Mice homozygous for the retinal degeneration slow (rds) mutation completely lack photoreceptor outer segments. The rds gene encodes rds/peripherin (rds), a membrane glycoprotein in the rims of rod and cone outer segment discs. rds is present as a complex with the related protein, rom1. Here, we generated transgenic mice that express a chimeric protein (rom/D2) containing the intradiscal D2 loop of rds in the context of rom1. rom/D2 was N-glycosylated, formed covalent homodimers, and interacted non-covalently with itself, rds, and rom1. The rds-rom/D2 interaction was significantly more stable than the non-covalent interaction between rds and rom1 by detergent/urea titration. Analysis of mice expressing rom/D2 revealed that rds is 2.5-fold more abundant than rom1, interacts non-covalently with itself and rom1 via the D2 loop, and forms a high order complex that may extend the entire circumference of the disc. Expression of rom/D2 fully rescued the ultrastructural phenotype in rds+/− mutant mice, but it had no effect on the phenotype in rds−/− mutants. Together, these observations explain the striking differences in null phenotypes and frequencies of disease-causing mutations between the RDS and ROM1 genes.

Rod and cone photoreceptors contain a structure, called the outer segment, comprising a stack of flattened membranous discs. These discs are the sites of photon capture and reactions of visual transduction. rds is a 36-kDa glycoprotein with four transmembrane segments located in the rims of rod and cone outer segment discs (1, 2). Although the function of rds is not known, the phenotype resulting from a null mutation in its gene has been described. Photoreceptors in retinal degeneration slow (rds−/−) mice completely fail to develop outer segments (3–5). Instead, rhodopsin-containing vesicles are present in the subretinal space between the photoreceptor cell bodies and retinal pigment epithelium (6–8). Disc addition at the base of the outer segment is an ongoing process in mature photoreceptors, to compensate for the diurnal shedding of distal outer segments. The addition of discs is an ongoing process in mature photoreceptors and retinal pigment epithelium (6–8). Disc addition at the base of the outer segment is an ongoing process in mature photoreceptors, to compensate for the diurnal shedding of distal outer segments (9). The rds−/− phenotype suggests that outer segment membranes are synthesized in these mutants but cannot fold into discs without rds. rds+/− heterozygotes have a partial phenotype of short and grossly disorganized outer segments, which results from haploinsufficiency (10, 11).

More than 50 mutations in the human RDS gene are responsible for several dominantly inherited retinal degenerations including retinitis pigmentosa (RP) (reviewed in Refs. 12 and 13). Most RDS mutations cause single-residue substitutions within the large intradiscal D2 loop of rds between transmembrane segments three and four (Fig. 1A). An unusual form of RP with digenic inheritance has been described (14). Affected individuals in three pedigrees were heterozygous for both a mutation in RDS, causing an L185P substitution in the D2 loop, and an early frameshift mutation in the unrelated gene for rom1 (14). Neither mutation alone caused disease.

rom1 is a protein homologous to rds with an overall identity of 37% and a similar predicted membrane topology (15). The distribution of rom1 in outer segment discs is identical to that of rds (15). Both rds and rom1 have been shown to form covalent homodimers (15, 16). Based on the results of immunoprecipitation and velocity sedimentation studies, it has been suggested that covalent homodimers of rds and rom1 interact non-covalently to form a heterotetrameric complex (17–21). The domain responsible for non-covalent interactions between rds and rom1 homodimers is not known. Given this apparent symmetry, mutations in the rom1 and rds genes should cause similar phenotypes. Curiously, this is not the case. No mutations in ROM1 alone have been convincingly associated with any human retinal diseases (22). Also, mice with a null mutation in the rom1 gene show only mild outer segment dysplasia (23), in contrast to complete nondevelopment of outer segments in rds−/− mutants (3–5).

In the current study, we generated transgenic mouse lines that expressed a chimeric protein, named rom/D2, containing the D2 loop of rds in the context of rom1 (Fig. 1A). Expression of rom/D2 in transgenic retinas allowed us to measure directly relative levels of rds and rom1 in photoreceptors, as well as the relative strength of interactions between these proteins. Based on our observations, we propose an alternative model for the interaction of rds and rom1 in outer segments.

