Mouse choroideremia gene mutation causes photoreceptor cell degeneration and is not transmitted through the female germline

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Choroideremia (CHM) is an X-linked progressive eye disorder which results from defects in the human Rab escort protein-1 (REP-1) gene. A gene targeting approach was used to disrupt the mouse chm/rep-1 gene. Chimeric males transmitted the mutated gene to their carrier daughters but, surprisingly, these heterozygous females had neither affected male nor carrier female offspring. The targeted rep-1 allele was detectable, however, in male as well as female blastocyst stage embryos isolated from a heterozygous mother. Thus, disruption of the rep-1 gene gives rise to lethality in male embryos; in female embryos it is only lethal if the mutation is of maternal origin. This observation can be explained by preferential inactivation of the paternal X chromosome in murine extraembryonic membranes suggesting that expression of the rep-1 gene is essential in these tissues. In both heterozygous females and chimeras the rep-1 mutation causes photoreceptor cell degeneration. Consequently, conditional rescue of the embryonic lethal phenotype of the rep-1 mutation may provide a faithful mouse model for choroideremia.

INTRODUCTION

Choroideremia (CHM) is an X-linked recessive eye disease which is characterized by progressive degeneration of the retinal pigment epithelium (RPE), choriocapillaris, and retina (1, 2). Affected males develop night blindness in their teenage years, which is followed by progressive constriction of visual fields and eventually complete blindness. Female carriers generally show no serious visual impairment (3). The gene that is mutated in CHM has been isolated by positional cloning (4-6). The open reading frame of the CHM gene consists of 15 exons spanning at least 150 kb of Xq21.2 and encodes a ubiquitously expressed protein of 653 amino acids.

Insight into the function of the CHM protein came with the biochemical purification of Rab geranylgeranyl transferase (Rab GGTase) from rat brain (7-9). This enzyme attaches geranylgeranylated groups to Rab proteins, a modification essential for their action in intracellular vesicular transport. Rab GGTase is a heterodimer composed of tightly associated α- and β-subunits. It requires an accessory component, the Rab escort protein (REP), for activity. Rat REP-1 was found to be identical with the protein encoded by the human CHM gene (8-10). REP-1 binds to newly synthesized Rab proteins, presents them to Rab GGTase and delivers the geranylgeranylated Rabs to their target membranes (10, 11).

Lymphoblasts of CHM patients have a markedly decreased but still detectable Rab GGTase activity (9) suggesting the existence of an additional REP protein. Indeed, a homologue of the CHML/REP-1 gene has been identified which was designated CHML for choroideremia-like. This intronless gene on chromosome 1q is expressed in a wide variety of tissues (12, 13). The CHML or REP-2 protein was shown to perform a function similar to that of REP-1. Therefore, it was hypothesized that this protein partially compensates for the loss of REP-1 activity in choroideremia patients, thereby preventing symptoms in tissues and organs other than the eye (14). Ocular symptoms might be due to
the presence of Rabs that are preferentially prenylated by REP-1. Recently, Seabra et al. (15) identified an unprenylated Rab protein in lymphoblasts from CHM patients that was prenylated more efficiently by REP-1 than by REP-2. This protein, Ram/Rab27, is present at high levels in the rat eye, particularly in the RPE and chorioalluparissains, suggesting that CHM may result from deficient geranylgeranylation of Ram/Rab27 or a closely related protein.

Despite the considerable knowledge about the molecular and biochemical defect causing choroideremia, the pathogenesis of the disease is still poorly understood. Against this background, and as a prerequisite for future therapeutic studies, we have generated a mouse model for choroideremia by targeted inactivation of the rep-1 gene.

RESULTS

Targeted disruption of the mouse rep-1 gene

Male E14 embryonic stem (ES) cells were transfected with a replacement-type targeting construct which, upon homologous recombination, results in disruption of exon 8 of the X-linked rep-1 locus by a neomycin resistance (neo+) gene (Fig. 1a). After selection with G418, 165 colonies were obtained and screened by Southern analysis. Five clones gave the diagnostic 3.0 kb BamHI fragment upon hybridization with the 5' probe (Fig. 1). Four of these also showed the expected 8.0 kb BglII fragment upon hybridization with the 3' probe confirming correct homologous recombination. Two successfully targeted ES cell lines (E14-IB3 and E14-IIE5) displaying a normal karyotype were injected into C57BL/6 blastocysts that were reimplanted into pseudopregnant foster mothers. The resulting chimeric males were bred to C57BL/6 females and their agouti offspring showed the expected 1:1 ratio of females and males (Fig. 2). As the rep-1 gene resides on the X chromosome, F1 females should be heterozygous for the rep-1 mutation and F1 males should be wildtype. This was confirmed by Southern or PCR analysis of tail DNA (Fig. 3).

