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Nonsense Mutations in the Rhodopsin Gene that Give Rise to Mild Phenotypes Trigger mRNA Degradation in Human Cells by Nonsense-Mediated Decay

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Abstract

Eight different nonsense mutations in the human rhodopsin gene cause retinitis pigmentosa (RP), an inherited degenerative disease of the retina that can lead to complete blindness. Although all these nonsense mutations lead to premature termination codons (PTCs) in rhodopsin mRNA, some display dominant inheritance, while others are recessive. Because nonsense-mediated decay (NMD) can degrade mRNAs containing PTCs and modulate the inheritance patterns of genetic diseases, we asked whether any of the nonsense mutations in the rhodopsin gene generated mRNAs that were susceptible to degradation by NMD. We hypothesized that nonsense mutations that caused mild RP phenotypes would trigger NMD, whereas those that did not engage NMD would cause more severe RP phenotypes—presumably due to the toxicity of the truncated protein. To test our hypothesis, we transfected human rhodopsin nonsense mutants into HEK-293T and HT-1080 human cells and measured transcript levels by qRT-PCR. In both cell lines, rhodopsin mutations Q64X and Q344X, which cause severe phenotypes that are dominantly inherited, yielded the same levels of rhodopsin mRNA as wild type. By contrast, rhodopsin mutations W161X and E249X, which cause recessive RP, showed decreased rhodopsin mRNA levels, consistent with NMD. Rhodopsin mutant Y136X, a dominant mutation that causes late-onset RP with a very mild pathology, also gave lower mRNA levels. Treatment of cells with Wortmannin, an inhibitor of NMD, eliminated the degradation of Y136X, W161X, and E249X rhodopsin mRNAs. These results suggest that NMD modulates the severity of RP in patients with nonsense mutations in the rhodopsin gene.

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Conflict of Interest

None

Keywords

Retinitis pigmentosa; Rhodopsin; Nonsense mutations; Nonsense-mediated decay; Patterns of inheritance

Retinitis pigmentosa (RP) is an inherited degenerative disease of the retina. Patients with RP initially start to lose peripheral and night vision, as rod photoreceptors degenerate. This early stage is followed by cone photoreceptor degeneration, leading to a loss of color vision and in some instances leaving the patients completely blind (Hartong et al., 2006). RP affects 1 in every 4000 individuals worldwide, with mutations in more than 40 different genes (Berger et al., 2010). Mutations in the rhodopsin gene account for ~30% of all RP cases. In fact, more than 150 mutations in rhodopsin cause RP (<http://www.sph.uth.tmc.edu/retnet/>; <http://www.hgmd.org>), making it the single most affected gene in this disease and a prime candidate for gene therapy (Chan et al., 2011; Wilson and Wensel, 2003).

Rhodopsin is a light-detecting G-protein coupled receptor expressed specifically in the rod photoreceptor cells of the retina. Its protein component, opsin, in combination with the chromophore, 11-*cis*-retinal, is responsible for efficient photon capture under dim light conditions. Upon capture of a photon, the chromophore is converted to all-*trans*-retinal, causing a conformational change in rhodopsin that activates the associated G-protein transducin, initiating the phototransduction cascade and ultimately triggering the sensation of vision. Rhodopsin is densely packed in the disks of rod outer segment, providing a critical structural component of rod photoreceptors, as well (Lem et al., 1999; Price et al., 2012). The intertwined roles of rhodopsin in rod cell structure and function may account for the large number of human rhodopsin mutations that have been found cause RP.

RP mutations in the rhodopsin gene include missense and nonsense mutations, insertions and deletions, as well as splice site mutations. These mutations can affect many aspects of rhodopsin function, including protein folding, post-translational modification, transport to the outer segment, and signaling capabilities. Nearly all mutations in rhodopsin cause dominant RP, with just five of more than 150 giving rise to recessive RP. Two of these recessive phenotypes are due to nonsense mutations W161X and E249X, which generate premature termination codons (PTCs) in the mRNA (Kartasasmita et al., 2011; Rosenfeld et al., 1992). But not all rhodopsin nonsense mutations cause recessive RP. Five rhodopsin nonsense mutations—Y60X, Q64X, Q312X, Q341X and Q344X—give rise to dominant RP (Cideciyan et al., 1998; Eisenberger et al., 2013; Macke et al., 1993; Sung et al., 1991; Zhao et al., 2001). In addition, nonsense mutation Y136X, although dominant, causes a very mild form of late onset RP (Sanchez et al., 1996). It is unclear whether the different phenotypes of these eight nonsense mutations result from varying toxicities of the truncated proteins or from the differential effects of nonsense-mediated decay (NMD) on the stability of the PTC-containing rhodopsin mRNAs.