EXPERIMENTAL PROCEDURES

Generation of Transgenic Mice—cDNA fragments encoding the C1–M3 domains of mouse rom1, the D2 domain of mouse rds, and the M4–C3 domains of mouse rom1 were amplified by polymerase chain reaction, and the resulting products were assembled into a coding unit for rom/D2. The sequence of rom/D2 is shown in Fig. 1A. The rom/D2 coding unit was assembled into a transgene construct containing 6.5 kilobase pairs from upstream of the mouse rhodopsin gene as a transcriptional regulator and the SV40 t-intron and early polyadenylation signal as a transcriptional terminator, similar to what we have used in other transgenic studies (21, 24, 25). The construct was confirmed by DNA sequence analysis prior to oocyte injection. ICR strain transgenic founders were out-crossed with wild-type C57BL/6 mice or with rds−/− homozygous mutants on a C57BL/6 background to yield mice of the desired genotype. To reduce possible photic damage, only pigmented mice were analyzed.

This paper is available online at http://www.jbc.org
(non-albino) animals were studied (26). Mice were maintained on a 12-h light-dark cycle at 40 lux and were sacrificed between 4 and 6 h after light onset. For all experiments, mice were studied at 3 weeks of age.

**Genotype Analysis**—Mice were analyzed for the presence of the transgenic rds-polypeptide chain reaction primers 5'-CCTGOGAGTGCTGCGCTTTG-3' and 5'-GTTCTTTTGTACGAGACCC, from the mouse rhodopsin coding region (27) and the rds coding region (28), respectively. Mice were analyzed for the presence of a wild-type rds gene by polymerase chain reaction using the primers: 5'-CCTCCATGCCCCTGCTCT, from the first intron of rds (11), and 5'-AGCAGAGCGGCTCTGA, from the rds coding region, and for the presence of the mutant rds gene with 5'-CCTGGACAGAAC, from the rds coding region, and 5'-CATCGCTGAGGCTCTGA, from the rds inserted element (11). Polymerase chain reaction was done on DNA from tail cuts under standard conditions.

**Nuclease Protection Analysis**—Total RNA was extracted from individual eyes using TRI Reagent (Molecular Research Center, Inc., Cincinnati, OH), according to the manufacturer's protocol. RNA samples were hybridized to a 32P-labeled RNA probe of 1050 nucleotides, which contained 899 nucleotides complementary to the rds mRNA and 321 nucleotides complementary to the transgenic mRNA. After hybridization and S1 nuclease digestion, protected fragments were analyzed by electrophoresis on 4% polyacrylamide gels containing 7 M urea as described (29). The 899- and 321-nucleotide protected fragments were quantitated on a Molecular Dynamics model 425F PhosphorImager and normalized for their content of radioactive nucleotide.

**Immunoblot Analysis**—Antiserum against the rds D2 loop (rds D2 Ab) was prepared by immunizing rabbits with peptide D2P4 (KEVRKDRKSNVDRG) (Fig. 1A) coupled to keyhole limpet hemocyanin. Antiserum against residues 296–346 from the carboxyl terminus of rds (rds C-term Ab) and residues 296–351 from the carboxyl terminus of rds1 (rom1 C-term Ab) were prepared as described (21). SDS-PAGE and immunoblot analysis were described previously (21). For analysis of protein dimers, dithiorthreitol was omitted in the sample buffer. For radioactive detection, 125I-labeled protein A (ICN Biomedicals, Irvine, CA) was used in a 6-h incubation at a concentration of 0.1 μCi/ml instead of secondary antibody. After five 8-min washes, the radioactive bands were visualized and quantitated on a Molecular Dynamics model 425F PhosphorImager. For quantitative immunoblotting, retinal extracts were deglycosylated, electrohoresed, and transferred to Immobilon P-SQ (Millipore) in buffer containing 0.05% SDS, immunolabeled, and detected with 125I-labeled protein A.

