Identification of an Alternative Exon in a GABA Receptor Gene

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Introduction

The Central Dogma and pre-mRNA Splicing

The central dogma of biology states that DNA is transcribed into mRNA, which is then translated into proteins. In order for translation to occur, pre-mRNAs first must be processed. In pre-mRNA processing, parts of the nucleotide sequence called introns are spliced out from the transcript, so the final mRNA is made up of entirely exons.

Alternative Splicing

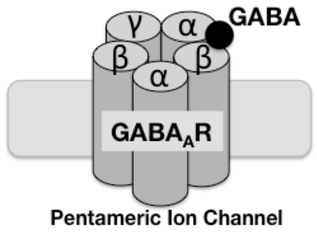
In alternative splicing, an exon is spliced out of the pre-mRNA transcript much like an intron. An mRNA transcript produced as a result of alternative splicing could produce a different protein than the mRNA without alternative splicing. Alternative splicing of an mRNA transcript could also result in a premature termination codon (PTC) within the mRNA sequence. This premature termination codon causes translation to stop before the full transcript has been translated, resulting in a truncated protein.

Nonsense Mediated Decay

Nonsense Mediated Decay (NMD) functions by degrading mRNA transcripts containing a PTC. NMD occurs during translation by an intricate series of protein-protein and protein-mRNA interactions that detect a PTC and result in the cleavage of PTC-containing mRNAs.

Our Genes of Interest: $GABA_A$ Receptor Genes

GABA is a neurotransmitter that binds to GABA receptor proteins. $GABA_A$ receptors ($GABA_A$Rs) are pentameric ion channels made up of five subunits from 20 possible closely related isoforms.



Hypotheses

Here, we present data on the alternative splicing of an exon in $GABA_A$R genes. We first discovered that a 68-bp exon in the *gabrr2b* gene (exon 3), which codes for the $GABA_A$R rho2b isoform, is alternatively spliced. Based on its close homology to exons in the other 19 $GABA_A$R genes, and the translation of *gabrr2b[-e3]*, we tested two hypotheses:

1) Similar 68-bp exons in the other $GABA_A$R genes are also alternatively spliced
2) mRNAs without the 68-bp exon are degraded via NMD

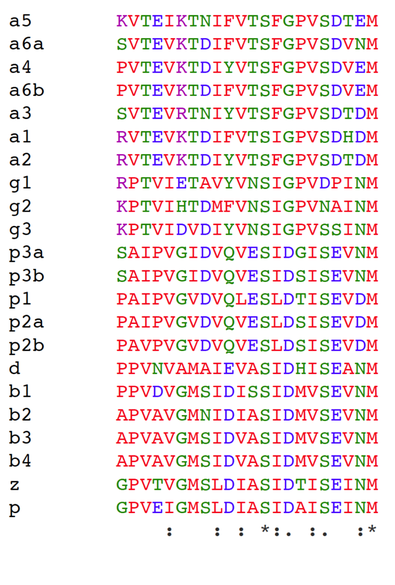
Results

Figure 1. 68-bp exon 3 is alternatively spliced in rho2b gene. Agarose gel showing products from GABA receptor subunit rho2b gene amplifications. mRNAs were extracted from 6 dpf larvae using RNeasy Mini Kit (Qiangen). RT-PCR was performed on rho2b gene with primers flanking exon 3 (Superscript IV First-Strand Synthesis System (Invitrogen), GoTaq Hot Start PCR Master Mix). Two resultant bands were extracted, gel purified, and sequenced. Sequence alignment revealed a new isoform of rho2b missing exon 3.

A

Gene	Subunit	Accession	68 bp Exon
gabrr1	ρ1	NM_001025553.1	exon 4
gabrr2a	ρ2a	XM_017351674.1	exon 3
gabrr2b	ρ2b	XM_692394.7	exon 3
gabrr3a	ρ3a	NM_001128760.1	exon 3
gabrr3b	ρ3b	X1WB79	exon 3
gabra1	α1	NM_001077326.1	exon 4
gabra2	α2	XM_009309207.2	exon 3
gabra3	α3	XM_009295708.1	exon 2
gabra4	α4	NM_001017822.1	exon 3
gabra5	α5	XM_005166082.3	exon 5
gabra6a	α6a	NM_200731.1	exon 3
gabra6b	α6b	XM_002667357.5	exon 3
gabrb1	β1	XM_002664133.4	exon 3
gabrb2	β2	NM_001024387	exon 3
gabrb3	β3	XM_005166079.3	exon 3
gabrb4	β4	XM_005173874	exon 3
gabrg1	γ1	A0A0R4IPF9	exon 3
gabrg2	γ2	NM_001256250	exon 3
gabrg3	γ3	XM_009302568.2	exon 3
gabrd	δ	XM_695007.7	exon 3
gabrz	ζ	NM_001114742.1	exon 4
gabrp	π	XM_005173293	exon 4