NMD is the cell's quality control mechanism for degrading PTC-containing mRNA transcripts, which can produce potentially deleterious truncated proteins (Popp and Maquat, 2013). In several cases, NMD is known to modulate the severity of the disease phenotype and the inheritance pattern (Holbrook et al., 2004; Khajavi et al., 2006). In the classic

example, nonsense mutations in the β -globin gene that engage NMD give rise to a recessive form of β -thalassemia, whereas mutations that escape NMD can cause a dominant version of the disease (Chang and Kan, 1979; Hall and Thein, 1994). Although it is not possible to predict with certainty whether a particular PTC-mRNA will engage NMD, PTCs that are beyond a critical boundary (50–55 nucleotides in front of the last splice junction) typically escape NMD, whereas PTCs upstream of that boundary usually engage NMD (Nicholson et al., 2010).

For our studies, we chose to test Q344X, one of the three dominant mutations downstream of the critical boundary, and Q64X, Y136X, W161X and E249X, four of the five mutations upstream of the boundary. The phenotypes of these mutations—Q64X and Q344X are severe, whereas Y136X, W161X, and E249X are mild—do not match the simplest expectations for NMD based on their positions relative to the critical boundary. As we show, however, their phenotypes are congruent with actual measurements of NMD: Q64X and Q344X escape NMD, whereas Y136X, W161X and E249X engage NMD.

Because NMD occurs in the context of mRNA splicing, we used the full-length rhodopsin gene, including all exons and introns, to build test plasmids. We showed previously that a full-length rhodopsin gene in a similar construct was expressed and spliced normally in HT1080 cells (Intody et al., 2000). The full-length rhodopsin gene was synthesized by GeneScript in two pieces: segment *a* extends from the beginning of exon 1 to the midpoint of intron 2; segment *b* extends from the midpoint of intron 2 to the end of exon 5. Each segment was cloned into the *Hind*III site of the pUC57 plasmid. Four constructs were generated by GeneScript: p*Ra*, p*Ra*-W161X, p*Rb* and p*Rb*-E249X. We introduced the Y136X point mutation separately in the p*Ra* plasmid using the QuickChangeII site-directed mutagenesis kit (Agilent #200521) to generate plasmid p*Ra*-Y136X. Appropriate segments were assembled into full-length rhodopsin genes by recombineering, using the In-FusionHD kit (Clontech #638909), following the manufacturer's protocol. We used the high fidelity KOD Hot Start DNA polymerase (EMD Millipore, 71086) for all In-Fusion PCR reactions, following the manufacturer's instructions. In-Fusion primers were designed using Clontech's Primer Design Tool for In-Fusion and synthesized by Sigma-Aldrich. We assembled full length sequences by In-Fusion cloning: p*Rb* was cloned into p*Ra* to generate p*Rab*-WT, p*Rb* was cloned into p*Ra*-Y136X to generate p*Rab*-Y136X, p*Rb* was cloned to p*Ra*-W161X to generate p*Rab*-W161X, and p*Rb*-E249X was cloned into p*Ra* to generate p*Rab*-E249X. Full length rhodopsin genes were then moved by In-Fusion cloning to *Hind*III-linearized pcDNA3.1(+) (Invitrogen #V790-20), leading to plasmids: phRhoWT, phRhoY136X, phRhoW161X and phRhoE249X, each of which is driven by the cytomegalovirus (CMV) promoter and carries a downstream Neomycin gene flanked by an SV40 promoter and an SV40 poly A site (Figure 1A). Rhodopsin mutations Q64X and Q344X were generated on phRhoWT by ProNovus Bioscience, who used site-directed PCR mutagenesis to produce plasmids phRhoQ64X and phRhoQ344X. All constructs were sequenced by Baylor College of Medicine's DNA Sequencing Core and Lone Star Labs to confirm the presence of the target mutations.

Plasmids containing the normal β -globin gene and the 39Ter (Q39X) mutation were provided by Dr. Lynn Maquat (Zhang et al., 1998). The normal and mutant β -globin genes

were cloned into pcDNA3.1 so that they were flanked by a CMV promoter and a bovine growth hormone (BGH) poly A site (Figure 1B).

Two human cell lines were used. HEK293 cells were maintained in plates containing DMEM with 4.5 g/L D-glucose (GIBCO, 11965) supplemented with L-glutamine (GIBCO, 25030). HT1080 cells were maintained in plates containing DMEM/F-12 (GIBCO, 11320), supplemented with 1% MEM nonessential amino acids (GIBCO, 10370). All cells were maintained in fresh growth medium supplemented with 10% HyClone Fetal Bovine Serum (Thermo Scientific, SH30071.03HI) at 37°C in a humidified incubator with 5% CO₂ for the duration of the experiments.