**Enzymatic Deglycosylation of rds and rom/D2**—Individual pairs of retina were homogenized in sodium phosphate buffer (NaP buffer) containing 0.05% sodium phosphate buffer, pH 7.3, 1% SDS, 0.1 M dithiorthreitol, 1 mM EDTA, 0.2 mM phenylmethylsulfonyl fluoride, and protease inhibitors), heated at 55 °C for 2 min in a homogenizer, and diluted 5-fold with the NaP buffer without SDS. After a 2-min clearing centrifugation, samples were incubated at 37 °C with cloned (protease-free) peptide N-glycosidase F (Roche Molecular Biochemicals) at 15 units per retina equivalent from 3-week-old mice were fixed overnight at 4 °C in 0.1 M cacodylate buffer (pH 7.4) containing 2.5% formaldehyde, 2.5% glutaraldehyde, and 0.5% picric acid. Fixed tissues were treated with 1% osmium tetroxide and 1.5% potassium ferrocyanide for 1 h and stained en bloc with 1% aqueous uranyl acetate for 1 h. The specimens were then dehydrated in ethanol and embedded in Spurr resin. 80-nm sections were cut on a Reichert Ultracut S ultramicrotome, collected on Formvar-coated copper oval slot grids, and stained with uranyl acetate and lead citrate. The sections were examined using a JEOL 1200EX II transmission electron microscope at 80 kV.

**Expression of the rom/D2 Transgene—**Six transgenic lines (rdm1–rdm6) were generated with the rom/d2 construct encoding the chimeric protein represented in Fig. 1, A and B. Fig. 1C shows the results of nuclease protection analysis performed on RNA from the eyes of 3-week-old transgenic offspring using a riboprobe that simultaneously detected the transgenic and endogenous rds mRNAs (21, 25). After correcting for probe length and UMP content, the ratio of transgenencidogenous rds mRNAs was determined. The levels of rom/d2 transgenic mRNA in these lines ranged between one and six times that of the wild-type endogenous rds mRNA (Fig. 1C).

We performed immunoblot analysis on retinal homogenates from the same mice using rds D2 and rom1 C-term antisera (Fig. 1D). As predicted, rds and rom/d2 were both detected by the rds D2 Ab, whereas rom/D2 and rom1 were both detected by the rom1 C-term Ab. The apparent molecular mass of rom/d2 was higher than that of rds and slightly lower than that of rom1. The endogenous rds mRNA and rds protein were at significantly reduced levels in high-expressing transgenic lines rdm2, rdm4, and rdm5 (Fig. 1, C and D), because of partial photoreceptor degeneration. Photoreceptor degeneration in these high-expressing lines was confirmed by light microscopic analysis of retinal sections (not shown). No reduction in these endogenous rds mRNA or protein levels were observed in lines rdm1, rdm3, and rdm6. The retinas of these animals were also normal by light microscopy (not shown). rom/D2 Is N-glycosylated—The D2 loop of rds, but not rom1, contains one site of N-glycosylation. To define whether rom/d2 is N-glycosylated in vivo, we incubated retinal homogenates from transgenic and non-transgenic rds+/+ mice with peptide N-glycosidase F, which cleaves asparagine-linked glycans (30). Homogenates were analyzed by immunoblotting with the rds D2 and rom1 C-term antisera (Fig. 2A). Both rds and rom/d2 migrated at reduced apparent molecular mass after digestion with N-glycosidase F, whereas the electrophoretic mobility of rom1 was unchanged. Thus, rom/d2 is N-glycosylated in vivo, similar to rds.

rom/D2 Covalently Dimersizes with Itself but Not with rds or rom1—Retinal homogenates were prepared under non-reducing conditions from transgenic and non-transgenic mice on rds+/+ and rds−/− genetic backgrounds. Samples were separated by SDS-PAGE in the absence of reducing agents and analyzed by immunoblotting with the rds D2 Ab. Similar to native rds in wild-type mice, most rom/d2 migrated as a dimer (Fig. 2B). Small amounts of monomer rds and rom/d2 were also detected, possibly representing synthetic intermediates.

