MOL231: Alternative splicing of the pre-mRNA processing factor 31 (PRPF31) in Retinitis pigmentosa

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Introduction:

The pre-mRNA processing factor 31 (PRPF31) is a ubiquitously expressed component of the U4/U6.U5 tri-snRNP (small nuclear ribonucleoprotein) subunit of the spliceosome that catalyses splicing of pre-mRNA (1). A *PRPF31* c.1115_1125del11 heterozygous mutation causes autosomal-dominant Retinitis pigmentosa, a non-syndromic retinal disease (1). This mutation is an 11-nucleotide long deletion, in the middle of exon 11, that creates a premature termination codon (PTC) in the 14th (last) PRPF31 exon (2).

A mis-splicing of the PRPF31 gene, caused by the given mutation, results in a long (LM) mRNA form and a short (SM) mRNA form (Figure 1) in addition to the full-length wildtype (WT) PRPF31 allele (2). The LM bears the deletion and a PTC in exon 14 and is NMD-insensitive (Nonsense-mediated mRNA decay), while the SM has a skipped exon 11, a PTC in exon 12 and is degraded by NMD (2). Localization of the PRPF31 WT is mostly nuclear while the LM is mislocalized and aggregated in the cytoplasm and shows no nuclear localization (3).

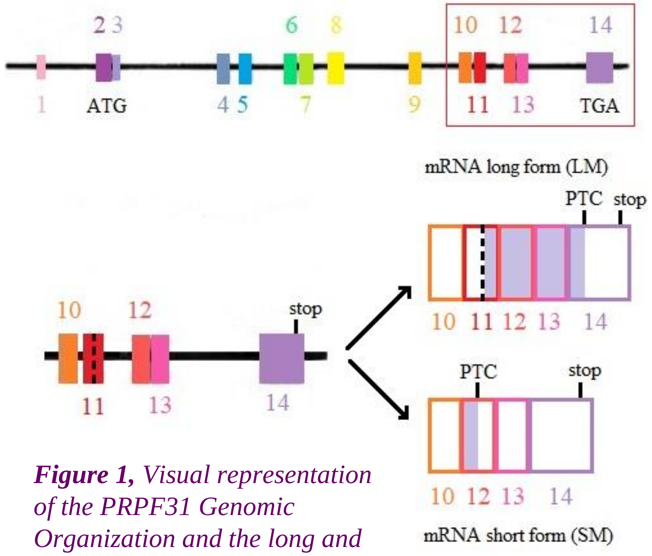
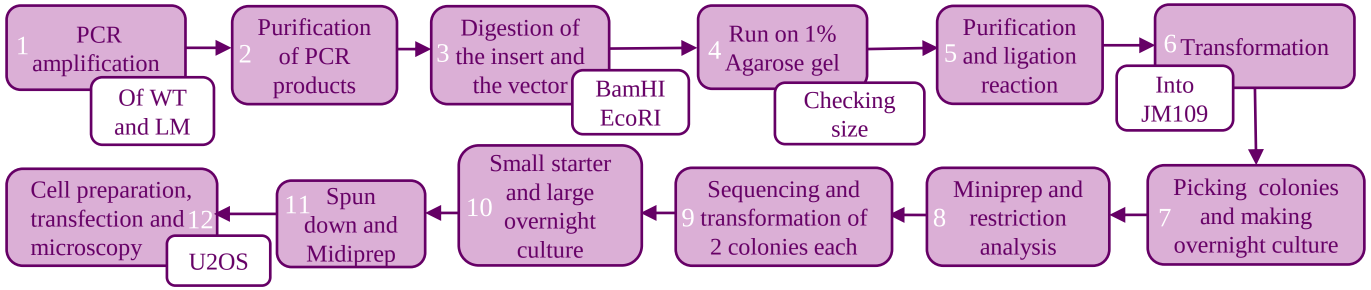


Figure 1, Visual representation of the PRPF31 Genomic Organization and the long and short form mutants; the light purple indicating the portion of the transcripts with out-of-phase reading frame

Aim:

The PRPF31 wildtype and the long form c.1115_1125del11 mutant are cloned into the EGFP-C1 vector with the aim of showing aggregation and mislocalization of the LM, with live-cell imaging and fluorescence microscopy.