Cells were transfected with 1 µg of plasmid per well of a six-well plate, using Lipofectamine 2000 (Invitrogen, 11668019) and following the manufacturer's protocol. Cells were trypsinized 48 hours after transfection and total RNA was extracted using a NucleoSpin RNA extraction kit (Macherey-Nagel, 740955), following the manufacturer's protocol, and supplemented with β-mercaptoethanol (Sigma-Aldrich).

Because NMD, once engaged, is known to be a rapid and quantitative process (Lejeune et al., 2003), it is standard practice to monitor it by measuring steady-state mRNA levels, and that is the approach we have taken, using quantitative RT-PCR. RNA quality and concentration were assessed using the Experion automated electrophoresis system with RNA StdSens chips (BioRad Laboratories, 700-7103), following the manufacturer's protocol. First strand synthesis was performed on 2 µg of total RNA per sample in a final volume of 20 µL, using the Omniscript RT kit (QIAGEN, 205111), following the manufacturer's instructions. We prepared the quantitative PCR reaction using the QuantiFast SYBR Green PCR Kit (QIAGEN, 204054) and 1 µL cDNA reaction per sample in a final volume of 25 µL, according to the manufacturer's protocol. PCR reactions contained 0.5 µM of each primer. Primer set 1 (5'-GTGAACGCTCCCGGCTTG and 5'-CCAGGTAGTACTGTGGGTAC) specifically amplified the human rhodopsin gene; primer set 2 (5'-GTGCCCTTAGTGATGGCCTG and 5'-GCCACCACCTTCTGGAAGG) specifically amplified the β-globin gene; and primer set 3 (5'-CTTTTCTGGATTCATCGACTGTG and 5'-CAAGAAGGCGATAGAAGGCG) specifically amplified the neomycin gene. As designed, each primer set amplified its target with 95–100% efficiency. The locations of these primer pairs are shown in Figure 1. Amplifications were performed on a CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories). The PCR cycling conditions were 5 minutes at 95°C, followed by 40 cycles of 95°C for 10 seconds and 60°C for 30 seconds. Following amplification, the temperature was ramped from 60°C to 95°C at a rate of 0.5°C every 5 seconds for a melt curve analysis. Each melt-curve analysis gave a single peak consistent with the melting of the expected PCR fragment.

Measurements of the levels of rhodopsin and β-globin mRNAs were obtained from two independent transfection experiments, each with three replicates. For each replicate, rhodopsin and β-globin mRNAs were measured relative to neomycin mRNA in the same sample. The relative levels of mRNA were calculated by comparing the number of cycles at which the PCR products became detectable above the basal threshold, which is defined as the crossover point (Livak and Schmittgen, 2001). The ratio of rhodopsin or globin mRNA

to neomycin mRNA was determined three times for each sample and averaged to obtain the mean for each replicate. The six means for each ratio determination were averaged and the standard deviation was calculated. In Figure 2, the data were plotted after normalizing to the mean ratio for wild-type rhodopsin/neomycin or wild-type β -globin/neomycin, which were defined as the controls. Statistical analyses of significance were conducted using Student's t-test to compare the mean and standard deviation of each experimental sample with the mean and standard deviation of the corresponding control, using GraphPad software.

To determine whether nonsense mutations in the rhodopsin gene can activate NMD, we compared rhodopsin transcript levels in human cells transfected with various forms of the human rhodopsin gene (Figure 1). To control for transfection efficiency, we measured rhodopsin mRNA relative to mRNA expressed from the neomycin gene on the same plasmid (Figure 1). We chose to test two different human cell lines—HEK293 and HT1080—both of which are competent to carry out NMD (Huang et al., 2012; Micale et al., 2009; Stoecklin et al., 2001). These cells were transfected with plasmids carrying the neomycin gene and a rhodopsin gene, and RNA was harvested 48 hours later. After conversion of mRNA to cDNA, we measured rhodopsin and neomycin transcript levels by quantitative reverse transcription PCR (qRT-PCR), using the primers indicated in Figure 1. In both cell lines, the Q64X and Q344X gave levels of rhodopsin mRNA that were indistinguishable from those obtained with wild type rhodopsin (Figure 2A). By contrast, Y136X, W161X, and E249X yielded significantly reduced levels of rhodopsin mRNA, approximately 40% lower than for wild type rhodopsin (Figure 2A). These observations suggest that the nonsense mutations Y136X, W161X, and E249X trigger mRNA degradation.

