Purposes: To identify and characterize gene expression changes associated with photoreceptor cell loss in a Bbs4 knockout mouse model of retinal degeneration.

Methods: Differential gene expression in the eyes of 5-month-old Bbs4−/− mice undergoing retinal degeneration were analyzed using gene microarrays (Affymetrix, Santa Clara, CA). Elevatedocular transcripts were confirmed by Northern blotting of RNA from Bbs4−/− and three additional mouse models of Bardet-Biedl Syndrome (BBS). TUNEL assays and transmission electron microscopy were used to study cell death and photoreceptor morphology in these mice.

Results: Three hundred fifty-four probes were differentially expressed in Bbs4−/− eyes compared with controls using a twofold cutoff. Numerous vision-related transcripts decreased because of photoreceptor cell loss. Increased expression of the stress response genes Edn2, Lcn2, Srp563n, and Socs3 was noted at 5 months of age and as early as postnatal week 4 in the eyes of four BBS mouse model strains. A burst of apoptotic activity in the photoreceptor outer nuclear layer at postnatal week 2 and highly disorganized outer segments by postnatal weeks 4 to 6 was observed in all four strains.

Conclusions: The specific loss of photoreceptors in Bbs4−/− mice allows us to identify a set of genes that are preferentially expressed in photoreceptors compared with other cell types found in the eye and is a valuable resource in the continuing search for genes involved in retinal disease. The molecular and morphologic changes observed in young BBS animal models are similar to those observed in human retinas. Understanding the specific function of these genes is important for the discovery of new BBS genes and will help us understand the pathogenesis of this disease.

Bardet-Biedl Syndrome (BBS) is a genetically heterogeneous autosomal recessive disorder with the primary clinical features of retinopathy, obesity, polydactyly, learning disabilities, renal abnormalities, and hypogonadism.1,2 BBS is also associated with increased susceptibility to hypertension, diabetes mellitus, and congenital heart defects.3-5 The retinal degeneration associated with BBS has an early onset and usually leads to blindness by the age of 20 years. It has been described as a syndromic retinal dystrophy of the photoreceptors with early but variable macular involvement.1,2-9

To date, 12 BBS genes (BBS1–BBS12) have been identified, and mutational analysis indicates that additional genes have yet to be discovered. The 12 BBS genes encode novel proteins with roles that are being elucidated by functional studies in a variety of organisms.10,11 Although mutations in each of the BBS genes result in the same spectrum of clinical features, these genes do not show significant homology to each other, nor do they, as a group, possess obvious functional domains. Exceptions are BBS6/MKKS, BBS10, and BBS12 which are members of a vertebrate-specific chaperonin-related superfamily.11-13 BBS3/ARL6, an ADP-ribosylation factor-like protein thought to be involved in membrane-associated intracellular trafficking14,15; and BBS11/TRIM32, an E3 ubiquitin ligase.16 BBS4 contains tetrapartide repeat (TPR) motifs that may play a role in protein–protein interactions and has been localized to the centriolar satellites of centrosomes and basal bodies of primary cilia, where it interacts with components of the dynein transport machinery in intraflagellar transport (IFT).17 These findings, together with studies of BBS animal models of the disorder,18-25 and bbs zebrafish knockdown models24 suggest that BBS proteins play a role in the structure or function of cilia and basal bodies and/or IFT.

After the identification of the BBS4 gene by our laboratory,25 we generated Bbs4−/− mice in an effort to gain better understanding of the specific function of this gene.19 Although polydactyly did not develop in the mice, they recapitulated the human BBS phenotypes of retinopathy and obesity and also exhibited phenotypes consistent with cilia dysfunction, including a deficit in olfaction,21 and male infertility due to the lack of sperm flagella. Postnatal 2-week Bbs4−/− mice demonstrated grossly normal eye and retinal morphology at the histologic level. By light microscopy, photoreceptor cells appeared normal with differentiated inner and outer segments. However, by 6 weeks of age, Bbs4−/− retinas exhibited an attenuation of the photoreceptor outer segment, a substantial loss of thickness of the outer nuclear layer, and an abnormally reduced electoretinogram. TUNEL assays at 5 months of age revealed an apoptotic photoreceptor cell outer nuclear layer that resulted in attenuation of the outer segments, culminating in the complete loss of the photoreceptor cell layer by 8 months.

