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RNA Granules and Cataract

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Cataract, the leading cause of blindness worldwide

Cataract - opacification of the transparent lens - is the leading cause of blindness and it affects 77 million individuals worldwide, with the costs of treatment in the U.S. alone exceeding 3.4 billion dollars annually [1][2]. Depending on onset, cataract can be classified as congenital or age-related [3][4][5], and can result from genetic, metabolic, or environmental causes. Congenital cataracts can occur in isolation or in association with other developmental defects, and collectively account for one-third of all childhood blindness. Between one-quarter to one-third of congenital cataract cases are inherited, typically with autosomal dominant inheritance [6][5]. To date, linkage and mutational analyses of candidate genes have provided the most successful strategies to identify genes that are mutated in congenital cataracts.

Approximately 30 loci have been mapped in cases where cataract is the primary phenotype, and more than 20 are associated with mutations in specific genes. Although mutations in genes encoding crystallin proteins constitute nearly half of these cases, the remainder are accounted for by mutations in genes that encode transcription factors, receptor proteins, gap-junction proteins, aquaporins, membrane proteins, cytoskeletal proteins and most recently, a protein encoding an RNA granule component called TDRD7 that is involved in post-transcriptional control of gene expression [7]. This latest addition to the growing class of molecules associated with cataract provides an exciting new direction for lens research, and is discussed further below.

What are RNA granules?

RNA granules (RGs) are ribonucleoprotein complexes found in the cytoplasm of eukaryotic cells from yeast to vertebrates that are implicated in the regulation of various aspects of mRNA control, including mRNA localization within the cell and its stabilization or degradation [8][9][10]. Although the full extent of RG composition and complexity in the cytoplasm of metazoan cells is incompletely understood, at present RGs are classified as: (1)

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processing bodies (PBs), (2) stress granules (SGs), (3) transport ribonucleoprotein (RNP) particles and (4) germ cell-specific granules (GCG). We briefly discuss each of these four classes.

PBs are constitutively found in cells but can also be stimulated to form by stress. They serve as cytoplasmic sites for mRNAs that are taken out of active translation and need to be temporarily stored or degraded. PBs are associated with components involved in microRNA-mediated silencing, nonsense-mediated decay (NMD) and with enzymes involved in mRNA decay processes [11]. The second class of RGs - SGs - are stalled translation pre-initiation complex aggregates that are formed in response to various conditions of stress [12][13]. Such stresses can be environmental, such as chemical or oxidative agents, or biological in nature, *e.g.*, the response to viral infection [14]. In conditions of stress, several pathways can trigger global translational silencing, during which SGs serve to store bulk mRNA but selectively refrain from harboring mRNAs that encode for proteins which function in the stress response.

In cells like oocytes, neurons and fibroblasts, a third, specialized type of RGs, termed transport RNPs, are assembled to transport mRNA to preferred sites of translation [15][16]. This mechanism transports mRNA in a translationally silent state, ensuring their localized translation at the sub-cellular site of function and allowing for a rapid response to stimuli, *e.g.*, during synapse formation in neurons. Lastly, GCGs, the fourth class of RG, are found specifically in the cytoplasm of metazoan germ cells, but share components with the PBs and SGs found in somatic cells [17][18]. GCGs also contain RNAs and proteins that are required for the differentiation of germ cells. One of the best-characterized GCGs is the so-called chromatoid body, or CB, which functions to mediate the post-transcriptional control of gene expression in male germ cell differentiation. As an interesting mechanism that may help to unify our understanding of the function of RGs, evidence exists that suggests that RNA exchange can occur between distinct classes of RGs, which may then determine whether they are directed to translational re-initiation or degradation, or transported to a specific location [19]. Taken together, these properties make RG mechanisms potentially attractive contributors to the post-transcriptional regulation of cellular differentiation in general, and to lens development in particular.

TDRD7 is an RNA granule component associated with lens development and cataract

RG components include several proteins that are likely to function in development, but their full significance in this process, particularly in vertebrates, is not well understood [20]. We recently identified TDRD7 (Tudor domain containing 7 protein), as an RG component with a strikingly enriched and evolutionarily conserved pattern of expression in the developing vertebrate ocular lens [7]. The TDRD7 protein contains three LOTUS/OST-HTH domains in its N terminus, and five Tudor domains distributed over the length of the protein that are thought to interact with double stranded RNA or with methylated arginine residues within other proteins, respectively. A majority of known Tudor domain proteins are associated with some aspect of RNA metabolism and control in the cell.

In the lens, TDRD7 functions as a component of a unique class of RNPs, termed TDRD7-RNA granules (TDRD7-RGs), which differentially associate with lens PBs as well as with other cytoplasmic RNPs that contain a well-characterized RG component, STAU1 (STAU1-RNPs). Furthermore, human *TDRD7* mutations result in the formation of bilateral posterior progressive cataracts, with posterior lenticonus and posterior capsule defects. These lens phenotypes are likely the result of the mis-regulation of developmentally important lens transcripts. In addition, a genetic mouse model exists that is deficient for the homologous

mouse *Tdrd7* gene. These mice, similar to humans, develop cataract shortly after birth, and also exhibit features of glaucoma (elevated intraocular pressure (IOP) and optic nerve damage), which may be either a primary defect or secondary to the cataract itself [7][21]. It is interesting to note that two of four human patients with the *TDRD7* mutation also developed features of glaucoma, although these patients were post-cataract surgery. Intriguingly, RNA co-immunoprecipitation experiments with TDRD7-specific or with STAU1-specific antibodies reveal the TDRD7-mediated regulation of several key lens-expressed mRNAs which encode: (i) Crystallin proteins (*e.g.*, CryβB3), (ii) proteins that bind and increase the stability of crystallins (*e.g.*, HSP27), or (iii) proteins essential for lens transparency (*e.g.*, EPHA2, SPARC). The interaction of TDRD7 with these mRNAs may involve direct or indirect interactions with other RG components. These findings provide a clear example of human lens defects that result from perturbation of an RG component, TDRD7, that exhibits lens-enriched expression and that is involved in post-transcriptional regulation in the lens.