Heterozygous F1 females were subsequently mated to C57BL/6 males. The litter sizes were small; on average 4–5 per pregnancy. Males and females were almost equally represented. Surprisingly, it became apparent upon genotyping of the F2 embryos that neither males nor females carried the targeted allele. Two blastocysts contained the wildtype as well as the targeted allele and thus were carrier females.

Nested polymerase chain reaction on blastocyst stage embryos

To decide between these two possibilities, blastocyst stage embryos were isolated from a heterozygous F1 female. A nested PCR assay was employed that allowed the identification of the wildtype as well as the targeted rep-1 gene (Fig. 4a). In the first round of amplification, a combination of two rep-1 primers and one neo primer was used in a single reaction. Subsequently, either two rep-1 primers or one rep-1 and one neo primer were used to detect the wildtype and the targeted allele, respectively. Nested PCR was performed on a total of nine blastocysts. In six of them the wildtype gene was present (Fig. 4b). In one blastocyst the targeted allele was amplified, indicating that this was a hemizygous male embryo. Two blastocysts contained the wildtype as well as the targeted allele and thus were carrier females.

With primers specific for the targeted allele an additional product, slightly larger than the expected 226 bp, was amplified from both blastocysts. Sequencing revealed that these products were indeed derived from the targeted rep-1 gene (data not shown). The increased length was due to incorrect amplification of a short repeat-like sequence. This PCR artefact does not compromise the conclusion that the two blastocysts were heterozygous female embryos. Thus, nested PCR demonstrated the presence of a targeted rep-1 gene in male as well as female preimplantation embryos isolated from a carrier mother, indicating that in mice, mutational inactivation of the rep-1 gene gives rise to embryonic lethality when transmitted through the female germline. Death presumably occurs early in embryonic development since stillborn embryos were never observed.
Ophthalmological analysis of adult mice

To search for ocular manifestations of the rep-1 mutation, F1 heterozygous females and chimeras were subjected to electroretinographic testing. Figure 5a shows electroretinograms (ERGs) recorded from dark adapted eyes using a flash intensity of 1 cd/s/m². Under these conditions both rods and cones contribute to the ERG signal. The basic response is a clearly detectable negativity (a-wave) followed by a positive deflection (b-wave) which is dominated by oscillatory potentials. Figure 5b shows the amplitudes of the b-waves of heterozygous females and chimeras. Variation of b-wave amplitudes is striking in chimeras, some of whom transmitted the ES cell genome to the F1 offspring. Two transmitting alleles. A total number of 21 chimeric males were bred to C57BL/6 females; six out of eight F1 carrier females were mated to C57BL/6 males. Relevant numbers of animals are given beneath the genotype.

the outer nuclear layer, resulting from loss of photoreceptor cell bodies, were filled by cells from the inner nuclear layer. At these sites, the outer plexiform layer was also replaced. The remaining photoreceptors appeared to be normal and possessed full-length outer segments. The pigment epithelium was intact and filled with pigment granula (Fig. 6c). The choroid was unremarkable except for the presence of melanocytes between the capillaries. The inner nuclear layer, the inner plexiform layer, and the ganglion cell layer were unaffected (Fig. 6c).

In chimeras, a patchwork of areas containing photoreceptors and areas with loss of photoreceptors was observed (Fig. 6d). Photoreceptors that were present appeared normal in terms of outer segment length. The transition between the outer nuclear layer and the outer plexiform layer was irregular due to a variable number of cell bodies in the outer nuclear layer. In large areas
photoreceptors were completely degenerated; only scattered remnants of cell nuclei but no photoreceptor outer and inner segments were found (Fig. 6d). At these locations, the inner nuclear layer was juxtaposed to the pigment epithelium. In the pigment epithelium, cells containing pigment granules alternated with those lacking granules (Fig. 6d) but in a pattern which did not correlate with the pattern of photoreceptor loss in the retina. Since 129 mice have a non-pigmented epithelium (Fig. 6a), while C57BL/6 mice have a pigmented epithelium (Fig. 6b) this phenomenon is simply a demonstration of the patchy distribution of 129 ES cell-derived and host-derived C57BL/6 cells. Irrespective of its origin, the retinal pigment epithelium was normal and intact. Choroidal pigment cells were found between the capillaries but the choroid was otherwise unaffected. The inner retina was also unremarkable (Fig. 6d).

