Characterization of alternatively-spliced isoforms in the PERK-coding gene *EIF2AK3* under ER stress

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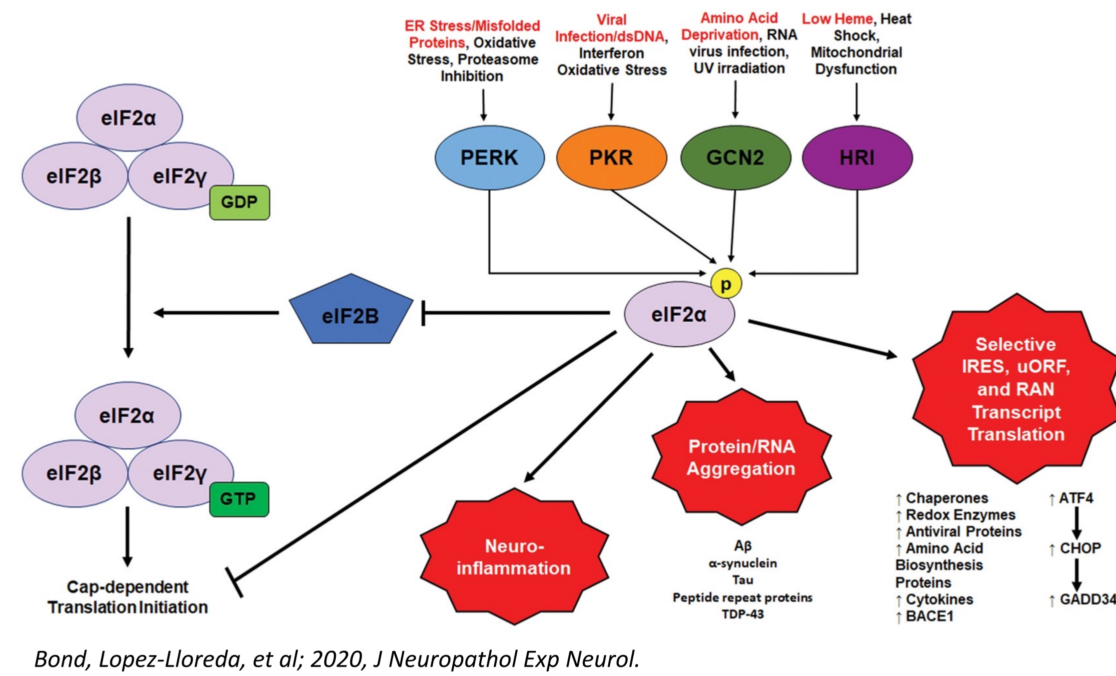
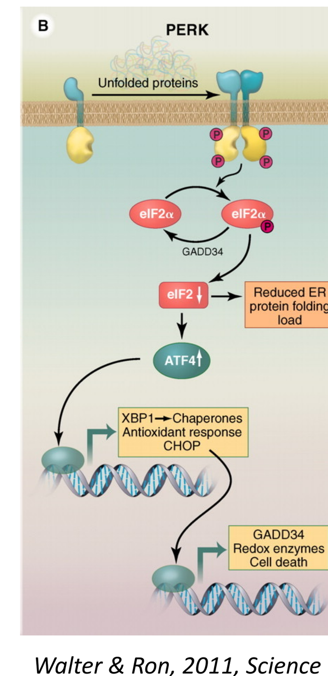
INTRODUCTION