To confirm that HEK293 and HT1080 cells can indeed carry out NMD, we compared transcript levels for wild-type β -globin with those for the well-characterized NMD-inducing β -globin 39Ter mutation (Lejeune et al., 2003). As described above for rhodopsin, we used qRT-PCR to quantify globin mRNA relative to mRNA expressed from a neomycin gene on the same plasmid (Figure 1). As shown in Figure 2A, β -globin 39Ter yielded about 75–85% less globin mRNA than wild type. These results indicate that both cell lines are capable of robust degradation of PTC-containing mRNAs, consistent with active NMD. To verify that β -globin 39Ter mRNA degradation was due to NMD, we chemically inhibited a critical component of the NMD pathway, SMG1 kinase, using Wortmannin (Yamashita et al., 2001). In response to a ribosome stalled at a PTC, SMG1 kinase phosphorylates UPF1, which leads to recruitment of enzymes that degrade the mRNA (Lejeune et al., 2003; Popp and Maquat, 2014). We treated cells with 10 μ M Wortmannin (Sigma) 42 hours after transfection for 6 hours and then immediately isolated total RNA for analysis. As shown in Figure 2B, Wortmannin treatment substantially increased expression of globin mRNA by the 39Ter nonsense mutant, indicating, as expected, that the degradation observed in the absence of Wortmannin was due to NMD.

We also used Wortmannin treatment to test whether the reduction in mRNA levels observed for rhodopsin mutants Y136X, W161X, and E249X was also due to degradation by NMD. In both HEK293 and HT1080 cells, Wortmannin eliminated the difference between wild type rhodopsin mRNA levels and the mRNA levels in the nonsense mutants (Figure 2B).

These results indicate that the NMD pathway is responsible for the degradation of rhodopsin mRNA in the Y136X, W161X, and E249X nonsense mutants.

With the exception of Q64X, these results are in accord with the empirical rule that PTCs more than 50–55 nucleotides upstream of the last splice junction induce NMD, whereas PTCs downstream of that boundary do not. In the case of Q344X, we have data in a Q344X mouse knockin that shows that the level of Q344X mRNA is the same as that in a comparable wild type knockin (Sandoval et al., 2014). How Q64X manages to avoid NMD is unclear. In the β -globin and triosephosphate isomerase (TPI) genes, where NMD has been extensively studied, PTCs near the AUG start codon—within 23 codons for β -globin and within 10 codons for TPI—typically evade NMD (Peixeiro et al., 2012; Zhang and Maquat, 1997). In both cases, avoidance of NMD is associated with reinitiation of translation downstream of the PTC, at Met14 in TPI and Met55 in β -globin. Efficient reinitiation and avoidance of NMD depend on the length of the upstream open reading frame (ORF) created by the PTC and the presence of a downstream AUG. The efficiency of reinitiation decreases with increasing length of the upstream ORF (Kozak, 2001), and in one study it was predicted to decrease to zero when the upstream ORF reached 55 codons (Luukkonen et al., 1995). Thus, Q64X would seem to create an ORF that is too long for efficient reinitiation. Further experiments will be required to resolve the mechanism by which Q64X evades NMD.

The key finding of this study is the strong correlation between induction of NMD and disease phenotype. Nonsense mutations that induce NMD give rise to recessive RP (in the case of W161X and E249X) or to a dominant form with a mild phenotype (in the case Y136X). For Y136X and E249X, it has previously been speculated that NMD might account for their mild phenotypes (Rosenfeld et al., 1992; Sanchez et al., 1996). By contrast, Q64X and Q344X, which evade NMD, give rise to dominant RP. It is known that the mammalian retina, including photoreceptors, has a functional NMD pathway; in a mouse model of age-related retinal degeneration, a nonsense mutation in the *Mdm1* gene caused a 40–80% reduction in *Mdm1* mRNA via NMD (Chang et al., 2008). The 40% reduction in PTC-containing rhodopsin mRNA observed in cultured cells, if it also occurs in rod photoreceptor cells, would reduce the levels of the encoded protein fragments, potentially contributing to the relatively healthy retinas observed in patients with the Y136X, W161X, and E249X rhodopsin nonsense mutations.

The high level of expression of rhodopsin, which accounts for 70% of the protein in the rod outer segment, is often cited as the reason why such a large fraction of rhodopsin mutations (>95%) are dominant. Whether a 40% reduction in rhodopsin mRNA is enough to modulate the severity of RP greatly depends on how toxic each of the products encoded by Y136X, W161X, and E249X is to rod photoreceptors. NMD-induced mRNA decreases of 35–95% have been linked to recessive inheritance of the phenotype in genetic diseases such as Robinow syndrome, β -thalassemia, human hereditary complement C3 deficiency, Alpers syndrome, Ehlers-Danlos syndrome, Hallopeau-Siemens type recessive dystrophic epidermolysis bullosa, and pseudoxanthoma elasticum (Baserga and Benz, 1988; Ben-Shachar et al., 2009; Chan et al., 2005; Christiano et al., 1997; Hu et al., 2003; Inacio et al., 2004; Neu-Yilik et al., 2011; Reis et al., 2004; Romao et al., 2000; Schwarze et al., 2004). Without further studies in mice, however, we cannot be sure whether the mild phenotypes of

Y136X, W161X, and E249X are due primarily to NMD-induced reductions in rhodopsin mRNA, or to the lower intrinsic toxicity of their encoded protein fragments, or to some combination of the two.