Methods:



Results:

Following the first 8 method steps, cloning of the WT and the LM into the EGFP-C1 vector was successful, and so the obtained plasmids were sequenced. The sequencing results showed an additional point mutation in addition to the wanted c.1115_1125del11 mutation making this a c.1115_1125del11 + c.1126A>G mutant (Figure 2). However, this newly-uncovered mutant (Mut) was still put through steps 9 to 12, and was transfected together with the WT. The localization of the PRPF31 WT was indeed mostly nuclear, however, the Mut also had nuclear localization and didn't show the expected mislocalization in the cytoplasm, characteristic of LM.

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Ensembl GTACCGCAAGATGAAGGAGCGGCTGGGGCTGACGGAGATCCGGAAGCAGGCCAACCGTAT
WT      GTACCGCAAGATGAAGGAGCGGCTGGGGCTGACGGAGATCCGGAAGCAGGCCAACCGTAT
Mut     GTACCGCAAGATGAAGGAGCGGCTGGGGCTGACGGAGATCC-----------GACCGTAT
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***Figure 2,** Multiple DNA sequence alignment of the Ensembl-retrieved PRPF31 sequence, the WT and LM;* indicating that there is an 11-base long deletion in position 1115-1125 together with an additional point mutation A>G at position 1126 in the mutant

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WT  KMKERLGLTEIRKQANRMSFGEIEEDAYQEDLGF
LM  KMKERLGLTEIQPYELRRDRGGRLP----GGPGI
Mut KMKERLGLTEIRPYELRRDRGGRLP----GGPGI
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***Figure 3,** Multiple protein sequence alignment of the WT, LM and Mut;* showing that, compared to the expected LM Q, the Mut contains an R like WT

***Table 1,** Percental representation of the different localizations of the PRPF31-EGFP-C1 WT and Mut*

Localization %	WT	Mut
Nuclear (N)	77	75
Cytoplasmic (C)	0	0
N + C	23	25

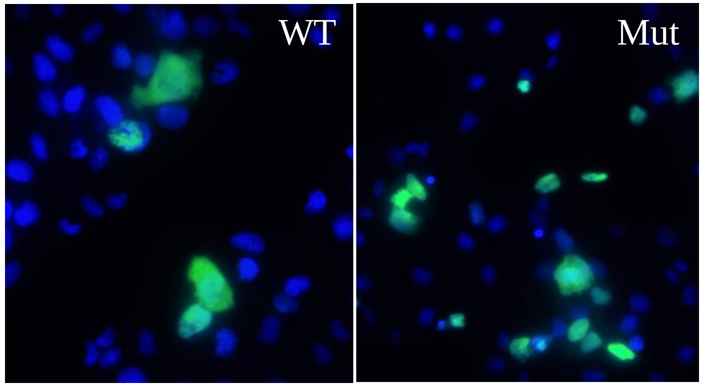

***Figure 4,** Fluorescent microscopy images of the DAPI stained U2OS cells containing the PRPF31-EGFP-C1 WT and Mut*

Conclusion and future prospects:

Given that there was no mislocalization and aggregation of the PRPF31 Mut in the cytoplasm, one can hypothesize that the additional substitution of A to G in position 1126, and its subsequent conversion of glutamine to arginine (that is known to suppress protein aggregation), were the reason behind this unexpected result. In order to confirm that the given point mutation reverts the effect of LM on the retina (that causes retinitis pigmentosa) one could transfect the RPE1 cells with the PRPF31 Mut and observe the concomitant response.

References:

1. Buskin A, et al. Disrupted alternative splicing for genes implicated in splicing and ciliogenesis causes PRPF31 retinitis pigmentosa. Nat Commun. 2018 Oct 12
2. Rio Frio T, et al. Premature termination codons in PRPF31 cause retinitis pigmentosa via haploinsufficiency due to nonsense-mediated mRNA decay. J Clin Invest. 2008 Apr 1;118(4):1519–31.
3. Valdés-Sánchez L, et al. Retinal pigment epithelium degeneration caused by aggregation of PRPF31 and the role of HSP70 family of proteins. Mol Med. 2019 Dec 31;26(1):1.