMed: 11943775]
- Zhou FX, Merianos HJ, Brunger AT, et al. Polar residues drive association of polyleucine transmembrane helices. *Proc. Natl. Acad. Sci. USA* 2001;98:2250–2255. [PubMed: 11226225]
- Zucker S, Hymowitz M, Rollo EE, et al. Tumorigenic potential of extracellular matrix metalloprotease inducer. *Am. J. Pathol* 2001;158:1921–1928. [PubMed: 11395366]

Fig. 1. Sequence alignment of basigin related proteins. Identical residues are highlighted in black and similar residues are highlighted in yellow. Only the C-terminus of *Drosophila* basigin 298 is shown (last line). *Drosophila* basigin 298 (accession number CG31605-PG) can be found at NCBI or flybase@bio.indiana.edu. The thick black underline indicates the putative transmembrane sequence. Residues that were changed in altered basigin proteins are marked with an asterisk. When more than one residue was changed at a time this is indicated by a line above residues marked with asterisks.

Fig. 2.

Testing *basigin* transgenes for their ability to rescue photoreceptor cell structural defects of *bsg*²⁶⁵. Frozen longitudinal head sections were labeled with anti-elav to visualize photoreceptor nuclear placement in the retina. re=retina. (A) Mosaic animal mutant in the eye for *bsg*²⁶⁵ but expressing wild-type *basigin* from an engineered transgene. Photoreceptor nuclei are placed as in wild-type animals (not shown). Labeled arrows show R1–R6, R7, and R8 nuclear placement. (B) Mosaic animals mutant in the eye for *bsg*²⁶⁵. Photoreceptor nuclei are found scattered throughout the retina. (C–L) Mosaic animal mutant in the eye for *bsg*²⁶⁵ but expressing the following *basigin* mutant proteins. (C) YE246SG. (D) PFL228LFTL.

Photoreceptor nuclear placement is normal. (E) T128A. (F) RVK218LVT. (G) EG132GV. (H) WKK64LKT. (I) Y103S. (J) E235G. (K) W151G. (L) DRGEY188AVRES.

Fig. 3.

Examination of protein stability from basigin mutant transgenes by immunoblot with anti-V5antibody. Our transgenic lines all make basigin protein with a V5 terminal tag. We expressed mutant proteins in the eye via an ey-Gal4 driver and performed an immunoblot with anti-V5 antibody. Arrows indicate the location of two MW markers. The proteins represented in order are (1) EG132GV. (2) T128A. (3) WKK64LKT. (4) EIE113GFG. (5) RVK218LVT. (6) wild-type basigin. (7) DRGEY188AVRES. (8) E235G. (9) W151G. (10) Truncated V5. Arrows show the positions of the nearest MW markers.

Fig. 4.

Expression of protein from *Drosophila basigin* transgenes in the visual system. Frozen longitudinal head sections were labeled with anti-basigin antibody. (A) Mosaic animal mutant in the eye for *bsg*²⁶⁵ but expressing basigin E235G from an engineered transgene. la= lamina, me=medulla. (B) Mosaic animal mutant in the eye for *bsg*²⁶⁵ but expressing basigin W151G from an engineered transgene. The expression pattern is the same as wild-type basigin (Curtin *et al.*, 2007).

Fig. 5.

Testing *basigin* mutant transgenes for their ability to rescue neuron-glia interaction defects of *bsg*²⁶⁵. Frozen longitudinal head sections were labeled with anti-repo, an antibody specific to glia cell nuclei. The post-synaptic lamina is shown. la=lamina. Three distinct layers of glia can be ascertained, each marked with distinctive arrows in panels (A) and (B). The arrow with the long stem and small head points to the epithelial glia, the ones found altered when the eye is mutant for *bsg*²⁶⁵. (A) Mosaic animal mutant in the eye for *bsg*²⁶⁵ show scattered epithelial glia. (B) Mosaic animals mutant in the eye for *bsg*²⁶⁵ but expressing wild-type basigin in the eye from an engineered transgene. Glia cell placement is like wild-type animals (not shown). (C) Mosaic animal mutant in the eye for *bsg*²⁶⁵ but expressing mutant basigin protein YE246SG from a transgene. Glia cell placement is normal. (D) Mosaic animal mutant in the eye for *bsg*²⁶⁵ but expressing mutant basigin protein P147L from a transgene. Glia cell placement is normal. (E) Mosaic animal mutant in the eye for *bsg*²⁶⁵ but expressing mutant basigin protein PFL228LFT from a transgene. Glia cell placement is like *bsg*²⁶⁵. (F) Mosaic animal mutant in the eye for *bsg*²⁶⁵ but expressing mutant basigin protein EIE113GFG from