**DISCUSSION**

The spectrum of known defects in the *REP-1* gene of choroideremia patients includes deletions, translocations, and a variety of subtle mutations (reviewed in 16). Most, if not all, of the subtle mutations lead to the introduction of a premature stop codon. To construct an animal model for this genetic disorder, the mouse *rep-1* gene was disrupted in exon 8 at nucleotide position 1172 of the 1995 bp open reading frame. RT-PCR analysis confirmed that this results in a premature termination codon (data not shown). Consequently, the targeted *rep-1* gene will either express a protein with a C-terminal truncation of 274 amino acids or no protein product at all. Recently, it has been shown that recombinant rat *rep-1* protein that lacks the 70 C-terminal amino acids is unable to assist in the geranylgeranylation of Rab proteins (F.P.M.Cremers and M.Seabra, unpublished data). It is therefore

![Figure 4. Nested PCR on blastocyst stage embryos. (a) Schematic outline of the PCR strategy. First, a combination of two *rep-1* primers and one neo primer allows the amplification of both the wildtype and the mutated allele in a single reaction. In separate second rounds, nested primers amplify a 226 bp fragment from the wildtype allele and a 226 bp fragment from the mutated allele. (b) Analysis of nested PCR products amplified from blastocysts that were isolated from a carrier female, a control blastocyst obtained from a wildtype female, targeted ES cell line DNA (E14-IB3), wildtype ES cell line DNA (E14) and tail DNA of a carrier female. The sizes of the PCR fragments are indicated.](image)

![Figure 5. Electroretinogram (ERG) recordings. (a) Original dark adapted ERGs recorded from a C57BL/6 female (upper trace) and two chimeras, one with a relatively normal response (middle trace) and one with a severely diminished response (lower trace). The triangle indicates the time of the flash (intensity 1 cd/m²), Vertical calibration 100 μV/division; horizontal calibration 20 ms/division. (b) Amplitude of the b-wave of dark adapted ERGs recorded from F1 heterozygous females, controls and chimeras using a flash intensity of 1 cd/m². Each point represents the average of three measurements on a single eye. Error bars indicate the mean ±SD for each group. The age in the control groups ranged from 6-12 months for the F1 wildtype males, was 17 months for the 129 females and 6-12 months for the C57BL/6 females. F1 carrier females were 12-20 months old, and the age of the chimeras varied from 10 to 22 months.](image)
clear that any protein resulting from the disrupted rep-1 gene will be non-functional.

Chimeric males transmitted the disrupted rep-1 gene to their female offspring. Surprisingly, these heterozygous F1 females had neither affected male nor carrier female offspring; none out of 71 F2 animals, born to six different heterozygous mothers, carried the rep-1 mutation. Nested PCR could demonstrate the targeted rep-1 allele in male as well as female blastocyst stage embryos isolated from a carrier mother. Thus, it appears that disruption of the rep-1 gene causes embryonic lethality in males; in females it is lethal if the mutated rep-1 allele is present on the maternal X chromosome but not if it is present on the paternal X chromosome. A plausible explanation for this observation would be that in mice expression of the rep-1 gene is required for functional survival of extraembryonic membranes. It has been extensively documented that in female mouse embryos the paternal X chromosome is preferentially inactivated in extraembryonic tissues (17,18). As a result, in female embryos that inherit the mutated X from their mother, the rep-1 gene is functionally absent in extraembryonic tissues because the wildtype gene on the paternal X is inactive. In contrast, female embryos carrying the targeted rep-1 gene on their paternal X would be healthy because this X chromosome is physiologically inactive; the only active rep-1 gene is the wildtype one on the maternal X. Affected male embryos would die because the rep-1 gene on their single X chromosome is mutated and they lack a functional protein product in their extraembryonic tissues.