The specific loss of photoreceptor cells in the Bbs4−/− mouse eye provides the opportunity to identify a set of genes that are preferentially expressed in this layer of the retina. Such genes are a valuable resource for the discovery of new BBS genes.
genes as well as new candidate genes for nonsyndromic retinal degeneration. New evidence of early apoptosis in the photoreceptor outer nuclear layer, together with increased stress gene expression and morphologic evidence of highly disorganized photoreceptor outer segments in young BBS model mice suggests that early events in photoreceptor cell development depend on the presence of functional BBS proteins.

**MATERIALS AND METHODS**

**RNA Isolation and Probe Labeling for Microarray Analysis**

All experiments were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Animal Care and Use Committee at the University of Iowa. Eyes were dissected from six 5-month-old Bbs4−/− mice and six age-matched control animals. One eye of each pair was placed in 4% paraformaldehyde and processed for histology as described later. The second eye was flash frozen in liquid nitrogen and stored at −80°C until use. We chose to perform microarray analysis on whole eyes because we have observed some retinal pigment epithelium (RPE) migration into the retina of Bbs knockout mice (RFM, unpublished data). Therefore, we felt that attempting to separate the RPE from the retina could result in significant cross-contamination in knockout mice that is not present in control animals and would confound the analysis. Moreover, splitting the eyes into individual fractions would increase the number of eyes required to perform the analysis, since insufficient RNA is obtained from three retinas to perform this experiment. Single eyes from each of the three Bbs4−/− mice, pooled, and single eyes of three of the six control mice were pooled for microarray analysis 1. The three remaining Bbs4−/− eyes (representing a single eye from a second set of three mice) were pooled as were the three remaining control eyes, and RNA was isolated for microarray analysis 2. Pooling was necessary because of the low RNA yield from individual eyes and also minimized the effects of biological variability between samples. RNA was extracted (TRizol reagent; Invitrogen, Carlsbad, CA) and purified through RNeasy columns (Qiagen, Chatsworth, CA). RNA integrity was assessed on a bioanalyzer (model 2100) with biozising software (Agilent Technologies, Palo Alto, CA). Similarly, RNA from the testes of pooled 5-month-old Bbs4−/− mice and pooled, age-matched control animals were assayed on replicate microarrays (total of six testes from Bbs4−/− mice and six from control mice).

Double-stranded cDNA was synthesized from 5 μg of total RNA (Superscript Choice system; Invitrogen, Carlsbad, CA) and T7- (dT)24 primer (Sigma-Genosys, The Woodlands, TX). In vitro transcription was performed, using double-stranded cDNA as a template in the presence of biotinylated UTP (Target-Labeling and Reagent Control Kit; Affymetrix, Santa Clara, CA).

**Microarray Hybridization**

The cRNA was fragmented and hybridized for 16 hours to the mouse 430 2.0 gene microarray using a gene chip instrumentation system (both from Affymetrix) at the DNA Core Facility of the University of Iowa. After stringent washes, the arrays were stained with streptavidin-phycocerythrin (Invitrogen-Molecular Probes, Eugene, OR) and then scanned on a gene array scanner (Hewlett Packard, Palo Alto, CA). Data were acquired using the data collection and analysis system (GCOS [Gene Chip Operating System]; Affymetrix).

**Data Analysis**

For microarray gene expression analysis, the RMA (robust multichip averaging) method20,21 was used to process .cel files from Affymetrix Microarray Suite (version 5.0) and obtain normalized summary expression values on a logarithmic base-2 scale for each probe set. The probe sets were then filtered to identify those probe sets that exhibited a twofold or greater change (increase or decrease) between the two pools in both replicates.

**Histology and TUNEL Assay**

One eye from 5-month-old control and Bbs4−/− mice was used for microarray analysis and one eye of postnatal 2-, 4-, and 6-week control and Bbs1MKS0N/N/MK0N knockout, Bbs2−/−, Bbs4−/−, and Mks6−/− mice in triplicate were enucleated and immersed in a solution of 4% paraformaldehyde in 10 mM phosphate-buffered saline (PBS; pH 7.4). After 2 to 4 hours of fixation, eyes were washed three times in 10 mM PBS followed by infiltration and embedding in acrylamide, as described.28 Cryostat sections, oriented along the superrior–inferior axis, were collected at a thickness of 7 μm, and stored at 4°C until use. Sections were stained with hematoxylin and cosin.