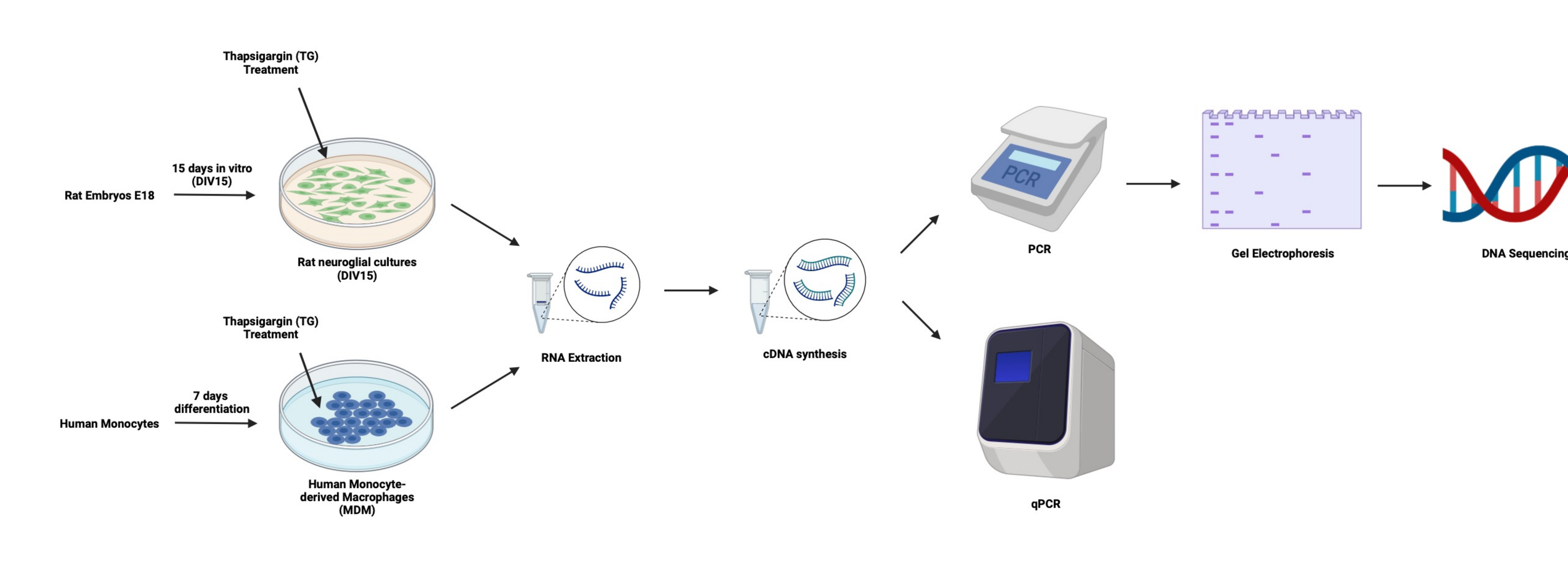
Bond, Lopez-Lloreda, et al; 2020, J Neuropathol Exp Neurol.



Walter & Ron, 2011, Science

- The integrated stress response (ISR) is an important regulatory pathway mediating cell stress and has been implicated in numerous neurodegenerative (ND) disorders.
- One branch of the ISR is the PERK protein, which responds to endoplasmic reticulum (ER) stress caused by protein misfolding. The activation of PERK leads to global translation inhibition, and this pathway specifically has been linked to ND disorders such as Alzheimer's Disease.
- While much has been studied on the role of PERK protein, there has been a gap in knowledge regarding regulation of the mRNA transcript resulting from the PERK-coding gene, *EIF2AK3*.

