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Mutations in the RNA Granule Component TDRD7 Cause Cataract and Glaucoma

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The precise transcriptional regulation of gene expression is essential for vertebrate development, but the role of posttranscriptional regulatory mechanisms is less clear. Cyttoplasmic RNA granules (RGs) function in the posttranscriptional control of gene expression, but the extent of RG involvement in organogenesis is unknown. We describe two human cases of pediatric cataract with loss-of-function mutations in TDRD7 and demonstrate that Tdrd7 nullizygosity in mouse causes cataracts, as well as glaucoma and an arrest in spermatogenesis. TDRD7 is a Tudor domain RNA binding protein that is expressed in lens fiber cells in distinct TDRD7-RG granules, which interact with Staufen1-ribonucleoprotein (RNP) particles and accumulate SGs in response to environmental stress. These findings demonstrate a role for RGs in vertebrate organogenesis.

In eukaryotic cells, cytoplasmic RNA granules (RGs) function in determining whether mRNAs undergo degradation, stabilization, or intracellular localization (1–3). Lower eukaryotes such as yeast harbor RGs that are classified as either processing bodies (PBs) or stress granules (SGs) (3), whereas metazoan cells harbor additional classes of RGs (4–7). Somatic cells contain both PBs and transport ribonucleoprotein (RNP) particles and accumulate SGs in response to environmental stress (8, 9). PBs contain components of mRNA decay processes like Xrn1-mediated 5′ to 3′ degradation, nonsense-mediated decay (NMD), and microRNA-mediated silencing, and serve as sites where mRNAs can either be stored or degraded (10). In neuronal cells and fibroblasts, RNPs function in the transport and localized translation of mRNAs involved in synapse formation or motility (8, 11). SGs store bulk mRNA during conditions of stress and can interact with PBs to exchange mRNAs that are then directed to translational reinitiation or degradation (9, 12). Lastly, germ cells contain germ cell-specific granules (GCCs) that have been implicated in germ cell specification (2, 7).

Proteins with roles in development have been associated with RGs (13), but the importance of this association and that of somatic cell RGs in metazoan organogenesis remains unknown. For example, the precise spatial and temporal expression of key transcription factors is essential for normal transcriptional regulation during development. However, whether a similar level of developmental control exists over the expression of factors involved in posttranscriptional mRNA regulation, and whether this control is required for organogenesis, is unclear. We report the identification of Tudor domain-containing 7 protein, or TDRD7, as an RG component with a highly enriched and conserved pattern of developmental expression in the vertebrate ocular lens. In lens cells, we identify TDRD7 as a component of a unique class of RNP complexes and show that these TDRD7-RNA granules (TDRD7-RGs) differentially associate with lens PBs and RNP complexes that contain a known RG component, Staufen1 (STAU1-RNPs). Furthermore, human TDRD7 mutations result in cataract formation via the misregulation of specific developmental critical lens transcripts, and Tdrd7 null mutant mice develop cataract as well as glaucoma, the latter defined by elevated intraocular pressure (IOP) and optic nerve damage.

The study of patients with balanced chromosomal rearrangements represents an important entry point to understanding disease mechanisms, and numerous examples exist of disease genes that have been identified by virtue of being disrupted by rearrangement breakpoints (14). As part of the Developmental Genome Anatomy Project (DGAP) (www.bwpathology.org/dgap), we ascertained a male patient, designated DGAP186, with juvenile cataract and hypospadias who has a de novo balanced paracentric inversion of chromosome 9, 46,XY,inv(9)(q22.33q34.11) (Fig. 1, A and B).