Cryostat sections were processed for the TUNEL assay by using a cell-viability kit (In Situ Cell Death Detection kit, TMR red; Roche Applied Science, Indianapolis, IN) was used according to the manufacturer's instructions. Nuclei were counterstained with 4',6-diamidino-2-phenindole (DAPI; Invitrogen-Molecular Probes). Sections were viewed on a microscope (model BX-41; Olympus, Lake Success, NY), and images were collected with a digital camera (SPOT RF, Diagnostic Instruments, Sterling Heights, MI).

The second eye from each of the postnatal 2-, 4-, and 6-week mice was flash-frozen in liquid nitrogen and stored at −80°C until use for RNA extraction.

**Northern Blot Analysis**

Two micrograms of mouse eye total cellular RNA was subjected to gel electrophoresis, blot analysis, and hybridization, as described previously.22 A BD-Clontech (Palo Alto, CA) mouse multiple tissue RNA blot containing 2 μg poly(A) RNA/lane was also used. After hybridization, the blots were stripped of radioactivity and were rehybridized with a cDNA probe for β-actin to verify equal loading. The mouse cDNA hybridization probes correspond to the 3' untranslated region (UTR) of each gene.

**Transmission Electron Microscopy**

Eyes were fixed in half-strength Karnovsky's fixative before osmium postfixation, dehydration, and embedding in Spurr’s resin. TEM was performed as described previously.20

**RESULTS**

**Histology of Bbs4−/− Retinas**

Previous studies in our laboratory have shown that Bbs4−/− mice undergo photoreceptor cell degeneration by an apoptotic mechanism.19 To analyze gene expression changes taking place in 5-month-old Bbs4−/− retinas, we used Affymetrix microarray technology to identify differentially expressed genes in control and knockout eyes. Extensive reduction of the outer nuclear layer of the Bbs4−/− retina and attenuation of the inner and outer segments was noted in comparison with wild-type control animals (Fig. 1). The inner retina, choroid, sclera, and anterior segment appeared normal in these animals.

**Identification of Genes with Decreased Expression during Retinal Degeneration**

Of the >39,000 probes (genes) on the Affymetrix microarray, 354 unique genes were differentially expressed in Bbs4−/− eyes compared with the control, when a twofold cutoff was used.

Most of the differentially expressed transcripts showed decreased expression. Three hundred and six genes (including 105 unknown genes; expressed sequence tags; ESTs) exhibited ≥twofold decreased expression in Bbs4−/− eyes, presumably because of the apoptotic death of photoreceptors (for a
complete listing, see Supplementary Table S1, online at http://www.iows.org/cgi/content/full/48/7/3329/DC1. As expected, most of the genes that showed decreased expression at 5 months are involved in phototransduction, sensory perception of light, visual perception, and response–detection of light. Functional classes of genes that also demonstrated significantly decreased expression included photoreceptor cell development, negative regulation of programmed cell death, primary/ protein metabolism, metal ion transport, lipid biosynthesis, regulation of transcription, signal transduction, cell communication, protein transport/localization, and generalized cellular metabolism, among others. Of the 125 cloned human retinal disease genes currently listed in the RetNet database (http://www.sph.uth.tmc.edu/RetNet/ provided in the public domain), most of the differentially expressed genes in knockdown eyes and testes are shown in Figure 3. As noted previously, most of the differentially expressed genes in Bbs4−/− eyes showed decreased expression, most likely as the result of photoreceptor cell death. In contrast to the finding in the eye at an earlier developmental stage, it is not expressed abundantly in the adult eye or may be expressed in a subpopulation of adult retinal cells that is below the level of detection.

In contrast to the large number of ocular transcripts that decreased in Bbs4−/− mice, microarray analysis of a second 5-month-old Bbs4−/− mouse tissue with an affected phenotype, the testes, revealed a much smaller set of only 20 genes with twofold or more differential expression. Scatterplot profiles of differential gene expression in Bbs4 control versus knockout eyes and testes are shown in Figure 3. As noted previously, most of the differentially expressed genes in Bbs4−/− eyes showed decreased expression, most likely as the result of photoreceptor cell death. In contrast to the finding in the eye, a smaller number of genes showed decreased expression in Bbs4−/− testes, nor any overlap between differentially expressed genes in these two tissues.