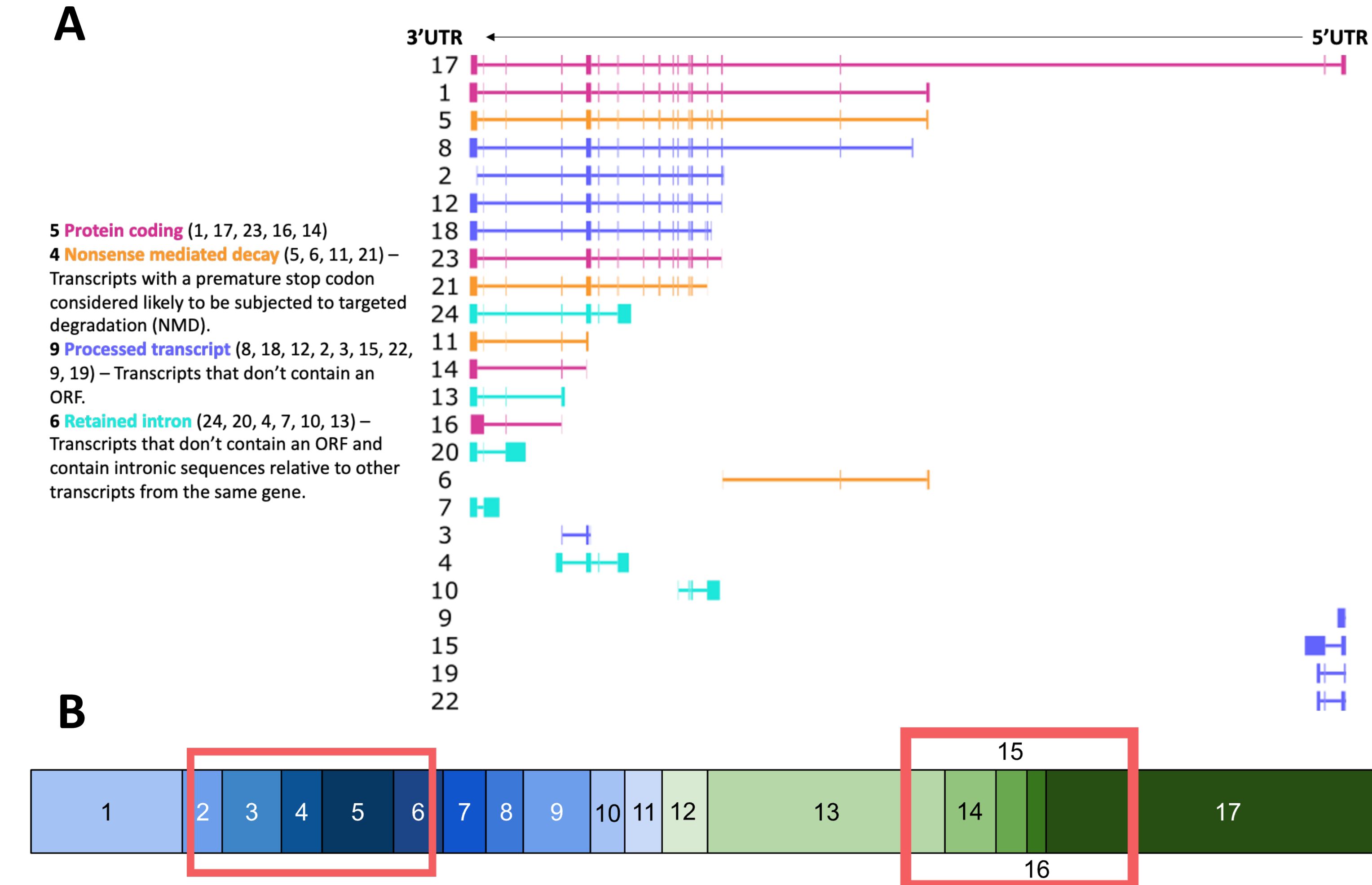
METHODS



- We identified two models to use for our study: 1) human monocyte-derived macrophage (MDM) cultures differentiated for 7 days (DIV7) and 2) primary rat cortical neuroglial (NG) cultures differentiated for 15 days in-vitro (DIV15).
- Both models were then treated with one of three treatments: vehicle (DMSO 150nM), 150nM thapsigargin (TG—known ER stress inducer) for 2 hours, or TG 150nM for 24 hours.
- RNA was extracted, synthesized into cDNA, and analyzed via PCR and qPCR. Gel electrophoresis and Sanger sequencing followed PCR.

RESULTS

Figure 1: Multiple EIF2AK3 transcripts were identified in the Ensembl genome browser, suggesting that alternative splicing may contribute to *EIF2AK3* transcriptional regulation



- **Figure 1A** shows the 24 previously identified human PERK isoforms visualized from the 'Ensembl' genome browser.
- **Figure 1B** shows the mRNA transcript produced from the PERK-coding gene *EIF2AK3*; the numbered spaces are the identified exons. The boxed regions represent the regions targeted by PCR primers, which constitute the exon 2-6 and exon 13-17 regions.