In humans, rep-1 mutations are transmitted normally, both by affected males and carrier females. The reason for this discrepancy is not immediately apparent. Though there is evidence for preferential paternal X-inactivation in the cytотrophoblast of human females (19,20), contradictory results have been obtained (21). Thus, it cannot be excluded that, regardless of the parental origin of the mutated rep-1 gene, in human carrier females there is expression of the functional rep-1 gene in extraembryonic tissues. However, human affected males lack a functional copy of the rep-1 gene as do murine affected males. The fact that affected males are viable in humans but not viable in mice may indicate differences in the REP/Rab GGTase system and/or its Rab substrates between the two species. As another possibility, pleiotropic enzymatic adaptations may obscure the phenotypic consequences of mutation, and do so in a species dependent manner.

Carrier females and chimeras were subjected to electroretinographic testing. However, control animals that match them with respect to age, sex and genetic background were not available. Interpretation of the data is further complicated by the fact that a possible phenotypic effect of the rep-1 mutation is influenced by X-inactivation in heterozygous females and depends on the contribution of targeted ES cells in chimeras. The ERG findings suggest that the heterozygous females have no obvious abnormalities in b-wave amplitudes. Nevertheless, histological examination demonstrated a clear but rather mild loss of photoreceptors in the retina of these mice. The remaining photoreceptors were well-developed and the RPE and choroid were both intact. In chimeras, a considerable variation in ERG b-wave amplitudes was observed. Some chimeras appeared to have a reduced response in the b-wave component, suggesting retinal dysfunction. Indeed, histology of the retina revealed areas that were relatively well-preserved and areas that were completely devoid of photoreceptors. Even in these regions the RPE and choroid were present. Together, these findings provide convincing evidence that the rep-1 mutation results in photoreceptor degeneration.

Histopathological studies in human female carriers of choroideremia are scarce and not consistent. One carrier female has been described in which the pigment epithelium showed alternating areas of atrophy and pigment clumping, with the retina and choroid otherwise appearing normal (22). Histopathological findings in another female carrier included areas with normal photoreceptors, areas in which the photoreceptors were reduced in number and outer segments were shortened or absent, and areas

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**Figure 6.** Morphological analysis of retinas from control (a) and C57BL/6 (b) females, a carrier female (c) and a chimera (d). In the retina of the carrier female (c), the outer nuclear layer is irregular in thickness. In (d), the transition between a relatively normal area and an affected area in the retina of a chimera is shown. In the affected region, there is complete loss of photoreceptors; the inner nuclear layer is in direct contact with the retinal pigment epithelium. gel, ganglion cell layer; inl, inner nuclear layer; ipl, inner plexiform layer; is, inner segments of the photoreceptors; onl, outer nuclear layer; opl, outer plexiform layer; os, outer segments of the photoreceptors; rpe, retinal pigment epithelium. Magnification is the same for all micrographs; the bar in (d) represents 10 μm.
containing no photoreceptors at all. The pigment epithelium was absent in some regions of profound atrophy and was irregular in thickness and pigmentation elsewhere. The choriocapillaris was normal in areas with normal receptor population but in regions in which the receptors were abnormal the choriocapillarids were fewer in number, had reduced luminal diameter, and fenestrate were sparse. In some areas of intense atrophy, the choriocapillaris was absent (23). In contrast, Ghosh et al. (24) reported on a carrier in which the choriocapillaris was present and the retinal pigment epithelium was intact, although there were areas showing depigmentation. Photoreceptors were present but there were widespread morphological changes of the outer segments. The retina contained specific areas of degeneration in which there was complete loss of photoreceptors. This resembles the findings in chimeric mice, although in these animals morphological changes of photoreceptor outer segments were not observed. Possibly, these changes precede degeneration of photoreceptors.

REP-1 mutations are viable in humans but, surprisingly, our studies showed that targeted disruption of the murine rep-1 gene causes embryonic lethality. In addition, our data indicate that the murine rep-1 gene is subject to preferential paternal X-inactivation in extraembryonic membranes and that, in mice, rep-1 plays an essential role in these tissues. Although attempts to generate affected males were unsuccessful, our studies have provided evidence that mutation of the murine rep-1 gene gives rise to ocular changes that are comparable to those found in human choroideremia. Thus, sophisticated transgenic strategies are required to render transmission of the rep-1 mutation through the female germline possible and to generate a useful animal model for choroideremia.