Identification of Genes with Increased Expression during Retinal Degeneration

Forty-eight probes (including 10 ESTs) exhibited ≥2-fold increased expression in the knockout eyes (for a complete listing, see Supplementary Table S2, online at http://www.iows.org/cgi/content/full/48/7/3329/DC1). The genes with known annotation fell into functional categories including nucleic acid metabolism (Entpd3), signal transduction (Gna14, Gprc5a, Osmr), immune response (Ifh3, Oasl2), cell adhesion (Comp), cytoskeletal organization (Krt11-3), and several other physiological processes. Several members of the complement activa-


**Table 1. Differentially Expressed Genes in 5-Month-Old Bbs4<sup>−/−</sup> Mouse Eyes that Overlap with Human Retinal Disease Genes<sup>a</sup>**

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Sets 1 and 2 represent comparisons of three wild-type and three knockout eyes, assayed on replicate arrays using RNA pools from two sets of mice. Normalized expression values are in log<sub>2</sub>.

<sup>a</sup> RetNet database: (http://www.sph.uth.tmc.edu/RetNet/).

### Early Expression of Stress Response Genes

Among the genes with increased expression in 5-month-old Bbs4<sup>−/−</sup> degenerating eyes were Edn2, Lcn2, Serpina3n, and Socs3 (Table 2). These genes are thought to be indicators of retinal stress and have been shown to be upregulated in several mouse models of retinal degeneration to be expressed in photoreceptors (Edn2), and in Müller cells and astrocytes (Lcn2 and Serpina3n).<sup>51</sup> It is not yet known whether an early stress gene response is part of the BBS retinal phenotype. To determine whether these four genes also showed elevated expression in early postnatal Bbs4<sup>−/−</sup> eyes before the overt retinal degeneration observed at later stages, we probed Northern blots prepared with RNAs isolated from postnatal 2-, 4-, and 6-week control and Bbs4<sup>−/−</sup> eyes, in triplicate. As seen in Figure 4, there was no detectable Edn2, Lcn2, or Socs3 expression in control eyes at any stage, or in postnatal 2-week Bbs4<sup>−/−</sup> eyes. Likewise, there was no increased Serpina3n expression above endogenous levels at 2 weeks. However, by week 4, transcripts of all four genes in Bbs4<sup>−/−</sup> eyes were elevated and remained so at 6 weeks of age. Edn2, Lcn2, Serpina3n, and Socs3 expression was also elevated in degen- erating 5-month-old Bbs4<sup>−/−</sup> eyes, as shown in Figure 2, and by 8 months when photoreceptor damage is complete, expression of these genes in Bbs4<sup>−/−</sup> eyes decreased to levels comparable to those of age-matched controls (data not shown). We did not observe changes in the expression of the other two endothelin genes, Edn1 and Edn3, or in the endothelin receptor genes Ednra or Ednrb.

Eyes of Bbs4<sup>−/−</sup> mice have increased expression of stress response genes before the appearance of gross retinal damage. To test whether this response is limited to Bbs4<sup>−/−</sup> mice or is shared by three other BBS mouse strains, we isolated RNA from the eyes of postnatal 2-, 4-, and 6-week Bbs1 knock-in mice (Bbs1<sup>Δ590R/Δ590R</sup>), which harbor the common human muta-

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tion (M390R) that converts a methionine to an arginine, and similarly staged eyes isolated from Bbs2−/− mice and Mkks−/− mice. In general, the temporal gene-expression patterns were comparable to those in the Bbs4−/− mice—that is, no increase at 2 weeks of age followed by increased expression by weeks 4 and 6 (Fig. 5). The exception was in Mkks−/− mice where Edn2 expression was elevated early (at 2 weeks) and Serpin3n was more marked at 6 weeks. Lcn2 and, in particular, Socs3 transcripts were difficult to detect in younger eyes of all BBS model mice compared with the levels seen at 5 months, suggesting that increased gene expression in later-stage BBS model mouse eyes may reflect a more robust response to advanced photoreceptor damage. Increased expression of the stress response genes appears to be limited to the eye, as the lungs and brains of Bbs4−/− animals showed no change.