Figure 2: PCR and Sanger sequencing results show alternative-spliced production of human isoform 5 and 11 in the context of ER stress in human MDM cultures

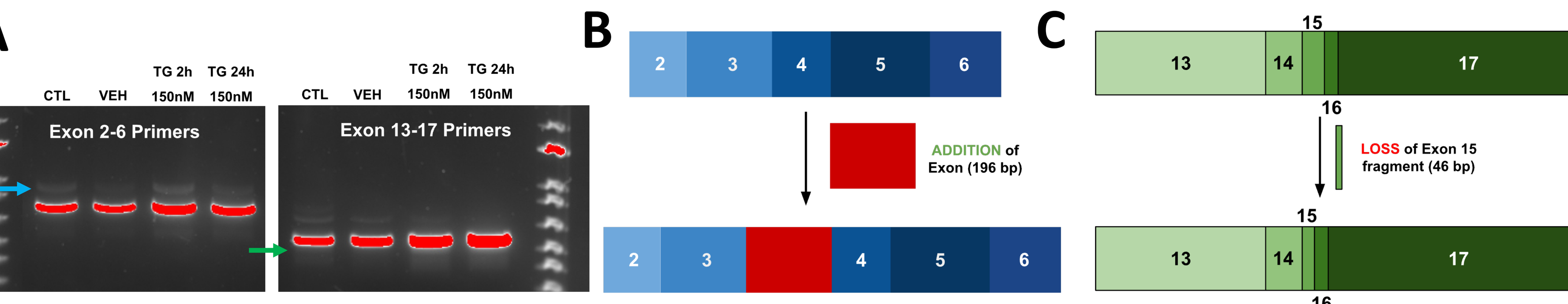


Figure 2A shows PCR results using primers targeting regions exon 2-6 and exon 13-17 of human MDM *EIF2AK3* from the four conditions (control, DMSO or vehicle, and TG 2h/24h). The presence of multiple bands suggests the presence of alternatively-spliced isoforms, which were confirmed based on the Sanger sequencing results shown in **Figures 2B and 2C**. **Figure 2B** shows sequencing of Isoform 5 from samples incubated with Exon 2-6 primers, which includes an addition of an exon region; **Figure 2C** shows sequencing of an Isoform 11-like sequence from samples incubated with Exon 13-17 primers which includes a small deletion within the exon 15 region. These are labeled on **Figure 2A** by the blue and green arrow, respectively.

Figure 3: PCR and Sanger sequencing results show three novel EIF2AK3 transcripts produced via alternative-splicing in the context of ER stress in rat NG cultures