It is possible that our knowledge of NMD of rhodopsin nonsense mutations could be exploited for gene therapy of RP patients. For example, drugs that promote ribosomal read-through of PTCs could in principle, be used to treat the dominant nonsense mutations Q64X, Q312X, Q341X, and Q344X, a strategy that has been studied for more than 40 different disease genes (Lee and Dougherty, 2012; Wang and Gregory-Evans, 2015) (Guerin et al., 2008). The major challenge of this approach is getting sufficient read-through to affect the disease phenotype. Would a 5–10% increase in full-length rhodopsin, and a corresponding decrease in the toxic fragment, be sufficient to ameliorate the phenotype in dominant diseases? Would a 5–10% increase be sufficient to improve the phenotype in recessive diseases? In neither case are the threshold levels of effective read-through known.

Alternatively, it may be possible to exploit NMD in a more general approach that would work for most, if not all, of the dominant rhodopsin mutations in the RP patient population. If a stop codon could be introduced into an NMD-sensitive region of a mutant rhodopsin gene, it may be possible to replace the deleterious mutant protein with low levels of a less toxic fragment. In principle, a stop codon could be introduced by homologous recombination with an appropriate fragment, by a nonhomologous end joining-induced shift in the reading frame after cutting with an engineered nuclease, or by incorporating an exon with a potent NMD-inducing PTC. Because of the difficulty in distinguishing the mutant gene from the normal one, this approach may require using a “kill and replace” strategy, in which both genes are targeted and a resistant rhodopsin gene is supplied at the same time (Hernan et al., 2012; Millington-Ward et al., 2011; Petrs-Silva et al., 2011). The main advantage of this approach is that a single set of proven reagents could be used to treat all dominant rhodopsin mutations.

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References

- Baserga SJ, Benz EJ Jr. Nonsense mutations in the human beta-globin gene affect mRNA metabolism. *Proc Natl Acad Sci U S A*. 1988; 85:2056–2060. [PubMed: 3353367]
- Ben-Shachar S, Khajavi M, Withers MA, Shaw CA, van Bokhoven H, Brunner HG, Lupski JR. Dominant versus recessive traits conveyed by allelic mutations - to what extent is nonsense-mediated decay involved? *Clin Genet*. 2009; 75:394–400. [PubMed: 19236432]
- Berger W, Kloeckener-Gruissem B, Neidhardt J. The molecular basis of human retinal and vitreoretinal diseases. *Prog Retin Eye Res*. 2010; 29:335–375. [PubMed: 20362068]
- Chan F, Hauswirth WW, Wensel TG, Wilson JH. Efficient mutagenesis of the rhodopsin gene in rod photoreceptor neurons in mice. *Nucleic Acids Res*. 2011; 39:5955–5966. [PubMed: 21478169]
- Chan SS, Longley MJ, Naviaux RK, Copeland WC. Mono-allelic POLG expression resulting from nonsense-mediated decay and alternative splicing in a patient with Alpers syndrome. *DNA Repair (Amst)*. 2005; 4:1381–1389. [PubMed: 16181814]