### Early Apoptosis and Photoreceptor Dysmorphology

Cell death during normal differentiation of the mouse retina occurs primarily during the first 2 weeks after birth, is essentially complete by the third week, and follows a temporal gradient staring at the central retina and progressing toward the periphery.34 By postnatal day 8 most of the photoreceptor nuclei have migrated through the outer plexiform layer or undergo programed cell death with an apoptotic peak between days 7 and 8. The wave of photoreceptor nuclear mi-

### Table 2. Partial List of Differentially Expressed Genes in 5-Month-Old Bbs4−/− Mouse Eyes

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</tbody>
</table>

Multiple listings for some BBS genes reflect multiple Affymetrix probes present on the arrays. Sets 1 and 2 represent comparisons of three wild-type and three knockout eyes, assayed on replicate arrays using RNA pools from two sets of mice. Normalized expression values are in log₂ scale.

### Table 3. Differential BBS Gene Expression in 5-Month-Old Bbs4−/− Mouse Eyes

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<tr>
<th>Gene ID</th>
<th>Locus</th>
<th>Symbol</th>
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<th>x-Fold Change</th>
<th>WT-2 KO-2</th>
<th>x-Fold Change</th>
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Northern blot confirmation of selected genes that exhibited increased, decreased, or no change in expression in 5-month-old Bbs4M390R/M390R knock-in, Bbs1+/−, and Mkks−/− retinas (Fig. 6A). Apoptosis appeared to be more pronounced in the peripheral retina near the anterior segment than in the central retina near the optic nerve. Apoptosis was not observed in 2-week-old Bbs4−/− retinas; however, increased TUNEL activity was noted at 4 weeks of age in the photoreceptor cell outer nuclear layer of the peripheral retina in these animals (Fig. 6B). These results, and the absence of detectable apoptotic activity in the age-matched control animals suggest that photoreceptor cell differentiation may be temporally delayed in BBS mouse model retinas. Alternatively, aberrant structure and function of the developing photoreceptor outer segments may lead to early cell death.

Photoreceptor cell inner and outer segments are connected by a nonmotile 9+0 sensory cilium that plays a critical role in the intracellular transport of proteins and lipids to their destination in the precisely stacked membranous disks that comprise the outer segment and play a fundamental role in phototransduction. Mouse photoreceptor outer segments, which are enlarged extensions of the connecting cilium, begin forming at postnatal day 5 as seen by electron microscopy. By postnatal day 14 when the mouse eye opens, the length of the outer segments has increased, reaching adult size before day 21.35,36