We determined that the 9q34.11 breakpoint disrupts the gene NRSF1 (Fig. 1). NRSF1 encodes a steroid nuclear receptor protein implicated in hypospadias in mouse and human mutants without cataract (15–17). NRSF1 disruption is therefore likely to explain the reproductive tract phenotype and unlikely to account for the cataract phenotype in DGAP186. Analysis of the 9q22.33 breakpoint revealed that TDRD7 is disrupted (Fig. 1, B and C, and fig. S1), and TDRD7 haploinsufficiency as a direct result of the allelic disruption was observed in DGAP186 lymphoblastoid cells at both the RNA and protein levels (Fig. 1D and fig. S1). To independently confirm the involvement of TDRD7 in pediatric cataract, we identified a family, F3R, with autosomal recessive congenital cataract. Homozygosity mapping (18) identified a single block of shared homozygosity between the four affected siblings that spanned the TDRD7 locus (Fig. 1E and fig. S2). Furthermore, bidirectional sequencing of TDRD7 uncovered a novel in-frame 3–base pair deletion that removes a highly conserved amino acid, V618 (Fig. 1F and fig. S2). This V618del variant, which was not detected in 320 ethnically matched controls (640 chromosomes), is predicted to disrupt the structure of TDRD7 and is therefore likely to represent a loss-of-function mutation (fig. S2). To assay the endogenous expression of TDRD7, we turned to mouse and chick embryos. In situ hybridization revealed strong and highly specific expression of Tdrd7 transcripts in the developing mouse lens (Fig. 2A and fig. S3). At embryonic day E12.5, Tdrd7 is expressed in differentiating fiber cells in the posterior lens, whereas the anterior epithelium of the lens (AEL) lacks detectable expression. Expression of chick TDRD7 is also high in the developing lens (Fig. 2B). To confirm a direct causal link between TDRD7 haploinsufficiency and cataract formation, we used a replication-competent avian sarcoma (RCAS) viral vector (19) to deliver short hairpins that

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specifically targeted chick *TDRD7* to achieve RNA interference–mediated gene knockdown. *TDRD7*-knockdown short hairpin–mediated RNA interference (shRNA) retrovirus were injected into E2 (Hamburger and Hamilton stage 11 (HH st. 11)) optic vesicles, and the embryos were analyzed for the presence of cataract at E16 (HH st. 42). Injections of green fluorescent protein–expressing control RCAS virus led to highly efficient uptake by lens cells (fig. S3). At E16, a cataract phenotype was observed at significant frequency (17/115, 15%) in chick embryos injected with *TDRD7*-shRNA RCAS virus but not in those that received control virus (1/48, 2%; P < 0.05) (fig. 2C and fig. S3). Quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis indicated that cataract formation was observed in lenses in which *TDRD7* transcripts were reduced to approximately 40% or less of control levels (fig. S3).

To gain further insight into *TDRD7* function, we analyzed mutant mice with an N-ethyl-N-nitrosourea (ENU)–induced recessive mutation in *Tdrd7*. These mice, identified during a screen for glaucoma phenotypes, develop cataracts and high IOP. A nonsense mutation c.2187C>T (Q723X) was found in *Tdrd7* null allele, as determined by western blot to of DGP 186lymphoblast cell. (*Westen blot of DGP 186lymphoblast cell.*)

We analyzed mutant mice with an N-ethyl-N-nitrosourea (ENU)–induced recessive mutation in *Tdrd7*. These mice, identified during a screen for glaucoma phenotypes, develop cataracts and high IOP. A nonsense mutation c.2187C>T (Q723X) produces a *Tdrd7* null allele, as determined by the absence of *TDRD7* protein in homozygous mutants (fig. S4). Within 4 weeks of birth, all *Tdrd7* homozygous mutants developed a posterior cataract that became severe with age (Fig. 2, D and E). At later stages, the lens fiber cell compartment developed vacuoles with lens capsule rupture and extrusion of fiber cell mass into the vitreous (Fig. 2, F and G). This feature of the mouse lens phenotype precisely recapitulates the unusual posterior lenticusen (a conical projection of the lens surface) and posterior capsule defects observed in the DGAP186 proband (15). In addition, in some *Tdrd7* mutant mice, the mass of fiber cells passed through the pupil into the anterior chamber of the eye (fig. S4). By 4 months of age, iris flattening was detected and anterior chamber depth increased (fig. S5). By 6 months of age, the IOP was elevated in some mutants, and the incidence of elevated IOP increased with age (Fig. 2H and fig. S5). The ocular drainage structures Schlemm’s canal and the trabecular meshwork normally influence IOP and are located in the angle of the anterior chamber—the angle formed between the iris and the cornea, where the aqueous humor flows out of the anterior chamber. If the egress of aqueous humor is impeded, the accumulated fluid leads to increased IOP, which in turn contributes to retinal ganglion cell (RGC) death and optic nerve atrophy, all of which are hallmarks of glaucoma. In *Tdrd7* mutants, the sizes are largely normal in morphology with an open-angle configuration, defined by absence of morphologic obstruction (Fig. 2, I and J); the open-angle configuration also predominates in human glaucoma. In *Tdrd7* mutant mice, severe optic nerve atrophy characterized by RGC axon loss and excitotoxic remodeling of the optic nerve were observed (Fig. 2, K to N, and fig. S5). Notably, in family F3R, two of the four affected individuals developed glaucoma with open angles and increased IOP after cataract extraction, pointing to the *Tdrd7* mutant mouse as a potential model for certain aspects of human glaucoma.