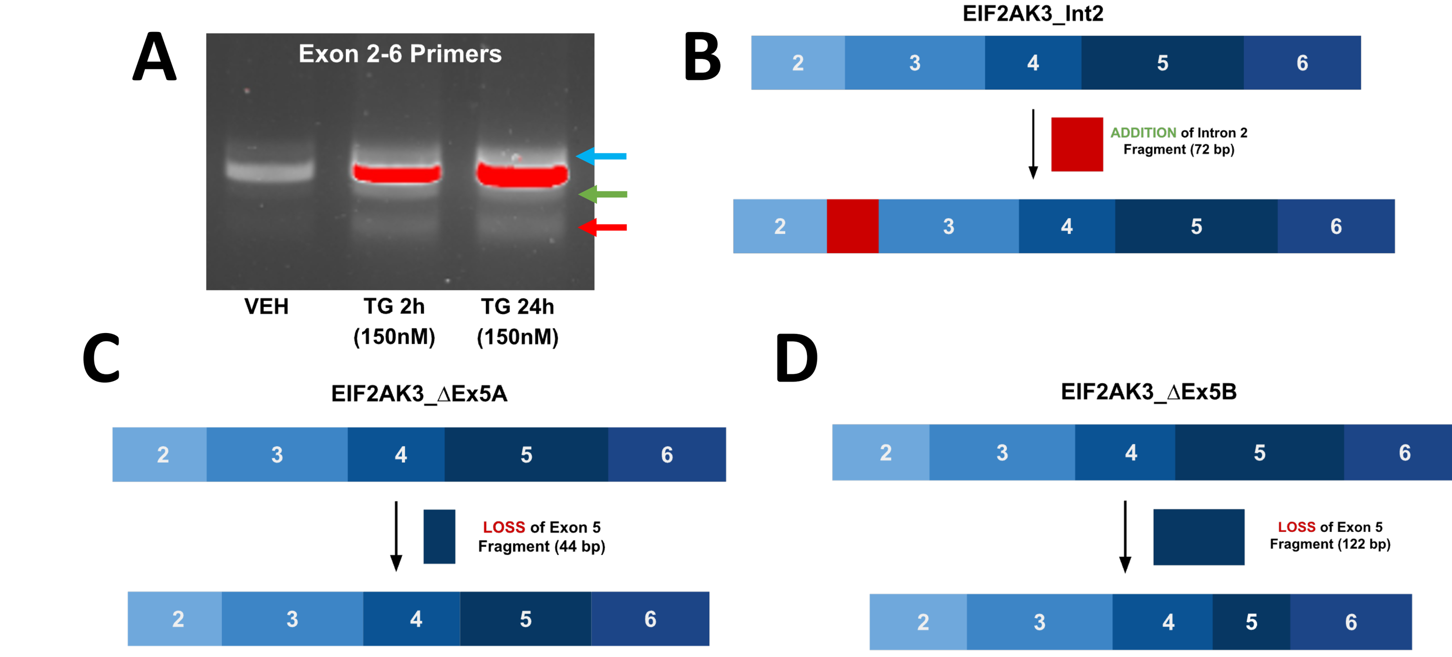
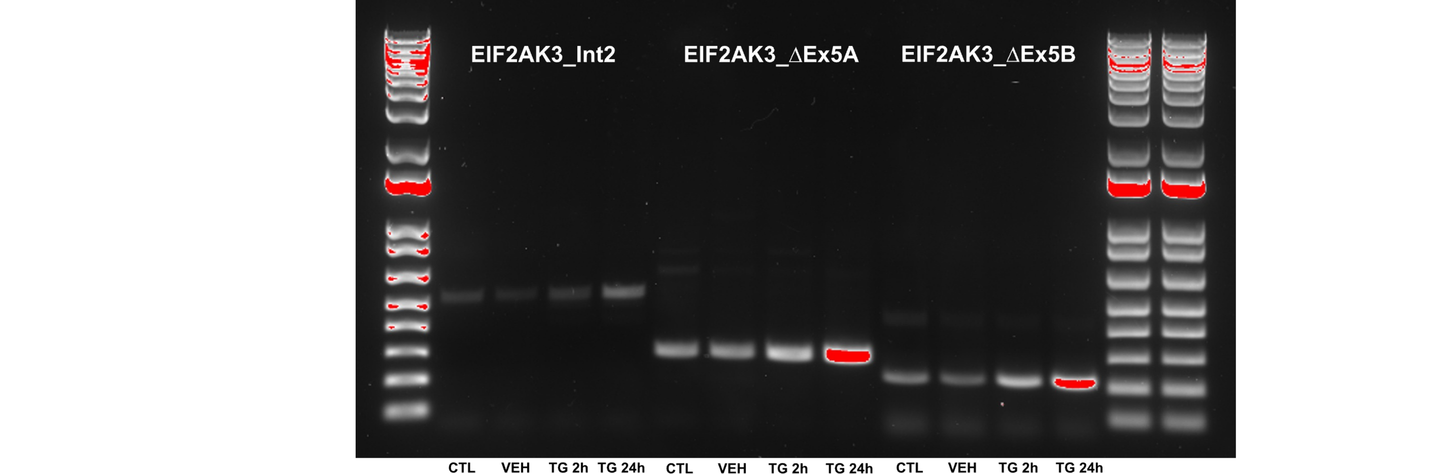


Figure 3A shows the PCR results using primers targeting regions exon 2-6 in rat cortical NG *EIF2AK3* in three different conditions (DMSO or vehicle, TG 2h and TG 24h). Blue, green, and red arrows are used to identify the three novel isoforms expressed in these samples, which have been named EIF2AK3_Int2, EIF2AK3_ΔEx5A, and EIF2AK3_ΔEx5B, respectively. The sequencing results for these isoforms are shown in **Figures 3B, 3C, and 3D**. Briefly, **Figure 3B** shows EIF2AK3_Int2, which has an addition of a fragment from intron 2. **Figure 3C** shows EIF2AK3_ΔEx5A, which has a loss of a minor fragment from Exon 5 while **Figure 3D** shows EIF2AK3_ΔEx5B, which has a loss of a major fragment from Exon 5.

Figure 4: Separation of rat NG isoforms via isoform-specific primers



Based on sequencing results, primers were created to target each of these distinct isoforms in rat NG cultures the PCR results are shown in **Figure 4**. These results also seem to show increasing concentrations of isoforms as TG incubation time increases.