Hypothesis: RNA granules and post-transcriptional control in lens biology

The lens is composed of anterior epithelial cells that exit the cell cycle in the transition zone region and that differentiate into posterior fiber cells that constitute the bulk of lens tissue. Lens fiber cells have several exceptional properties. First, they are highly elongated and polarized, and upon maturation, they undergo degradation of their nuclei and organelles. Moreover, lens fiber cells express unusually high levels of a highly restricted set of proteins, namely crystallins and other structural proteins (estimated concentration ~450 mg/mL), which need be produced prior to the loss of transcriptional and translation capability due to organelle degradation. At first glance, these features may appear unique to lens fiber cells, but closer inspection reveals that they are actually shared by many other cell types in the body. For example, neurons are elongated, migratory fibroblasts are polarized, and differentiating sperm cells undergo unusually high levels of chromatin compaction that eventually limit their transcriptional and translation capacity. Interestingly, these diverse cell types share all harbor RGs which can function in the generation of these specialized cellular phenotypes [17][16][8][10]. Therefore, we hypothesize that lens fiber cells also use an RG-based mechanism to mediate the post-transcriptional regulation of gene expression and to acquire their unusual state of differentiation. Such control of mRNAs may be necessary in lens fiber cells, which are elongated, polarized and under stress from translating high levels of proteins as transcriptional capacity is progressively lost.

Recent data supporting the hypothesis that TDRD7 controls the unique attributes of lens fiber cell morphology by regulating the stability, degradation or transport of lens-specific mRNAs includes several findings. First, TDRD7-RGs interact with STAU RNPs as well as with PBs in lens fiber cells [7]. STAU1 function in RNA transport is conserved in diverse cell types like oocytes and neurons, and in organisms as distant as Drosophila and human [22]. Therefore, the association of TDRD7-RGs and STAU1 RNPs may represent a mechanism to actively transport RNA in elongated lens fiber cells, where the high protein content required would render a purely diffusion-dependent RNA transport mechanism insufficient to generate the protein levels necessary to achieve high refractive index. In addition, STAU1 functions in Staufen 1 (STAU1)-mediated mRNA decay (SMD) by directly binding to translationally active mRNAs in their 3'-untranslated regions (3' UTRs) and channeling them for degradation [23]. Thus, in addition to PBs and TDRD7-RGs, SMD may render specificity to the process by which mRNAs are stabilized and translated in lens fiber cells, a stressful cellular environment in which access to translational machinery is likely to be highly competitive. It is possible that the direct binding of TDRD7 to crystallin mRNAs may be required for their high level translation. In addition, HSP27 is a known SG component that encodes a chaperone protein in the stress response pathway; it is also

implicated in mRNA decay. It is possible that the association of TDRD7 with *HSP27* mRNA reflects a function of TDRD7 similar to that of SGs in stressed cells.

Interestingly, moving beyond lens fiber cells, RGs other than TDRD7-RGs may be involved in the translational regulation of lens epithelial cell transcripts [24]. For example, it is possible that in the lens epithelium, mRNAs are bound by RG components like PBs, and released for translation only upon receipt of an appropriate signal. Finally, it is also possible that RGs similar to SGs may neutralize various stress conditions in the lens, *e.g.*, oxidative stress resulting from elevated oxygen levels [25]. It can also be argued that the features of glaucoma observed in *Tdrd7* null mice and in human patients with *TDRD7* mutation result from defects in a TDRD7-mediated protective stress response.

The future: identification of novel post-transcriptional regulators in the lens

A comprehensive understanding of the gene regulatory network (GRN) that underlies lens development also requires decoding the post-transcriptional network in the lens, which in turn depends on the identification of additional components of this regulatory pathway. To efficiently identify genes and GRNs that connect to lens function and disease, we recently developed a novel bioinformatics approach termed integrated Systems Tool for Eye gene discovery (iSyTE) (Lachke and Maas, unpublished)[26]. iSyTE is based on microarray analysis of gene expression from several critical stages of mouse embryonic lens development, and as proof of principle, it successfully identifies the majority of known critical regulators of mammalian lens development, including transcription factors, signaling molecules and structural proteins. iSyTE also successfully identifies the majority of known genes associated with human congenital cataract, including the newly described genes, *TDRD7* and *PVRL3* [7][27]. We anticipate that iSyTE will be a useful tool for the discovery of novel lens-expressed genes that encode post-transcriptional regulators and other RG components that, when mutated, are also excellent candidates to cause cataract. Characterization of these candidate genes may reveal potential new therapeutic targets, and will likely provide insight into the control of gene regulation that extends our understanding of cellular differentiation and its associated diseases.

Conclusion

In this article, we outline the function of RNA granules (RGs) and post-transcriptional control in metazoan cells, and provide arguments supporting their function in lens and lens fiber cell biology and in the formation of cataract. Our recent identification of the Tudor and LOTUS/OST-HTH domain containing protein TDRD7 in lens development and cataract formation in chicken, mouse and human provides initial experimental evidence in support of this hypothesis. We anticipate that studies over the next five years will identify several new genes in this pathway, and further elucidate the complexity of RNA granule function in lens development and disease.

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• of interest

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