MATERIALS AND METHODS

Construction of the targeting vector

A mouse 129/SvEv genomic library in EMBL-3 (kindly provided by Dr G. Grosvenor, St Jude Children’s Research Hospital, Memphis, USA) was screened with a mouse rep-1 cDNA probe (van den Hurk et al., unpublished data). One of the positive phages (TCD-4) contained a 17 kb DNA fragment that included three exons of the rep-1 gene that were orthologous to human REP-1 exons 6–8. A targeting vector was constructed from TCD-4 sequences using standard cloning procedures. The final construct (Fig. 1a) consisted of pGEM4 (Promega), a 7.6 kb Sall–Clal genomic fragment of the rep-1 gene that included exons 7 and 8, and a 1.1 kb blunted XhoI–BamHI neo’ cassette derived from pMC1neoPolA (Stratagene) that was introduced in the BalI site of exon 8. The neo' cassette was positioned in the opposite transcriptional orientation to the rep-1 exons. The targeting construct contained a unique Sall-site for linearization.

Generation of targeted ES cell clones

129/ola-derived male E14 ES cells (kindly provided by Dr A. Berns, Netherlands Cancer Institute, Amsterdam, The Netherlands) were grown on irradiated SNLH9 (25) feeder layers in Dulbecco’s modified Eagle’s medium supplemented with 15% fetal calf serum, 2 mM L-glutamine, 1 mM sodium pyruvate, and 0.1 mM β-mercaptoethanol at 37°C, 5% CO2. Aliquots of ~5 x 106 E14 ES cells were resuspended in 0.8 ml culture medium and electroporated with 20 μg linearized rep-1 targeting vector DNA using the BioRad Gene Pulser set at 250 V/0.4 cm, 500 μF. ES cells were plated onto 10 cm diameter tissue culture dishes containing irradiated feeder cells. Twenty-four hours later, the medium was replaced by selection medium containing 300 μg/ml G418 (Geneticin, Gibco) and refreshed every second day. After 9–11 days individual resistant colonies were picked, trypsinized, and transferred into 96-microwell plates coated with irradiated feeder cells. Following growth to confluency, three quarters of each clone was frozen in culture medium containing 10% DMSO, and the remainder was expanded on gelatin-coated 96-microwell plates for the isolation of genomic DNA (26). ES cell clones were screened for homologous recombination events by Southern analysis of BamHI-digested genomic DNA using an 0.5 kb Aval–Sall genomic rep-1 fragment, located immediately upstream of the sequences in the targeting construct, as 5’ probe (Fig. 1). ES cell clones that gave the diagnostic 3.0 kb BamHI fragment were thawed and expanded under the conditions specified above. Part of each clone was re-frozen for storage, while the rest was passaged onto gelatin-coated plates. Genomic DNA was isolated from these cells (27), digested with BglII, and analyzed with the rep-1 5’ probe (Fig. 1), which is a 0.3 kb Clal–BglII fragment immediately downstream of the sequences in the targeting vector, and with a neo’ specific fragment. Finally, correctly targeted ES cell lines were subjected to cytogenetic analysis (28).

Blastocyst injections, breeding, and genotyping

Blastocysts were flushed from the uterine horns of naturally mated C57BL/6 females at 3.5 days post coitum. Per blastocyst, ~10 ES cells of targeted clones were injected into the blastocoele cavity, and groups of 8–16 blastocysts were transferred into C57BL/6 x CBA/Ca F1 pseudopregnant females (29). Chimerism of the resulting offspring was estimated on the basis of coat colour. Males with more than 50% agouti coat colour were mated to C57BL/6 females. Resulting F1 heterozygous females were bred to C57BL/6 males. Genotyping of mice was performed by Southern or PCR analysis of genomic DNA prepared from tail tips (30). For Southern analysis, DNA was digested with BamHI or BglII and hybridized with the rep-1 5’ or 3’ probe, respectively. For PCR analysis, a combination of two rep-1 primers was used: forward 1075: CGATAAATTTTTCCATATTTCAATAC, and reverse 1761: CGACAAAGAGCCTGGGCGG. The PCR mixture contained 62.5 ng of each primer, 50 ng DNA, 0.5 mM dNTPs, and 1.25 U AmpliTaq DNA polymerase in 25 μl of 6 mM MgCl2, 50 mM KCl, 5 mM DTT, and 10 mM Tris 1 HCl pH 8.3. After 35 cycles of amplification, each consisting of 94°C for 1 min, 62°C for 1 min and 72°C for 1.5 min, products were analyzed on a 2% agarose gel stained with ethidium bromide.