- Chang B, Mandal MN, Chavali VR, Hawes NL, Khan NW, Hurd RE, Smith RS, Davisson ML, Kopplin L, Klein BE, et al. Age-related retinal degeneration (arrd2) in a novel mouse model due to a nonsense mutation in the Mdm1 gene. *Hum Mol Genet.* 2008; 17:3929–3941. [PubMed: 18805803]
- Chang JC, Kan YW. beta 0 thalassemia, a nonsense mutation in man. *Proc Natl Acad Sci U S A.* 1979; 76:2886–2889. [PubMed: 88735]
- Christiano AM, Fine JD, Uitto J. Genetic basis of dominantly inherited transient bullous dermolysis of the newborn: a splice site mutation in the type VII collagen gene. *J Invest Dermatol.* 1997; 109:811–814. [PubMed: 9406826]
- Cideciyan AV, Hood DC, Huang Y, Banin E, Li ZY, Stone EM, Milam AH, Jacobson SG. Disease sequence from mutant rhodopsin allele to rod and cone photoreceptor degeneration in man. *Proc Natl Acad Sci U S A.* 1998; 95:7103–7108. [PubMed: 9618546]
- Eisenberger T, Neuhaus C, Khan AO, Decker C, Preising MN, Friedburg C, Bieg A, Gliem M, Charbel Issa P, Holz FG, et al. Increasing the yield in targeted next-generation sequencing by implicating CNV analysis, non-coding exons and the overall variant load: the example of retinal dystrophies. *PLoS One.* 2013; 8:e78496. [PubMed: 24265693]
- Guerin K, Gregory-Evans CY, Hodges MD, Moosajee M, Mackay DS, Gregory-Evans K, Flannery JG. Systemic aminoglycoside treatment in rodent models of retinitis pigmentosa. *Exp Eye Res.* 2008; 87:197–207. [PubMed: 18644591]
- Hall GW, Thein S. Nonsense codon mutations in the terminal exon of the beta-globin gene are not associated with a reduction in beta-mRNA accumulation: a mechanism for the phenotype of dominant beta-thalassemia. *Blood.* 1994; 83:2031–2037. [PubMed: 8161774]
- Hartong DT, Berson EL, Dryja TP. Retinitis pigmentosa. *Lancet.* 2006; 368:1795–1809. [PubMed: 17113430]
- Hernan I, Gamundi MJ, Borrás E, Maseras M, Garcia-Sandoval B, Blanco-Kelly F, Ayuso C, Carballo M. Novel p.M96T variant of NRL and shRNA-based suppression and replacement of NRL mutants associated with autosomal dominant retinitis pigmentosa. *Clin Genet.* 2012; 82:446–452. [PubMed: 21981118]
- Holbrook JA, Neu-Yilik G, Hentze MW, Kulozik AE. Nonsense-mediated decay approaches the clinic. *Nat Genet.* 2004; 36:801–808. [PubMed: 15284851]
- Hu X, Peek R, Plomp A, ten Brink J, Scheffer G, van Soest S, Leys A, de Jong PT, Bergen AA. Analysis of the frequent R1141X mutation in the ABCC6 gene in pseudoxanthoma elasticum. *Invest Ophthalmol Vis Sci.* 2003; 44:1824–1829. [PubMed: 12714611]
- Huang X, Tian M, Hernandez CC, Hu N, Macdonald RL. The GABRG2 nonsense mutation, Q40X, associated with Dravet syndrome activated NMD and generated a truncated subunit that was partially rescued by aminoglycoside-induced stop codon read-through. *Neurobiol Dis.* 2012; 48:115–123. [PubMed: 22750526]
- Inacio A, Silva AL, Pinto J, Ji X, Morgado A, Almeida F, Faustino P, Lavinha J, Liebhaber SA, Romao L. Nonsense mutations in close proximity to the initiation codon fail to trigger full nonsense-mediated mRNA decay. *J Biol Chem.* 2004; 279:32170–32180. [PubMed: 15161914]
- Intody Z, Perkins BD, Wilson JH, Wensel TG. Blocking transcription of the human rhodopsin gene by triplex-mediated DNA photocrosslinking. *Nucleic Acids Res.* 2000; 28:4283–4290. [PubMed: 11058128]
- Kartasmita A, Fujiki K, Iskandar E, Sovani I, Fujimaki T, Murakami A. A novel nonsense mutation in rhodopsin gene in two Indonesian families with autosomal recessive retinitis pigmentosa. *Ophthalmic Genet.* 2011; 32:57–63. [PubMed: 21174529]
- Khajavi M, Inoue K, Lupski JR. Nonsense-mediated mRNA decay modulates clinical outcome of genetic disease. *Eur J Hum Genet.* 2006; 14:1074–1081. [PubMed: 16757948]
- Kozak M. Constraints on reinitiation of translation in mammals. *Nucleic Acids Res.* 2001; 29:5226–5232. [PubMed: 11812856]
- Lee HL, Dougherty JP. Pharmaceutical therapies to recode nonsense mutations in inherited diseases. *Pharmacol Ther.* 2012; 136:227–266. [PubMed: 22820013]
- Lejeune F, Li X, Maquat LE. Nonsense-mediated mRNA decay in mammalian cells involves decapping, deadenylating, and exonucleolytic activities. *Mol Cell.* 2003; 12:675–687. [PubMed: 14527413]

