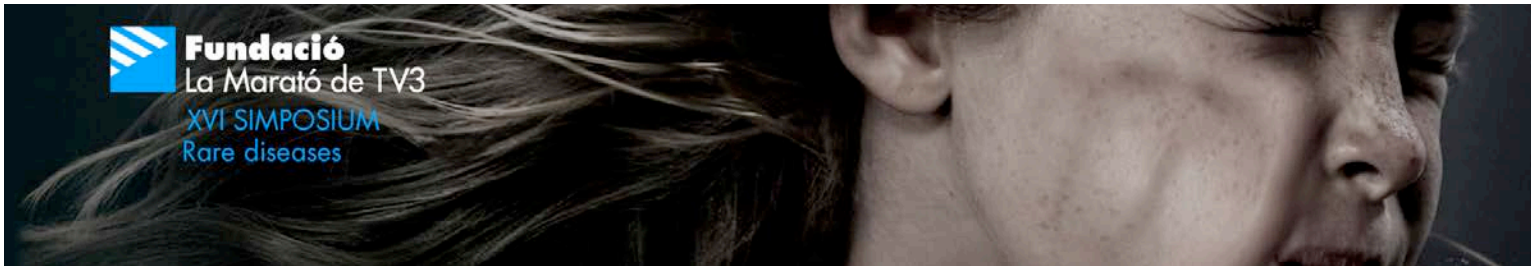

Functional role of splicing factors in autosomal dominant retinitis pigmentosa (ad RP): decoding the new molecular mechanisms in *Caenorhabditis elegans* to explore new therapies

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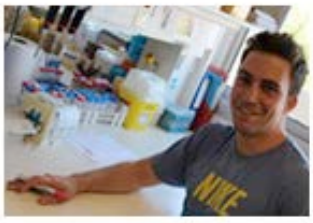
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1. Project Summary

Retinitis pigmentosa (RP) is a rare degenerative disease that causes progressive blindness. Among the rare diseases RP is quite frequent as it affects 1 in 4,000. This means that there are approximately one and a half million people affected worldwide. Retinitis pigmentosa patients are born with mutations in certain genes that predispose them to develop a condition known as tunnel vision, which in some cases leads to complete blindness.

This hereditary disease can be transmitted in several ways. Among the 50 genes that have been associated with retinitis pigmentosa, we studied 6 that are transmitted dominantly from parents to children (a mutation in one of the two copies of the gene is enough to produce the disease).

These six genes are PRPF3, PRPF4, PRPF6, PRPF8, PRPF31 and SNRNP200. They all encode proteins that are a key part of a cellular mechanism called splicing. When splicing takes place, the messenger RNA, which comes from a specific gene, is processed so it can be used as a template to produce a functional protein. These genes are all very well conserved through evolution, which means that the human genes are very similar to those of the worm *Caenorhabditis elegans* (*C. elegans*). Moreover, they carry similar functions in the cells of both organisms.

Despite the more than 9,000 published scientific studies about retinitis pigmentosa, no efficient treatment to avoid the blindness produced by this disease has been proposed. This absence of effective treatment may be due to the diversity of genes that may be involved. For this reason, a personalized medicine approach that identifies the mutation for each patient might be the best strategy to use. As the genes that we are studying share the same cellular function, it is highly possible that they would also share a similar treatment. As a study model we used *Caenorhabditis elegans*, a 1-millimeter long worm that is commonly used

for biomedical research around the world. Studies in *C. elegans* have resulted in three Nobel Prizes in the last decades, two of them in Medicine and Physiology.

A paradox of the retinitis pigmentosa subtype that we have studied is that the mutations that cause this disease are present in genes known to be expressed in every cell of the organism. However, despite their global genetic activity, these mutations cause disease only in the retina and do not affect other organs of the body.

Some questions guided our research from these background facts:

- Is it possible to generate a model for the study of retinitis pigmentosa using *C. elegans*, an organism that has proved to be very efficient and successful in biomedical research?
- What is the mechanism by which mutations in these six splicing genes produce a disease exclusively in the retina?
- Could the understanding of the mechanism of the disease orientate and improve the search for efficient therapies for patients with this subtype of retinitis pigmentosa?

2. Results

A classic method to study the function of a particular gene is its inactivation and subsequent analysis of its consequences. In this sense, *C. elegans* has the peculiarity of allowing us to inactivate genes flexibly and efficiently using RNA interference by feeding. Briefly, we can inactivate the function of a gene of interest in different grades by regulating the feeding (bacterial clone that produces the specific double strand RNA for each gene) in a population of worms. Our group have generated and validated tools for the partial inactivation of these six genes in

C. elegans. In this way we were able to model the partial loss of function presented in retinitis pigmentosa patients as a consequence of mutations.

In addition to the discovery of the consequences of the inactivation of these genes in *C. elegans*, we have studied the transcriptomes (RNA-sequencing) of worm populations with a partial loss of function of each one of these six genes. The most relevant finding was the observation of an increase in the expression of two very well conserved genes as a cellular response to the inactivation of these splicing genes. These genes are *atl-1*, human homolog of ATL which participates in the DNA damage response and replicative stress, and *egl-1*, human homolog of apoptosis activator genes.