Figure 5: Greater fold change of PERK and EIF2AK3_Int2 isoforms as TG incubation time increases

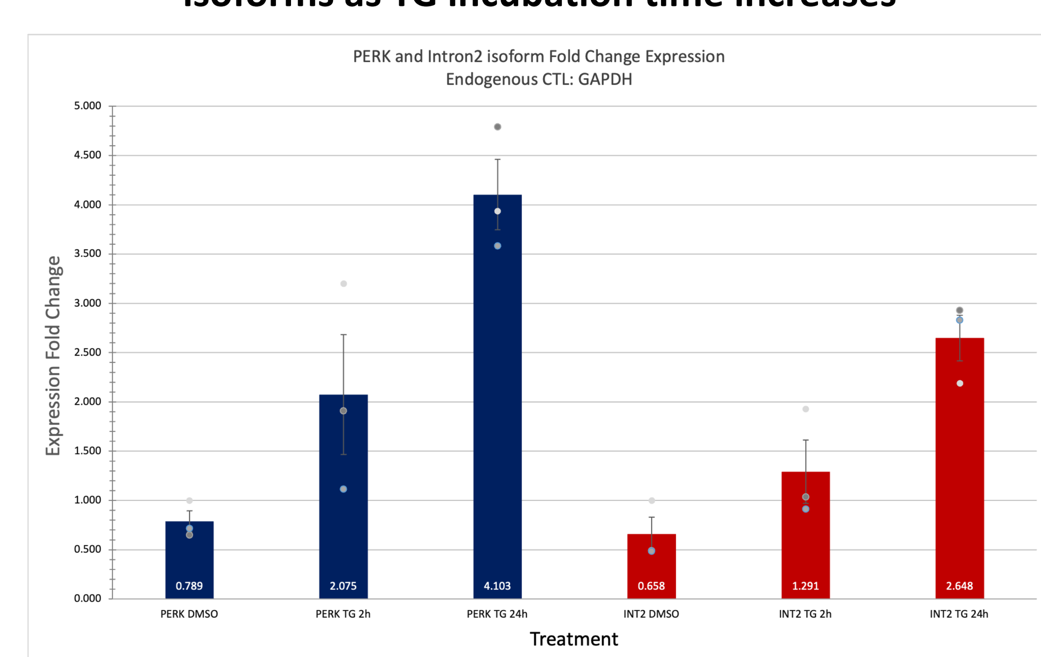


Figure 5 shows the qPCR results using TaqMan probes of the normal PERK isoform and custom-made 'INT2' isoform, which directly targets the EIF2AK3_Int2 isoform. Data shows statistically significant ($p<0.001$) increases in PERK isoform fold change with incubation of TG 2h, and even greater fold change with 24h presence of TG. Data also shows statistically significant ($p<0.05$) differences in EIF2AK3_Int2 DMSO and TG 24h conditions (performed by 2-sample t test)

DISCUSSION

Table 1: Lengths of alternatively-spliced rat NG transcripts expressing PTCs

	PCR fragment size (nt)	Transcript size (nt)	Resulting ORF length (aa)
Transcript 1 (Main)	776	1140	379
EIF2AK3_Int2 (Higher)	848	441	146
EIF2AK3_ΔEx5A (Lower)	732	765	254
EIF2AK3_ΔEx5B (Lowest)	654	801	266

- Overall, we have observed prevalence of alternative splicing in both of our models, with identification of isoforms 5 and 11 in our human MDM model and three novel PERK isoforms in our rat NG model.
- Sequencing results showed that the three rat NG isoforms contained pre-mature termination codons (PTCs), which may suggest that these isoforms are under nonsense-mediated decay (NMD) regulation (**Table 1**) which degrades PTC-expressing isoforms to more tightly regulate gene expression

In the future, we hope to:

- Create qPCR TaqMan probes for the other two EIF2AK3 isoforms identified and compare results.
- Explore the functional role of these alternative isoforms (i.e., investigate if NMD is responsible)
- Utilize different models to better characterize these transcripts, such as PERK KO model or conducting experiments that analyze if any alternatively-spliced transcripts are protein-coding