For a better understanding of the time course of the pathophysiology underlying the BBS mutant mouse eyes, we analyzed the photoreceptor outer segments of the four BBS mouse strains, by using transmission electron microscopy. As seen in Figure 7A, the outer segments of postnatal 4-week control mouse eyes exhibit well-organized, stacked membranous disks that were perpendicular to the apical surface of the retinal pigment epithelium (RPE). At postnatal weeks 4 to 6, the outer segments of all four BBS model knock-in and knockout mice were highly disorganized. Outer segments of 6-week-old Bbs4M390R/M390R retinas were an admixture of normal-appearing outer segments and grossly abnormal outer segments (Fig. 7B) that were no longer perpendicular to the apical surface of the RPE (Fig. 7C). Normal-looking outer segments were comparatively rare in 4-week-old Bbs2+/− retinas (Fig. 7D). Normal-appearing outer segments were also rare in 4-week-old Bbs4−/− and 5-week-old Mkks−/− retinas (Figs. 7E, 7F). The outer segment material was highly disorganized, consisting of layered and folded sheets of membrane, whorls, and other amorphous structures. Stacks of membranous disks were observed, but these were randomly oriented and were not assembled into discrete outer segments. Although we previously described highly disorganized outer segments in 5-month-old Bbs2+/− mice28 and a similar finding was subsequently noted in 13-week-old Mkks−/− mice,37 this is the first report of highly disorganized photoreceptor outer segments in young BBS mouse retinas.
The present study also underscores the utility of microarray analyses, to discover the predicted commonalities of the genomic response resulting from a diverse set of insults to the retina that culminate in retinal degeneration. Microarray analysis of degenerating 5-month-old Bbs4/H11002/H11002 eyes revealed increased expression of four stress response genes (Edn2, Lcn2, Serpina3n, and Socs3) that belong to a limited set of genes shown recently to have increased expression in three different genetic mouse models of retinal degeneration (Prcad/H11002/H11002, Rds/H11002/H11002, and Rd7), a mouse model of light-induced retinal degeneration, and a mouse model of physical retinal injury. The increased stress gene response observed in 5-month-old Bbs4/H11002/H11002 eyes prompted us to refine our analysis of young BBS model mouse eyes and test whether the young eyes whose retinas appeared sound by gross histologic analysis were in fact undergoing changes that preceded the overt damage observed at later stages. Indeed, this was the case. Increased Edn2, Lcn2, Serpina3n, and Socs3 gene expression was observed as early as postnatal weeks 4 and 6 in Bbs1/M390R/M390R knock-in, Bbs2/H11002/H11002, Bbs4/H11002/H11002, and Mkk8/H11002/H11002 eyes. Because of the lack of cross-species reactivity of endothelin 2, lipocalin 2, serpina3n, and SOCS3 antibodies, we were unable to localize or quantify the stress response proteins in BBS mouse eyes using immunohistochemistry or Western blots. However, a new study of Rds/H11002/H11002 mice undergoing retinal degeneration demonstrated a comparable increase in Edn2 mRNA in photoreceptors that was paralleled by increased endothelin 2 protein as detected by HPLC and radioimmunoassay. The endothelins are stress-responsive regulators that work in paracrine and autocrine fashion in a variety of organs with both beneficial and detrimental roles. Endothelin 2 is a potent regulator of vasoconstriction that has been shown recently to play a role in light-induced photoreceptor degeneration. Lipocalin2 and serine protease inhibitor 3 are acute-phase proteins associated with inflammation in several disorders. Suppressor of cytokine signaling 3 (SOCS3) is involved in the negative regulation of cytokines that signal through the JAK/STAT pathway in many cellular functions, including inflammation. Continued study...
of the stress response in the retinas of a variety of animal models of retinal degeneration including BBS may provide clues for therapeutic measures to delay or decrease the severity of photoreceptor cell loss. In addition, the early expression of the stress response genes in the photoreceptor component of the BBS phenotype can be used as early markers for the purpose of studying possible gene–gene interactions involved in potential complex inheritance of BBS.56
The early stress gene response also prompted us to re-evaluate the young BBS mouse model eyes to gain insight into the underlying pathophysiology of the retinal degeneration phenotype. A burst of apoptotic activity was observed in postnatal 2-week-old Bbs1M390R/M390R knock-in and Bbs2−/− and Mkks−/− photoreceptor outer segments. A comparable apoptotic burst was also observed in the postnatal 4-week Bbs4−/− outer nuclear layer, although the significance of this delay is not yet known. One explanation for the observed early photoreceptor cell death is that it may reflect a temporal delay of photoreceptor cell differentiation in the BBS model mice. However, examination of postnatal 4- and 6-week BBS mouse model retinas by transmission electron microscopy showed that at postnatal week 4, the young photoreceptor outer segments are already highly disorganized. The temporal separation of the early burst of photoreceptor apoptosis and the beginning of the prolonged stress gene response observed in BBS model mice suggests that the stress response does not initiate photoreceptor cell death, but rather may respond to photoreceptor damage and play a role in the progression of retinal degeneration.

Rhodopsin mislocalization, photoreceptor apoptosis, and disorganized photoreceptor outer segments have been observed in all BBS model mice to date and are consistent with other animal models of photoreceptor cilia dysfunction.57–65 Based on our current knowledge of BBS proteins and their proposed functions, the highly disorganized photoreceptor outer segments in the young BBS mouse eyes may result from a combination of cellular events, including defects in cargo loading at the basal body organizing center located at the base of the photoreceptor connecting cilium, impaired intracellular transport of phototransduction proteins and lipids from the photoreceptor inner segment to the outer segment along the connecting cilium, or incorrect protein folding that leads to malformed and dysfunctional photoreceptor outer segments resulting in cell death. The photoreceptor has a high rate of cellular metabolism, continuously shedding and restoring membranous discs from the outer segment apical tip and trafficking an estimated 2000 photopigment molecules per minute through the connecting cilium.64 Thus, precise photoreceptor assembly and function must be highly sensitive to connecting cilia/intracellular transport dysfunction starting at the earliest stages of retinal development, and function and may result in the early photoreceptor cell outer segment morphology and apoptosis in the BBS model mouse eyes that lack functional BBS proteins.