*TDRD7* contains five conserved Tudor class domains and three OST-HTH (Oskar-TDRD7-Helix-Turn-Helix)/LOTUS domains (Fig. 1B), which bind methylated arginine residues and RNA, respectively (20). TDRD1, TDRD6, and TDRD7 have been associated with a GCG known as the chromotid body (CB) that is found in mammalian male germ cells (21, 22). To gain insight into *TDRD7*’s cellular function, we generated a mouse *TDRD7* antibody and analyzed protein expression during mouse embryogenesis. *TDRD7* expression between E11.0 and E12.5 becomes markedly enriched in lens fiber cells, where it is expressed in numerous cytoplasmic granules of 0.3 to 0.8 μm diameter (Fig. 3, A and B, and fig. S6). A similar pattern was observed with a second independent *TDRD7* antibody (fig. S6). *TDRD7* granules were also found in differentiating secondary fiber cells in postnatal day 1 (P1) lens (fig. S6). To determine whether these *TDRD7* positive granules contained RNA, we stained mouse E12.5 lens sections with SYTO RNASelect or Pyronin Y, two RNA-specific stains (Fig. 3C and fig. S7). Co-staining with *TDRD7* antibody revealed that *TDRD7* granules colocalize with RNA in lens fiber cell cytoplasm, and thus constitute bona fide RGs, denoted TDRD7-RGs (Fig. 3, C to E).

To establish whether *TDRD7* is associated with a specific class of RGs in mouse embryonic lens, we examined the expression of protein markers for different classes of somatic cell RGs (23). Immunostaining of mouse embryonic lens sections with antibodies against the PB markers DCP1A and Ge-1 (23) demonstrated the presence of numerous cytoplasmic PBs in lens fiber cells (Fig. 3F and fig. S8). These structures were also found to contain DX6/RCK and were dissociated by cycloheximide (CHX) (fig. S9), indicating that they are functional PBs. Co-staining of these sections with *TDRD7* antibody demonstrated that *TDRD7*-RGs interact with PBs (Fig. 3F and fig. S8). In addition to colocalization, PBs and *TDRD7*-RGs were occasionally juxtaposed to each other.

[Fig. 1. *TDRD7* mutations in human pediatric cataract. (A) Cataract in DGAP186 (left eye, white arrowhead). (B) Ideogram of normal and inverted chromosome 9 [inv(9)]. Inversion breakpoints are denoted by purple boxes. (C) Chromosomal spread of DGAP186 lymphoblastoid cells analyzed by fluorescence in situ hybridization shows split *TDRD7*-specific red probe, whereas green anchor probe remains intact in inv(9). White arrowheads indicate inverted genomic region that hybridizes to *TDRD7*-specific probe; white arrow represents noninverted region (see fig. S1). (D) *TDRD7* haploinsufficiency is demonstrated by Western blot of DGAP186 lymphoblastoid cells. (E) Pedigree of consanguineous family F3R with congenital cataract (solid symbols, affected status; half-solid symbols, carrier status). (F) Sequence chromatogram shows c.1852_1854del (p.617delVal) mutation (boxed) in family F3R (see fig. S2).]
in an arrangement similar to the docking-type interactions observed for DCP1A and STAU1-RNPs in neurons (fig. S8) (24). Quantification of both colocalizing and docking configurations between TDRD7 granules and PBs indicated a modest but significant degree of overall interaction (12 ± 4%, mean ± SEM, n = 250 PBs; P < 1.5 × 10⁻⁵, compared to a model of random distribution throughout the lens (15)).