Next, we attempted to identify the protein species that dimerizes with rom/d2. To test for the formation of rds-rom/D2 covalent dimers, we performed immunoprecipitation analysis on 1% SDS plus 8.0 M urea homogenates of retina from transgenic and non-transgenic rds+/+ and rds−/− mice with immobilized rds or rom1 C-term Abs. The rds C-term Ab precipitated rds but not rom1 or rom/d2, whereas the rom1 C-term Ab precipitated both rom1 and rom/d2 but not rds (Fig. 3A). These data rule out the presence of rom1-rom/D2 covalent heterodimers. To test for rdm1-rom/D2 covalent heterodimers, we precipitated SDS-urea homogenates from transgenic and
non-transgenic rds+/+ retinas with the rds D2 Ab. The bound fraction plus starting retinal homogenate were analyzed by immunoblotting using the rom1 C-term antiserum for detection (Fig. 3B). Here, the rds D2 Ab precipitated rom/D2 but not rom1, excluding the presence of rom/D2-rom1 covalent heterodimers. Together, these data show that rom/D2 covalently dimerizes with itself but does not form dimers with rds or rom1.

The rds/rom1 Complex Is More Stable than the rds/rom/D2 Complex and Involves Interactions of the D2 Loop—We tested the stability of the rds/rom1 and rds/rom/D2 non-covalent interactions by defining the conditions required to dissociate the complexes. SDS and increasing concentrations of urea were added to Triton X-100 homogenates of non-transgenic and transgenic retinas. Samples were diluted 20-fold and immunoprecipitated with the rds C-term Ab followed by immunoblot analysis (Fig. 3B). Here, the rds D2 Ab precipitated rom/D2 but not rom1, excluding the presence of rom/D2-rom1 covalent heterodimers. Together, these data show that rom/D2 covalently dimerizes with itself but does not form dimers with rds or rom1.

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antiseras, we used this protein as an internal standard to determine the ratio of rds to rom1 in the same transgenic retinal homogenate. Accordingly, we performed quantitative immunoblot analysis of retinal homogenates from 3-week-old transgenic rdm6 (TG) and non-transgenic (nTG) rds+/+ mice were digested with peptide N-glycosidase F (N-Gly F), where indicated, prior to SDS-PAGE and immunoblotting. Identical blots were reacted with the rds D2 and rom1 C-term Abs and detected by chemiluminescence. The positions of molecular mass standards are indicated to the left in kDa. Note the increased electrophoretic mobilities of rds and rom/D2, but not rom1, after digestion with N-glycosidase F, B; covalent dimerization. Retinal homogenates were prepared from 3-week-old transgenic rdm6 (TG) and non-transgenic (nTG) mice on both rds+/+ and rds−− genetic backgrounds in the absence of sulfhydryl-reducing agents. After separation by SDS-PAGE, the samples were analyzed by immunoblotting with the rds D2 Ab. The immunoblot was reacted with 125I-labeled protein A and developed on a PhosphorImager. The positions of molecular mass standards are indicated to the right in kDa. Note that the preponderant immune signals in both non-transgenic and transgenic retinal homogenates migrate at approximately twice the molecular mass of rds and rom/D2 monomers.

Fig. 2. Immunoblot analysis of rom/D2 post-translational processing. A, N-glycosylation of rds or rom/D2. Retinal homogenates from transgenic rdm6 (TG) and non-transgenic (nTG) rds+/+ mice were digested with peptide N-glycosidase F (N-Gly F), where indicated, prior to SDS-PAGE and immunoblotting. Identical blots were reacted with the rds D2 and rom1 C-term Abs and detected by chemiluminescence. The positions of molecular mass standards are indicated to the left in kDa. Note the increased electrophoretic mobilities of rds and rom/D2, but not rom1, after digestion with N-glycosidase F, B; covalent dimerization. Retinal homogenates were prepared from 3-week-old transgenic rdm6 (TG) and non-transgenic (nTG) mice on both rds+/+ and rds−− genetic backgrounds in the absence of sulfhydryl-reducing agents. After separation by SDS-PAGE, the samples were analyzed by immunoblotting with the rds D2 Ab. The immunoblot was reacted with 125I-labeled protein A and developed on a PhosphorImager. The positions of molecular mass standards are indicated to the right in kDa. Note that the preponderant immune signals in both non-transgenic and transgenic retinal homogenates migrate at approximately twice the molecular mass of rds and rom/D2 monomers.