The sequence of loss of photoreceptor cone and rod function in patients with BBS is difficult to analyze because of the rapid and often severe onset of the retinal degeneration. Using BBS mice as models of the disease, recent electroretinogram (ERG) data from postnatal 4-week Bbs4−/− eyes indicate that the knockout mice have both rod and cone dysfunction, yet the cone dysfunction is earlier in onset and greater in severity.23 These studies support the cone–rod dystrophy observed in some patients with BBS65,66 and in young Bbs1M390R/M390R knock-in mice (Philp AR et al. IOVS 2006;47:ARVO E-abstract 5777). Retinal imaging studies of patients with BBS1 bearing the M390R mutation revealed a wide spectrum of retinal abnormalities.67 The authors67 concluded that the retinal disease initially affects rod and cone photoreceptor function, but that there is a subsequent effect on inner retinal function, mainly evident in the rod retinal pathway. Although we did not observe any significant changes in rod- or cone-specific gene expression in postnatal 4-week-old Bbs4−/− eyes by microarray analysis (RES and DYN, unpublished data), we noted that the s-cone opsin gene showed the greatest decrease in expression in retinas of Bbs4−/− mice undergoing late-stage retinal degeneration at 5 months of age compared with all other
known genes on the microarray. Further examination of photoreceptor cell expression changes in BBS animal model eyes in the early stages of retinal degeneration coupled with functional ERG measurements will add to the characterization of the sequence of rod and cone loss in the BBS eye phenotype.

In our microarray studies, we found a significantly decreased expression of several BBS genes in the 5-month-old Bbs4-/- mouse eye. The decrease in the Bbs1, -2, -3, -5, -7, -8, -9, and -12 gene expression is most likely caused by apoptosis of photoreceptor cells that express multiple BBS genes rather than by a more complex inhibitory feedback mechanism orchestrated by the lack of Bbs4 protein. This conclusion is strengthened by the observation that in Bbs4-/- mouse testes, another tissue with an affected phenotype, the loss of Bbs4 resulted in differential expression of a very small set of genes that did not include any of the other BBS genes. It is interesting that Trim32 and Mkks expression did not change in 5-month-old Bbs4-/- eyes. Based on immunostaining of wild-type adult mouse retinas, Mkks has been reported in the connecting cilium, the inner nuclear layer and outer nuclear layer, but not to the inner or outer segments of the photoreceptor cells.68 In contrast, the retinal phenotypes of our Bbs1M390R/M390R knock-in, Bbs2-/-, Bbs4-/-, and Mkks-/- mice did not include degradation of the inner nuclear layer. One explanation for the

**Figure 7.** Transmission electron microscopy of BBS mouse model photoreceptor outer segments. (A) Electron micrographs from postnatal week 4 wild-type retinas have well-organized outer segments that are perpendicular to the apical surface of the RPE. (B, C) Six-week-old Bbs1M390R/M390R knock-in retinas. (D) 4-week-old Bbs2-/- retinas, (E) 4-week-old Bbs4-/- retinas, and (F) 5-week-old Mkks-/- retinas show highly disorganized photoreceptor outer segments composed of amorphous material that lies parallel to the apical surface of the RPE. RPE, retinal pigment epithelium; OS, photoreceptor outer segment. Scale bar (A, C, D–F) 2 μm; (B) 0.2 μm.
microarray result is that apoptotic loss of photoreceptors in the 
*Bbs4*−/− mouse leads to aberrantly increased Mkks transcription 
in the inner nuclear layer of the retina or perhaps in other 
layers of the retina that masks decreased Mkks transcription in 
injured and dying photoreceptor cells. The lack of differential 
*Mkks* and *Trim32* expression in *Bbs4*−/− knockout mouse 
eyes bears further investigation that will clarify this and may 
also yield useful information on the function of these proteins 
in the retina.

In summary, microarray analysis of *Bbs4*−/− mouse eyes 
undergoing retinal degeneration identified a set of genes that 
are preferentially expressed in the photoreceptor cells. The 
analysis will be a valuable resource in the continuing search for 
genes involved in retinal disease. It also revealed increased 
expression of an overlap set of stress response genes that 
respond to the early stages of retinal degeneration in several 
mouse models of the disorder. Further examination of young 
eyes from four different BBS mouse strains revealed early apo-
ptosis and disorganized photoreceptor cell outer segments. 
Continued study of young BBS mouse model retinas will pro-
vide useful information regarding the early pathophysiology of 
this disorder that might be useful in the design of therapeutic 
treatments to delay or decrease retinal degeneration.

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cardial hypoplasia, microphthalmia, and function in the mouse. *Nat 

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