- Lem J, Krasnoperova NV, Calvert PD, Kosaras B, Cameron DA, Nicolo M, Makino CL, Sidman RL. Morphological, physiological, and biochemical changes in rhodopsin knockout mice. *Proc Natl Acad Sci U S A*. 1999; 96:736–741. [PubMed: 9892703]
- Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods*. 2001; 25:402–408. [PubMed: 11846609]
- Luukkainen BG, Tan W, Schwartz S. Efficiency of reinitiation of translation on human immunodeficiency virus type 1 mRNAs is determined by the length of the upstream open reading frame and by intercistronic distance. *J Virol*. 1995; 69:4086–4094. [PubMed: 7769666]
- Macke JP, Davenport CM, Jacobson SG, Hennessey JC, Gonzalez-Fernandez F, Conway BP, Hecklenlively J, Palmer R, Maumenee IH, Sieving P, et al. Identification of novel rhodopsin mutations responsible for retinitis pigmentosa: implications for the structure and function of rhodopsin. *Am J Hum Genet*. 1993; 53:80–89. [PubMed: 8317502]
- Micale L, Muscarella LA, Marzulli M, Augello B, Tritto P, D'Agruma L, Zelante L, Palumbo G, Merla G. VHL frameshift mutation as target of nonsense-mediated mRNA decay in *Drosophila melanogaster* and human HEK293 cell line. *J Biomed Biotechnol*. 2009; 2009:860761. [PubMed: 20145706]
- Millington-Ward S, Chadderton N, O'Reilly M, Palfi A, Goldmann T, Kilty C, Humphries M, Wolfrum U, Bennett J, Humphries P, et al. Suppression and replacement gene therapy for autosomal dominant disease in a murine model of dominant retinitis pigmentosa. *Mol Ther*. 2011; 19:642–649. [PubMed: 21224835]
- Neu-Yilik G, Amthor B, Gehring NH, Bahri S, Paidassi H, Hentze MW, Kulozik AE. Mechanism of escape from nonsense-mediated mRNA decay of human beta-globin transcripts with nonsense mutations in the first exon. *RNA*. 2011; 17:843–854. [PubMed: 21389146]
- Nicholson P, Yepiskoposyan H, Metze S, Zamudio Orozco R, Kleinschmidt N, Muhlemann O. Nonsense-mediated mRNA decay in human cells: mechanistic insights, functions beyond quality control and the double-life of NMD factors. *Cell Mol Life Sci*. 2010; 67:677–700. [PubMed: 19859661]
- Peixeiro I, Inacio A, Barbosa C, Silva AL, Liebhaber SA, Romao L. Interaction of PABPC1 with the translation initiation complex is critical to the NMD resistance of AUG-proximal nonsense mutations. *Nucleic Acids Res*. 2012; 40:1160–1173. [PubMed: 21989405]
- Petrs-Silva H, Yasumura D, Matthes MT, LaVail MM, Lewin AS, Hauswirth WW. Suppression of rds expression by siRNA and gene replacement strategies for gene therapy using rAAV vector. *Adv Exp Med Biol*. 2011; 723:215–223. [PubMed: 22183336]
- Popp MW, Maquat LE. Organizing principles of mammalian nonsense-mediated mRNA decay. *Annu Rev Genet*. 2013; 47:139–165. [PubMed: 24274751]
- Popp MW, Maquat LE. The dharma of nonsense-mediated mRNA decay in mammalian cells. *Mol Cells*. 2014; 37:1–8. [PubMed: 24552703]
- Price BA, Sandoval IM, Chan F, Nichols R, Roman-Sanchez R, Wensel TG, Wilson JH. Rhodopsin gene expression determines rod outer segment size and rod cell resistance to a dominant-negative neurodegeneration mutant. *PLoS One*. 2012; 7:e49889. [PubMed: 23185477]
- Reis ES, Nudelman V, Isaac L. Nonsense-codon-mediated decay in human hereditary complement C3 deficiency. *Immunogenetics*. 2004; 55:667–673. [PubMed: 14639503]
- Romao L, Inacio A, Santos S, Avila M, Faustino P, Pacheco P, Lavinha J. Nonsense mutations in the human beta-globin gene lead to unexpected levels of cytoplasmic mRNA accumulation. *Blood*. 2000; 96:2895–2901. [PubMed: 11023527]
- Rosenfeld PJ, Cowley GS, McGee TL, Sandberg MA, Berson EL, Dryja TP. A null mutation in the rhodopsin gene causes rod photoreceptor dysfunction and autosomal recessive retinitis pigmentosa. *Nat Genet*. 1992; 1:209–213. [PubMed: 1303237]
- Sanchez B, Borrego S, Chaparro P, Rueda T, Lopez F, Antinolo G. A novel null mutation in the rhodopsin gene causing late onset autosomal dominant retinitis pigmentosa. *Hum Mutat*. 1996; 7:180.
- Sandoval IM, Price BA, Gross AK, Chan F, Sammons JD, Wilson JH, Wensel TG. Abrupt onset of mutations in a developmentally regulated gene during terminal differentiation of post-mitotic photoreceptor neurons in mice. *PLoS One*. 2014; 9:e108135. [PubMed: 25264759]