In parallel, we discovered that one of the six splicing genes related with the disease, PRP-8, has a functional interaction with another protein, RSR-2, which participates in the coupling of the splicing and transcription processes (*Fontrodona et al., Plos Genetics 2013*).

Finally, we are proposing three ways, two of them novel, in which the viability of the cells in the retina may be affected when these six splicing genes are not active: (i) failure in the splicing process *per se* or the messenger RNA maturation, (ii) the transcriptional activity efficiency, and (iii) the activation of ATL as a consequence of replicative stress and the subsequent induction of apoptosis (Figure 1).

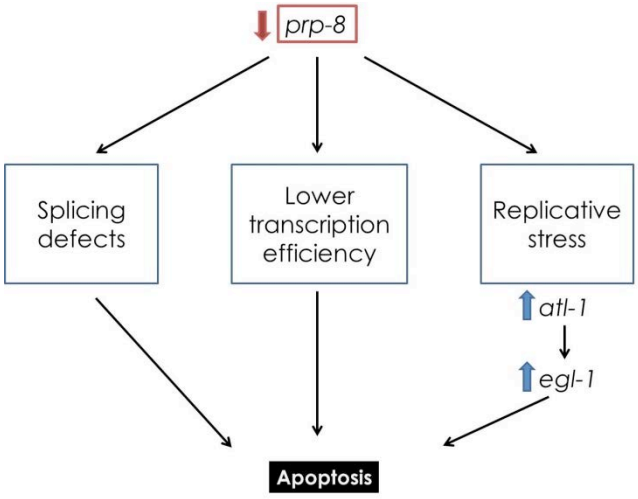

Figure 1

The molecular details of how a partial loss of function of these six genes can produce apoptosis would be matter of a longer discussion, but we can anticipate that in our model the apoptosis might be induced by the cotranscriptional splicing and the formation of R-loops in the DNA replication forks, and this occurs exclusively in cells with a high transcriptional activity.

3. Relevance and possible implications

The fact that the apoptosis activator *egl-1* was induced after the partial inactivation of either of these six splicing genes was a relevant finding as the retina cells of RP patients also die by this same mechanism. Furthermore, we observed that this apoptosis was occurring mainly in the hypodermal cells of the worm. So in the worms the apoptosis is taking place in a particular cell type, just as happens in humans with retinal cells.

The fact that there is a clear resemblance between the cells in the human retina and the hypodermal cells of the worm is very interesting because both cell types present a higher transcriptional activity than other cell types of each organism. So we propose a very attractive model for the study of retinitis pigmentosa that will certainly be used by other research groups and by ourselves in the near future.

Moreover, the activation of *atl-1* expression implies that we have identified a new pathway responsible for the apoptosis in these cells. If we are able to modulate or inhibit this pathway pharmacologically, we could probably increase the retina cells' survival and delay, or avoid, the progression of the blindness.

CRISPR and personalized medicine

The novel CRISPR/Cas9 technique allows the genome editing of cells and model organisms with a flexibility and efficiency never seen before. To date, our group has already used this technique in other projects to introduce point mutations or small insertion/deletions. In this scenario, CRISP/Cas9 and our *C. elegans* model for retinitis pigmentosa all together will allow us to introduce specific mutations from RP patients into the worm's genome, and therefore each person diagnosed with a mutation in these splicing genes could own a 'personalized multicellular assay tube' in which to test possible therapies.

4. Literature generated

Publications

Title: Modeling of autosomal dominant Retinitis Pigmentosa in *Caenorhabditis elegans* uncovers a nexus between impaired function of splicing factors and apoptosis.

Authors: Laura Fontrodona, David Aristizábal-Corrales, Karinna Rubio, Montserrat Porta-de-la-Riva, Silvia Torres, Francisco Javier García, Eric Cornes, Julián Cerón

Journal: *RNA* journal (paper in second revision)

Title: RSR-2, the *Caenorhabditis elegans* Ortholog of Human Spliceosomal Component SRm300/SRRM2, Regulates Development by Influencing the Transcriptional Machinery.

Authors: Laura Fontrodona; Montserrat Porta de-la-Riva; Tomás Morán; Wei Niu; Mònica Díaz; David Aristizábal Corrales; Alberto Villanueva; Simó Schwartz; Valerie Reinke; Julián Cerón.

Journal: PLoS Genetics. 9 - 6, pp. e1003543. 06/2013. ISSN 1553-7404

Master projects

Title: Modelando la retinopatía humama Retinosis Pigmentaria autosómica dominante en *C. elegans*

University: Universitat Autònoma de Barcelona (UAB). Máster in Advanced Genetics

Student: Karinna Rubio Peña. Date: 19/07/2013

Title: Desarrollo del modelo de Retinitis Pigmentosa en *C. elegans*

University: Universitat de Barcelona (UB). Master in Biomedicine.

Student: Silvia Torres Manjón. Date: 18/07/2014