B



C

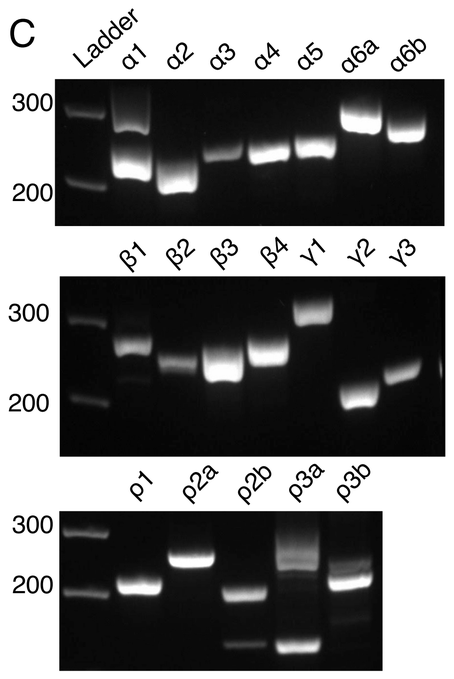
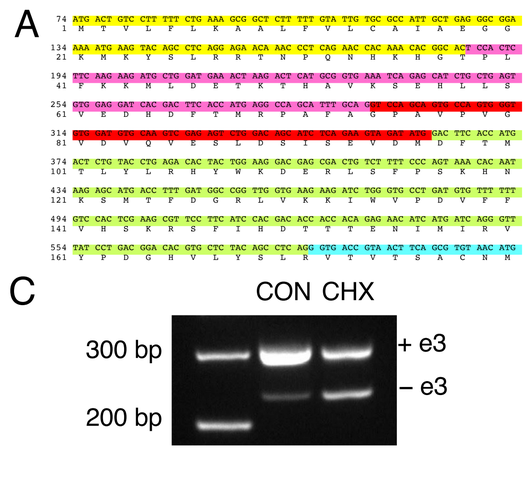


Figure 2. Highly conserved 68-bp exon is only alternatively spliced in rho2b gene. A) 20 $GABA_A$R genes all contain a 68 bp exon. B) Clustal Omega alignment demonstrates conservation between translated 68 bp exons. C) 2% agarose gels showing products from GABA receptor subunit gene amplifications. mRNA transcripts were extracted from 6 dpf larvae using RNeasy Mini Kit (Qiagen). RT-PCR was performed on the various GABA subunit genes with specific primers designed to flank the 68-bp exon in each gene (Superscript IV First-Strand Synthesis System (Invitrogen), GoTaq Hot Start PCR Master Mix). The subunits tested are labeled above gels. No products were detected at the appropriate size for mRNAs missing exon 3, except in the rho2b gene.



C

D

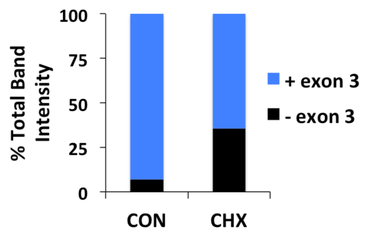


Figure 3. mRNAs without exon 3 may be degraded via NMD. Translated mRNAs of rho2b with (A) and without (B) exon 3 show that when exon 3 (red in (A)) is excluded, a frame-shift occurs in exon 4 resulting in a PTC (red in (B)). C) Zebrafish larvae were treated in a petri dish with DMSO only (CON) or 0.1% cycloheximide (a translation inhibitor) in DMSO (CHX) for 24 hours before RNA Extraction,and RT-PCR using primers that flanked alternative exon 3. Expected sizes for products were: + e3: 317 bp, – e3: 249 bp. 2% agarose gel shows shift in percentage of transcripts containing exon 3 when NMD pathways are inhibited. (D) Quantification of relative band intensity of PCR products shown in (C).

Conclusions

1. We did not find any evidence of alternative splicing of the highly conserved exon within any of the other genes coding for $GABA_A$R subunits, and therefore we do not have evidence to support our first hypothesis.
2. Our data suggests that rho2b mRNAs excluding exon 3 are degraded by NMD, and therefore do not code for novel protein isoforms.

Future Directions

Preliminary data from cycloheximide treated larvae shows evidence that nonsense mediated decay degrades mRNA transcripts that are produced as a result of alternative splicing. Our next steps are to repeat experiments with CHX and other translation inhibitors (e.g., puromycin) for different incubation times in an attempt to verify our findings.

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