STAU1 and STAU2, mammalian homologs of the Drosophila RNA-binding protein Staufen, are components of transport RNPs (25–27). Staining with STAU1 antibody revealed the presence of numerous STAU1-positive RNPs in lens fiber cells (Fig. 3G). These colocalized to a high degree (29 ± 5%, mean ± SEM, n = 258 granules) with TDRD7 (Fig. 3G and fig. S10). Expression of TDRD7 and STAU1 proteins could be detected as early as E10.5 at the lens vesicle stage (fig. S10). When tested at E11.5, the extent of TDRD7-STAU1 colocalization was highest along the anterior edge of the elongating fiber cell compartment (Fig. 3H), which apposes the AEL at later developmental stages. Similar results were obtained with the second TDRD7 antibody (fig. S10). We also tested for other known components of RGs, namely TIA-1, TIAR1 (TIAR), STAU2, and HuR (ELAVL1) (23), and found that these were not components of mouse lens RGs at E12.5 (fig. S11).

Previous studies have indicated that RGs dynamically interact with each other, and they may share components or participate in mRNA exchange (12). STAU1 is especially relevant, as it is a component of all four RG classes and functions in diverse aspects of RNA metabolism, including mRNA transport and mRNA decay (24, 28, 29). We therefore hypothesized that TDRD7 granules, either alone or through their interaction with STAU1-RNPs and PBs, might regulate the number of RGs in the cell as well as the expression levels of specific lens transcripts (fig. S12). We further postulated that the misregulation of specific mRNAs due to reduced TDRD7 dosage might cause cataract formation. To test these hypotheses, we first performed TDRD7 knockdowns in a U2OS cell line–based assay previously developed to identify genes critical for RG formation (13). These experiments revealed that Tdrd7 knockdown led to significant reductions in the numbers of SGs and PBs (fig. S13). To determine whether this effect was conserved in lens cell lines with higher numbers of SGs and PBs, we examined the human lens cell line SRA01.04 and quantified the results. In these cells, TDRD7-knockdown produced a dramatic reduction in SGs, indicating that TDRD7 is critical for an appropriate response of these cells to stressful stimuli (Fig. 4A and fig. S14). In contrast, TDRD7 knockdown produced only minimal effects on PB numbers. Statistically significant reductions in SG numbers were also observed in Tdrd7-null mutants and littermate controls, ~6% of expressed genes were differentially regulated at a threshold of 1.3-fold or greater (table S1). Of particular interest, key genes that encode SG and PB components (G3bp, Hspb1, and Ddx6), mouse homologs of known human cataract genes (Crygs and Eph2a), and genes involved in fiber cell differentiation (Proxl) were all significantly downregulated (Fig. 5A and fig. S15).

To extend these results in vivo, we further analyzed the Tdrd7 null mouse lens. As expected from Western blot analyses (fig. S4), immunofluorescence confirmed a complete absence of TDRD7 in the lens (Fig. 4B). We then undertook a microarray expression analysis of isolated lenses from Tdrd7 null mutants and littermate controls, focusing on postnatal days P4, three weeks before overt cataract appearance, and P30, when...
cataракты are fully penetrant. To accurately identify Tdrd7-dependent genes that function in lens development, we compared the P4 and P30 Tdrd7 mutant mouse lens microarray data with that obtained from the Tdrd7-KD 21EM15 mouse lens cell line. Comparison of differentially regulated genes (DRGs) from the P4 and P30 Tdrd7 null lens microarray data sets (each versus control), and the DRGs in Tdrd7-KD 21EM15 cells (versus control) identified several biologically relevant genes that were concordantly regulated in all three data sets (Fig. 5, A and B). These DRGs were classified into five distinct functional categories, denoted Classes I to V: Class I, genes involved in 5G assembly or function; Class II, genes involved in P-body function and/or encoding helicases; Class III, genes linked to cataract or other ocular phenotypes; Class IV, crystallin genes (apart from Class III); and Class V, genes normally down-regulated during lens fiber cell differentiation (Fig. 5A). Whereas Tdrd7 null lenses and Tdrd7-KD 21EM15 lens cells exhibit overlapping expression, each also exhibits unique categories of gene expression (e.g., Classes IV and V, respectively), and thus constitute complementary systems for revealing the full spectrum of DRGs attributable to Tdrd7 loss-of-function.