In this study, we generated transgenic mice that express a chimeric protein comprising rom1 substituted with the large intradiscal D2 loop of rds. The D2 loops of rds and rom/D2 were identical except for a single, conservative amino acid substitution (Arg to Lys) adjacent to membrane-spanning segment M3. We demonstrated that rom/D2 was N-glycosylated, underwent covalent homodimerization, participated in non-covalent complex formation with rds, and effected rescue of the outer segment phenotype in rds mutant mice. In rds−− heterozygotes, we observed virtually complete rescue of the outer segment disorganization by expression of rom/D2 (Fig. 6, D and E). In contrast, expression of rom/D2 did not affect the phenotype of absent outer segments in rds+/− null mutants (Fig. 6F). This lack of rescue was observed in lines rdm6, rdm1, and rdm4 on rds−−, indicating that the level of transgene expression was not a determinant. Together, these observations suggest that rom/D2 can only rescue the rds phenotype in the presence of at least a small quantity of normal rds.

**DISCUSSION**

In this study, we generated transgenic mice that express a chimeric protein comprising rom1 substituted with the large intradiscal D2 loop of rds. The D2 loops of rds and rom/D2 were identical except for a single, conservative amino acid substitution (Arg to Lys) adjacent to membrane-spanning segment M3. We demonstrated that rom/D2 was N-glycosylated, underwent covalent homodimerization, participated in non-covalent complex formation with rds, and effected rescue of the outer segment phenotype in rds+/− mutants. These features suggest that rom/D2 was stable, was correctly processed and efficiently translocated to outer segments, and was functional. We also showed that rds, rom/D2, and rom1 all form covalent homodimers but that mixed dimers between these proteins do not occur (Figs. 2 and 3). We used retinas from mice expressing rom/D2 to make several observations about the function of rds and rom1.

The non-covalent interaction between rds and rom/D2 was more stable than the interaction between rds and rom1 in a dissociating titration with increasing concentrations of urea (Fig. 3C). Because rom/D2 and rom1 differ only in their D2 loop domains, this observation provides the first indication that the non-covalent interaction between rds and rom1 is via their D2 loops. Further evidence to support this conclusion is that rds could only be immunoprecipitated with the rds D2 Ab after dissociation of the complex (Fig. 3D), implying that the D2P4 epitope is normally sterically blocked. The D2P4 epitope is inside a highly conserved stretch (Fig. 1B), which may represent an interacting structural element. The D2 loop of rds, but not rom1, is N-glycosylated. However, in a previous study we showed that this modification does not contribute to the function of rds in vivo (21). For example, non-glycosylated rds interacted normally with rom1 by immunoprecipitation and fully rescued the rds−− null phenotype. Therefore, the D2-loop structure itself, and not the glycan moiety, is responsible for this interaction.

between rds and rom1, we performed reciprocal co-immunoprecipitation from wild-type retinal homogenates with antibodies against rds and rom1. After precipitation, we analyzed both the pellets and supernatants for the presence of rds and rom1 (Fig. 5). As predicted, the antibody against either rds or rom1 efficiently precipitated its cognate protein. The rom1 C-term Ab also precipitated more than 90% of rds. Efficient co-immunoprecipitation of rds and rom1 was reported previously (15, 19). Given the 2.5-fold greater abundance of rds over rom1, quantitative precipitation of rds by rom1 implies that these proteins exist in a multimeric complex of higher order than tetramer.
We exploited the shared epitopes and different electrophoretic mobilities of rds, rom/D2, and rom1 to show that rds is 2.5-fold more abundant than rom1. This result was unexpected, given the similarity of rds and rom1 mRNA-levels by Northern blot analysis and by the isolation frequencies of cDNA clones (15, 28). Nearly identical results were obtained by quantitating both proteins in the same transgenic retinal homogenate and by comparing levels in transgenic and non-transgenic homogenates (Fig. 4), indicating no transcriptional cross-regulation between the transgene and endogenous rds gene. Formally, it is possible that the observed differences in abundance between transgenic and non-transgenic rds1/1 and rds2/2 genetic backgrounds.