- Schwarze U, Hata R, McKusick VA, Shinkai H, Hoyme HE, Pyeritz RE, Byers PH. Rare autosomal recessive cardiac valvular form of Ehlers-Danlos syndrome results from mutations in the COL1A2 gene that activate the nonsense-mediated RNA decay pathway. *Am J Hum Genet.* 2004; 74:917–930. [PubMed: 15077201]
- Stoecklin G, Stoeckle P, Lu M, Muehlemann O, Moroni C. Cellular mutants define a common mRNA degradation pathway targeting cytokine AU-rich elements. *RNA.* 2001; 7:1578–1588. [PubMed: 11720287]
- Sung CH, Davenport CM, Hennessey JC, Maumenee IH, Jacobson SG, Heckenlively JR, Nowakowski R, Fishman G, Gouras P, Nathans J. Rhodopsin mutations in autosomal dominant retinitis pigmentosa. *Proc Natl Acad Sci U S A.* 1991; 88:6481–6485. [PubMed: 1862076]
- Wang X, Gregory-Evans CY. Nonsense suppression therapies in ocular genetic diseases. *Cell Mol Life Sci.* 2015
- Wilson JH, Wensel TG. The nature of dominant mutations of rhodopsin and implications for gene therapy. *Mol Neurobiol.* 2003; 28:149–158. [PubMed: 14576453]
- Yamashita A, Ohnishi T, Kashima I, Taya Y, Ohno S. Human SMG-1, a novel phosphatidylinositol 3-kinase-related protein kinase, associates with components of the mRNA surveillance complex and is involved in the regulation of nonsense-mediated mRNA decay. *Genes Dev.* 2001; 15:2215–2228. [PubMed: 11544179]
- Zhang J, Maquat LE. Evidence that translation reinitiation abrogates nonsense-mediated mRNA decay in mammalian cells. *EMBO J.* 1997; 16:826–833. [PubMed: 9049311]
- Zhang J, Sun X, Qian Y, Maquat LE. Intron function in the nonsense-mediated decay of beta-globin mRNA: indications that pre-mRNA splicing in the nucleus can influence mRNA translation in the cytoplasm. *RNA.* 1998; 4:801–815. [PubMed: 9671053]
- Zhao K, Xiong S, Wang L, Cui Y, Wang Q. Novel rhodopsin mutation in a Chinese family with autosomal dominant retinitis pigmentosa. *Ophthalmic Genet.* 2001; 22:155–162. [PubMed: 11559857]

Highlights

- Dominant nonsense mutations Q64X and Q344X do not induce NMD
- Recessive nonsense mutations W161X, and E249X induce NMD
- Weakly dominant nonsense mutation Y136X induces NMD
- Wortmannin treatment, which inhibits NMD, eliminates Y136X, W161X, and E249X mRNA degradation
- Inheritance patterns for rhodopsin nonsense mutations correlate with induction of NMD

Figure 1.

Constructs used in transfections to assess NMD. **A.** Rhodopsin-Neomycin constructs. The full-length human rhodopsin gene was included in all rhodopsinneomycin constructs. The coding regions of the rhodopsin and neomycin genes are shown as black boxes. The locations of the nonsense mutations used in this study are indicated above the exons of the rhodopsin gene. **B.** β-Globin-Neomycin constructs. The β-globin coding regions are shown as black boxes, with the position of the nonsense mutation 39Ter indicated. Transcription of the β-globin genes and the various versions of the rhodopsin gene is driven by the cytomegalovirus (CMV) promoter and terminated at a bovine growth hormone poly A site (BGHpA). Transcription of the Neomycin genes in both types of construct is driven by the SV40 promoter and terminated at an SV40 poly A site (SV40pA). The locations of primers used in real-time RT-PCR are shown as horizontal arrows below the genes. Primer pair 1 is specific for the human rhodopsin gene; pair 2, for β-globin; pair 3, for the neomycin gene.

A HEK293 Cells

HT1080 Cells

B HEK293 Cells (Wortmannin)

HT1080 Cells (Wortmannin)

Figure 2.

Expression of nonsense mutations of rhodopsin and β -globin in cultured human cells in the absence or presence of Wortmannin. **A.** Expression in HEK293 cells and in HT1080 cells in the absence of Wortmannin. **B.** Expression in Wortmannin-treated HEK293 cells and in Wortmannin-treated HT1080 cells. In all cases, mRNA expression in the transfected nonsense mutants was normalized to expression in the transfected wild type counterpart, which was defined at 100%. Error bars represent standard deviations. Statistical significance was determined for nonsense mutants relative to their wild type counterpart; significance was assessed by Student's two-tailed *t* test: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.