a transgene. Glia cell placement is like *bsg*²⁶⁵. (G) Mosaic animal mutant in the eye for *bsg*²⁶⁵ but expressing mutant basigin protein E235G from a transgene. Epithelia glia are located at the proximal margin of the lamina. (H) Mosaic animal mutant in the eye for *bsg*²⁶⁵ but expressing mutant basigin protein W151G from a transgene. Epithelia glia are located at the proximal margin of the lamina.

Table 1

Basigin transgenes coding altered basigin proteins were tested for rescue of the photoreceptor defect in *bsg*²⁶⁵ mutant eyes as assayed by misplaced photoreceptor nuclei labeled with anti-elav. Percent misplaced photoreceptor nuclei calculated by dividing average number of misplaced nuclei/eye by 160 (average number of nuclei/eye) $\times$ 100. Also examined was the ability of each transgene to rescue the neuron-glia interaction defects of *bsg*²⁶⁵ as assayed by examining epithelia glia cell placement with anti-repo antibody. All mutant proteins that fail to rescue the cell structure defects of *bsg*²⁶⁵ also fail to rescue the neuron-glia interactions. Mutant proteins that partially rescue the photoreceptor nuclear defects do not rescue the neuron-glia interaction defects. p-values >0.05 represent mutants not significantly different from the respective control. Partial rescue mutants fall below 0.05 when compared with either control.

Transgene	Putative Domain Location	% Misplaced Photoreceptor Nuclei (S.E.)	Photoreceptor Placement	p-value positive control	p-value negative control	% Misplaced Glia	Glia Placement
KRR 248	Intracellular	1%	rescue	ND	ND	0%	rescue
MGG	Intracellular	1%	rescue	1.0000	<0.0001	0%	rescue
YE 246	Intracellular	1%	rescue	1.0000	<0.0001	0%	rescue
SG	Intracellular	1%	rescue	1.0000	<0.0001	0%	rescue
PL47 L	1 st domain	2%	rescue	0.9764	<0.0001	0%	rescue
D 188 N	2 nd domain	3%	rescue	0.6642	<0.0001	0%	rescue
I 178 T	2 nd domain	3%	rescue	0.8627	<0.0001	0%	rescue
PFL 228	TM	3%	rescue	0.8780	<0.0001	0%	rescue
LFT	TM	3%	rescue	0.8780	<0.0001	0%	rescue
EG 132	Between 1 st and 2 nd domain	7%	partial rescue	0.0002	<0.0001	26%	no rescue
GV	Between 1 st and 2 nd domain	7%	partial rescue	0.0002	<0.0001	26%	no rescue
T 128 A	Between 1 st and 2 nd domain	10%	partial rescue	<0.0001	<0.0001	27%	no rescue
WKK 64	1 st domain	10%	partial rescue	<0.0001	<0.0001	47%	no rescue
LKT	1 st domain	10%	partial rescue	<0.0001	<0.0001	47%	no rescue
EIE 113	Between 1 st and 2 nd domain	12%	partial rescue	<0.0001	<0.0001	27%	no rescue
GFG	Between 1 st and 2 nd domain	12%	partial rescue	<0.0001	<0.0001	27%	no rescue
RVK 218	Between 2 nd and TM	13%	partial rescue	<0.0001	<0.0001	32%	no rescue
LVT	Between 2 nd and TM	13%	partial rescue	<0.0001	<0.0001	32%	no rescue
Y 103 S	1 st domain	14%	partial rescue	<0.0001	<0.0001	29%	no rescue
W 151 G	2 nd domain	17%	no rescue	<0.0001	0.9986	100%	no rescue
E 235 G	TM	18%	no rescue	<0.0001	0.9934	100%	no rescue
Truncated V5	TM	18%	no rescue	<0.0001	0.9986	100%	no rescue
DRGEY 188	2 nd domain	19%	no rescue	<0.0001	1.000	100%	no rescue
AVRES	2 nd domain	19%	no rescue	<0.0001	1.000	100%	no rescue
None		18%		<0.0001	1.000	100%	