FIG. 3. Immunoprecipitation of rds and rom/D2. A, SDS-urea homogenates of retinas were prepared under non-reducing conditions from non-transgenic (nTG) and transgenic rds6 (TG) mice on rds1/+ and rds−/− genetic backgrounds. Extracts were immunoprecipitated with the rds C-term or rom1 C-term Abs (precipitating Ab). After washing and elution, the bound proteins were analyzed as monomers by immunoblotting with the rds D2 and rom1 C-term Ab (detecting Ab) as indicated. Note that the rds C-term Ab precipitated rds but not rom/D2 or rom1 and that the rom1 C-term Ab precipitated rom1 and rom/D2 but not rds. B, SDS-urea homogenates of retina prepared under non-reducing conditions from non-transgenic (nTG) and transgenic rds6 (TG) mice on rds1/+ were treated with peptide N-glycosidase F and immunoprecipitated with the rds D2 Ab. The starting retinal homogenate (homog) and immunoprecipitates (rds D2 ppt) were analyzed by immunoblotting with the rom1 C-term Ab. Blots were reacted with 125I-labeled protein A. Note that the rds D2 Ab precipitated rom/D2 but not rom1. C, retinas from non-transgenic (nTG) and rds6 transgenic (TG) rds1/1 mice were homogenized under non-reducing conditions in buffers containing 1% Triton X-100 plus the indicated concentrations of SDS and urea. After dilution, samples were immunoprecipitated with the rds C-term Ab. Bound proteins were eluted and analyzed by immunoblotting with the rds and rom1 C-term Abs as indicated. Note the large reduction in rom1 after immunoprecipitation when 1% SDS was added to the homogenization buffer. In contrast, note the presence of rom/D2 in the homogenate treated with 1% SDS or 1% SDS plus 2 M urea. D, retinas from non-transgenic rds1/1 mice were homogenized under non-reducing conditions in Triton X-100 plus or minus 1% SDS and 8 M urea as indicated. After dilution, samples were immunoprecipitated with the rds D2 Ab. Bound proteins were eluted and analyzed by immunoblotting under reducing conditions with the rds D2 Ab. Note that rds was only present in the immunoprecipitate from the SDS-urea-containing homogenate.

FIG. 4. Immunoblot quantitation of rds and rom1. Retinal homogenates prepared under reducing conditions from 3-week-old rds6-transgenic (3 w TG), 3-week-old non-transgenic (3 w nTG), and 2-month-old non-transgenic (2 m nTG) rds1/+ mice were digested with peptide N-glycosidase F (N-Gly F) for the indicated times prior to SDS-PAGE. Each lane contained 2% of a retinal homogenate. The transblots were analyzed with the rds D2 or rom1 C-term Abs as indicated. The blots were reacted with 125I-labeled protein A, and the intensities of each band were quantitated on a PhosphorImager.

We exploited the shared epitopes and different electrophoretic mobilities of rds, rom/D2, and rom1 to show that rds is 2.5-fold more abundant than rom1. This result was unexpected, given the similarity of rds and rom1 mRNA-levels by Northern

FIG. 5. Co-precipitation of rds and rom1. Retinal homogenate prepared under non-reducing conditions from a non-transgenic rds1/+ mouse was immunoprecipitated with either rds C-term or rom1 C-term Abs (precipitating Ab). Equivalent volumes of the starting homogenate (homog), pellet, and supernatant (sup) were separated by SDS-PAGE and analyzed by immunoblotting, using the rds C-term or rom1 C-term Abs (detecting Ab). The blots were reacted with 125I-labeled protein A and developed on a PhosphorImager. Note complete co-precipitation of rds with rom1 by the rom1 C-term Ab.
rds, rom/D2, and rom1 may be due to context-dependent differences in antibody binding. However, because the proteins were analyzed under denaturing conditions and because the D2 and C-term epitopes are both far removed from one another and from the rom1-rds junction-regions in rom/D2, this explanation is unlikely.