Of 12 DRGs whose expression was significantly altered in Tdrd7-KD 21EM15 lens cells and that were relevant to lens development or RG function, six (Hspb1, Ddx6, Ddx26, Eph2a, Prox1, and Crygg) were also concordantly down-regulated in Tdrd7 null lens data sets. Two other genes that were down-regulated in both P4 and P30 Tdrd7 null lenses, Sparc and Crybb3, are associated with cataracts and are thus also of interest (31, 32) (Fig. 5B). Thus, from comparative analyses of the microarray data, we identified eight genes that were significantly down-regulated in the Tdrd7 null lens, Tdrd7-KD 21EM15 lens cells, or both, and that have biologically plausible links to lens development or RG function.

To determine whether the altered expression of these eight genes resulted from the direct or indirect action of TDRD7, we used qRT-PCR to confirm the microarray expression changes in P30 Tdrd7 null lens RNA. This analysis confirmed the down-regulation of Crybb3, Hspb1, Sparc, and Eph2a and of several other genes (fig. S15). We then performed RNA immunoprecipitation (RIP) experiments with TDRD7 antibody, followed by RNA isolation and RT-PCR. Because 21EM15 cells substantially recapitulate the expression profile of the developing lens, and there is considerable concordance in DRGs between the corresponding Tdrd7 loss-of-function state, we employed 21EM15 lens cells for these experiments. Crybb3 and Hspb1 transcripts were markedly enriched in the TDRD7 immunoprecipitations, whereas Eph2a transcripts were only modestly enriched and Sparc transcripts were not enriched (Fig. 5C). Thus, based on their strong Tdrd7-expression dependence and evidence for direct binding from the RIP experiments, we conclude that Crybb3 and Hspb1 mRNAs are likely to be direct targets of TDRD7 regulatory function in the lens.

An attractive model emerges from these results. During lens development, AEL cells migrate into the lens “equatorial zone,” where they differentiate into fiber cells. During fiber cell differentiation, because of the nuclear degradation process that helps promote lens clarity, most AEL-expressed genes cease transcription. By binding and stabilizing specific transcripts, TDRD7 and other RG components may facilitate the translation of crystallin mRNAs necessary to achieve the high protein levels and tight packing that provides for ocular transparency at high refractive index. TDRD7 also functions by maintaining mRNA expression levels of the heat shock gene Hspb1, which encodes a stress response chaperone protein that is a stress granule component that functions in mRNA decay (33, 34). Down-regulation of Hspb1 mRNA levels is one of the earliest and quantitatively most striking gene expression changes we detect in Tdrd7 null lenses.

HSPB1 down-regulation at the protein level was detected in P4 and P22 lenses (Fig. 4B and fig. S15). Interestingly, HSPB1 also interacts with several lens crystallin proteins (33) and stabilizes B-crystallin (36). Moreover, destabilizing mutations in B-crystallin are a known cause of congenital cataract (OMIM ID 123590). Thus, Crybb3 and Hspb1 are excellent candidates to contribute to cataract formation in Tdrd7 loss-of-function mutants.