Nested PCR on blastocysts

Blastocysts were recovered from a superovulated F1 heterozygous female that had mated with a C57BL/6 male 3.5 days earlier. Blastocysts were kept at 37°C for 1 h, washed several times in PBS, and visually inspected for the presence of contaminating maternal cells. Next, 5 μl lysimax containing 0.9% TWElREN 20, 0.9% Triton X-100, 0.2 μg/ml proteinase K, 2 mM DTT, 5 mM EDTA, and 10 mM Tris–HCl pH 7.5 was added to each blastocyst. Likewise, 5 μl lysimax was added to each of the following control samples: a blastocyst obtained from a C57BL/6
female at 3.5 days post coitum, 2 μl E14-HB3 ES cell DNA (60 pg), 2 μl E14 ES cell DNA (60 pg), 2 μl tail tip DNA (60 pg) from a F1 heterozygous female and 2 μl Milli Q. Samples were incubated at 65°C for 10 min, then at 95°C for 10 min, and subsequently allowed to cool to room temperature. Thereafter, 43 μl PCR mixture I was added to give final concentrations of 6 mM MgCl2, 50 mM KCl, 5 mM DTT, 10 mM Tris–HCl pH 8.3, 0.5 mM dNTPs, 0.03 U/μl AmpliTaq DNA polymerase and 2.5 ng/μl of each primer. For the first round of amplification, two rep-1 intronic primers (rep-1 forward 1075, rep-1 reverse 1090; GGAGGATCCTACCCAGGCTCC) and one neo primer (3’ neo 742) were used. Conditions of amplification were 94°C for 5 min, then 4 cycles at 94°C for 3 min, 62°C for 1 min and 72°C for 1.5 min, followed by 28 cycles at 94°C for 1 min, 62°C for 1 min and 72°C for 1.5 min, and finally 72°C for 5 min and cooling to 4°C. A 4 μl sample of the reaction mixture was subsequently added to 46 μl PCR mixture II giving final concentrations as described above. For the second round of amplification, either two rep-1 primers (rep-1 forward 1056; CATAGGGAACACATCATTTC, rep-1 reverse 761) or one rep-1 primer (rep-1 forward 1056) and one neo primer (3’ neo 1089; CGCACGGGTTGTTGGGTCG) were used under the same conditions as described for the first round. Products were analyzed on a 2% agarose gel stained with ethidium bromide.

**Electroretinogram recordings**

Details on electroretinogram (ERG) recordings are described elsewhere.31 In brief, mice were dark adapted for at least 12 h. Pupils were dilated with 1% atropine and 0.5% tropieamide. Mice were anesthetized with a mixture of xylazine (20 mg/kg) and ketamine (40 mg/kg) injected subcutaneously. ERGs were measured with a corneal contact lens electrode; silver needles applied subcutaneously below the eyes and at the forehead served as reference and ground electrodes, respectively. A Ganzfeld stimulus device was used to present flashes of light (6000 K, –50 μs) with an intensity of 1 cd/m².

**Histology**

Mice were anesthetized with ether and decapitated. Eyes were enucleated, hensinated along the ora serrata, and the cornea and lens were removed. The posterior eye cup with the retina left in place was fixed in ice-cold 1% paraformaldehyde, 2.5% glutaraldelyde in PB (0.05 M phosphate buffer pH 7.2) at 4°C overnight. Thereafter, eye cups were washed in PB, postfixed in 1% osmium tetroxide for 1 h, and dehydrated in ascending ethanol series. In the 70% ethanol fraction an en bloc staining was carried out with 2% uranyl acetate overnight. Specimens were subsequently embedded in resin (TAAB LABORATORY). For light microscopy, radial semithin sections (60 nm) were stained either with toluidine blue or cresyl violet. Ultrathin sections (60 nm) were poststained with lead citrate and examined with a Zeiss EM 10.

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**ABBREVIATIONS**

CHM, choroideremia; CHML, choroideremia-like; ERG, electroretinogram; ES cells, embryonic stem cells; REP, Rab escort protein.

**REFERENCES**