The currently accepted model for the rds-rom1 complex is a heterotetramer composed of paired covalent homodimers (17, 18, 20, 21). This model demands revision on two accounts. First, the 2.5-fold difference in abundance between rds and rom1 requires that rds homodimers interact primarily with themselves. Non-covalent interactions between rds and rom1 must be rare. Second, quantitative co-precipitation of rds and rom1 by the rom1 C-term Ab (Fig. 5, Refs. 15 and 19) requires that both proteins be part of a higher order complex rather than a simple tetramer. The rds-rom1 complex may extend along the entire disc circumference. The distance between luminal faces of the terminal loop in bullfrog outer segments is ~130 Å (32), a plausible distance to be spanned by paired 130-residue globular domains. The disc rim represents a thermodynamically unfavorable fold in the membrane with a radius of curvature only about twice the thickness of the bilayer (32). Because rds and rom1 are situated within this terminal loop, and because discs fail to form without rds despite ongoing outer segment membrane and protein synthesis (6–8), rds may serve to stabilize this sharp fold in the disc membrane.

Expression of rom/D2 at a level similar to that of endogenous rds resulted in rescue of the outer segment disorganization in rds<sup>1/2</sup> heterozygotes (Fig. 6). However, up to 4-fold overexpression of rom/D2 had no effect on the ultrastructural phenotype in rds<sup>2/2</sup> homozygotes. In an earlier study, we completely rescued the rds<sup>−/−</sup> phenotype by expressing normal rds in transgenic retinas (24). Because rom/D2 contains the complete D2 loop of rds, these observations suggest that a second domain of rds may be required in trans for its normal function. By definition, this domain must be functionally absent in rom1. Possible functions of this rds domain are: (i) trafficking of the rds complex to outer segment discs, as has been shown for the carboxyl terminus of rhodopsin (33); (ii) coupling of the disc rim to the outer segment plasma membrane via direct protein-lipid interactions (34) or via interactions between rds and the cytoskeleton; or (iii) interactions between rds complexes of adjacent discs to maintain outer segment alignment.

In this work, we have identified three characteristics that distinguish rds from rom1. Together, these differences explain...
the mild phenotype in rom1−/− mutant mice and the paucity of disease-causing mutations in the human ROM1 gene. First, rom1 is significantly rarer than rds. Thus, even if rds and rom1 were functionally equivalent, we would predict a much milder phenotype in rom1−/− null mutants compared with rds+/− mutants. However, this cannot be the whole story. If we assume that rds and rom1 are functionally equivalent and differ only by their abundance, then the rds+/− phenotype is seen in the context of 64% total protein expression and the much milder rom1−/− phenotype (23) in the context of 71% total protein expression. It is unlikely that the disparity of phenotypes between these mutants could be explained by a mere 7% difference in total protein expression.

The second distinguishing characteristic between rds and rom1 is the difference in stability of their respective non-covalent interactions with rds. Given the apparent weakness of the rom1-rds interaction, loss of rom1 may have little effect upon outer segment structure. Can we then conclude that rom1 has no function in outer segments? Probably not. The reported digenic form of RP in humans, because of a nonsense mutation in ROM1 and a simultaneous mis-sense mutation in RDS (14), provides genetic evidence that rom1 plays a role. This clinical observation was corroborated by our analysis of a transgenic model for digenic RP. In mice doubly heterozygous for an rds L185P and rom1 null allele, we saw accelerated photoreceptor degeneration compared with the two “monogenic” controls.2

Finally, the ability of rom/D2 to rescue the rds+/− but not rds−/− phenotype independent of transgene expression level suggests a second functional domain in rds outside of the D2 loop that is absent from rom1. Thus, we suggest that rds is a bifunctional protein that interacts on both its intradiscal and cytoplasmic surfaces. rom1, on the other hand, may interact only via its intradiscal D2 loop.

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