TDRD7 may also regulate certain transcripts indirectly. For example, TDRD7 deficiency results in reduction of Sparc transcripts, and Sparc null mice develop late-onset cataracts that resemble Tdrd7 null mutant cataracts (31). These results place Tdrd7 function upstream of Sparc mRNA expression and, because Tdrd7 is involved in the regulation of multiple cataract genes (fig. S15), can explain the earlier cataract onset in Tdrd7 mutants compared with Sparc mutants. In addition, Sparc and Eph2a mRNAs have been described as direct targets of STAU1-RNPs, and
Epha2 mutations cause cataracts in both mouse and human (26, 37). Because STAU1-NPs and TDRD7-RGs interact in the lens, Sparc and Epha2 down-regulation in Tdrd7 mutant lenses may reflect the disruption of this interaction between distinct RNPs and provide an additional mechanism of action for TDRD7 in the lens. A model encompassing both direct and indirect mechanisms of TDRD7 action in the developing lens is depicted in Fig. 5D.

In sum, TDRD7 is an RNA granule component that is highly enriched in the developing lens; TDRD7 perturbation in chick, mouse, and human causes cataract. In the lens, TDRD7-RGs play an essential role in the regulation of specific genes that are critical for lens development, including those responsive to stress, such as Hspb1. Tdrd7 deficiency distorts this regulatory mechanism and leads to cataract. The RNA granule function of TDRD7 may also be deployed in other tissues and cell types at various developmental stages. For example, TDRD7 is a component of a testis-specific RNA granule, the chromatoid body (CB) (21), and we observe that Tdrd7 null mice exhibit male sterility due to an arrest in spermatogenesis at the round spermatid stage, likely due to a CB defect (fig. S16). Lastly, Tdrd7 null mice develop elevated IOP and other features of glaucoma as they age. IOP elevation and glaucoma can result from oxidative and other stresses that...
damage the aqueous humor drainage tissues (38). It is possible that the IOP elevation in Tdrd7 null mice and in the two human patients who developed glaucoma results, at least in part, from abnormalities in the protective stress response or in other TDRD7-related functions in the drainage tissues. Thus, the data demonstrate that human organogenesis defects can result from perturbation of a distinct, tissue-specific RG component that posttranscriptionally regulates the levels of developmentally critical mRNAs.

References and Notes
15. Materials and methods are available as supporting material on Science Online.
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Reports

Baryons at the Edge of the X-ray–Brightest Galaxy Cluster

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Studies of the diffuse x-ray–emitting gas in galaxy clusters have provided powerful constraints on cosmological parameters and insights into plasma astrophysics. However, measurements of the faint cluster outskirts have become possible only recently. Using data from the Suzaku x-ray telescope, we determined an accurate, spatially resolved census of the gas, metals, and dark matter out to the edge of the Perseus Cluster. Contrary to previous results, our measurements of the cluster baryon fraction are consistent with the expected universal value at half of the virial radius.

Galaxy clusters provide critical constraints on cosmological parameters that are independent from those determined using type Ia supernovae, galaxy surveys, and the primordial cosmic microwave background radiation (CMB) (1–3). In particular, knowledge of their baryon content is a key ingredient in the use of clusters as cosmological probes (4, 5). Most baryons reside in the hot, diffuse, x-ray–emitting intracluster medium (ICM). Until recently, x-ray observations have generally targeted only the inner parts of clusters, where the emission is brightest, leaving a large fraction of their volumes practically unexplored. Estimates of the gas mass and total mass at large radii have relied on simple model extrapolations of the thermodynamic properties measured at smaller radii.

X-ray spectroscopy of the outer regions of galaxy clusters was made possible only recently, with the use of the Suzaku satellite. Because of its much lower instrumental background than that of other x-ray observatories, Suzaku can measure the characteristics of the faint emission from cluster outskirts more reliably. Even so, few such observations have been published, and the thermodynamic profiles at large radii are not well resolved (6–11). The Perseus Cluster of galaxies is the brightest extragalactic extended x-ray source. It is a relaxed system, both closer (at a redshift of 0.0183) and substantially brighter than any of the other clusters for which Suzaku has previously been used to study the ICM properties at large radii. Its large angular size mitigates the impact of residual systematic uncertainties in modeling the effects of Suzaku’s complex point-spread function (PSF), making the Perseus Cluster an ideal target in which to study cluster outskirts.

A large mosaic of Suzaku observations of the Perseus Cluster, with a total exposure time...