

Which GABA Receptors are Expressed in the Zebrafish Lateral Line?

Kyler Hayes, Tenzin Yangchen, Megan Schwehr, Cecilia Toro
Biology Department, Linfield College, McMinnville, OR



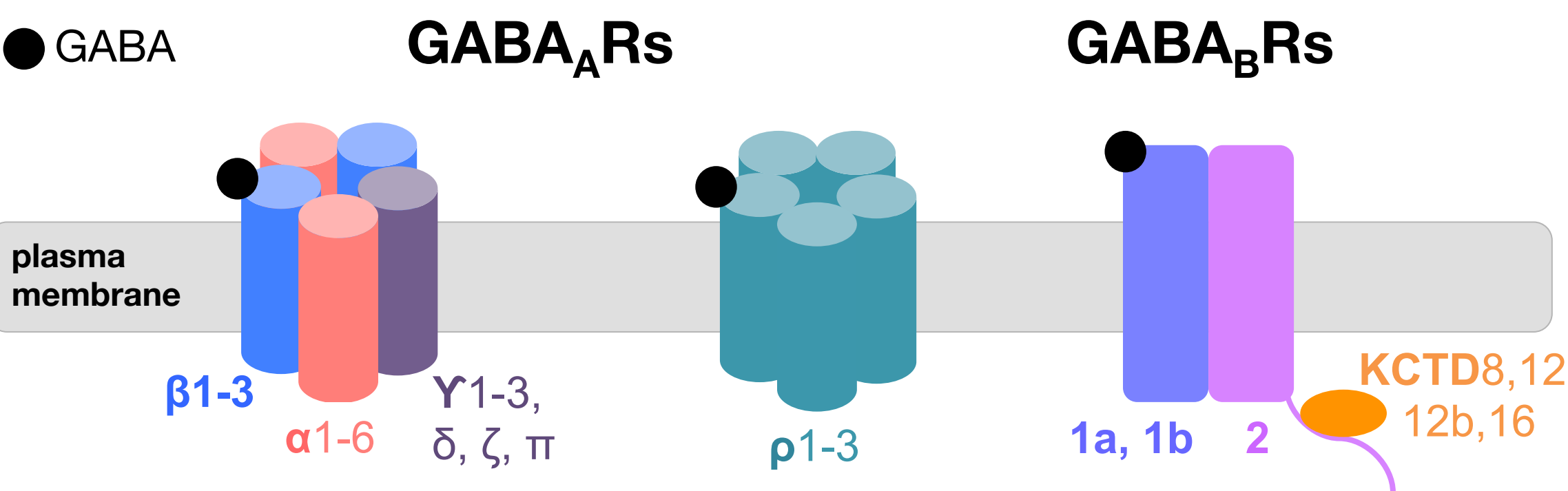
Abstract

The presence of the neurotransmitter GABA in the mammalian inner ear is well established, yet its role in regulating inner ear cell function is less clear. We seek to understand the role of GABA in the inner ear by using the model organism zebrafish. Zebrafish possess a sense that humans do not: they can detect water movement with their lateral line system. Zebrafish sense water movement with cells that project out from the body of the fish into the environment. These so-called hair cells are remarkably similar to the sensory cells of the cochlea and semicircular canals. Because they are on the outside of zebrafish, and not behind a bony skull, lateral line hair-cells are easily accessible for study. Therefore, we are determining if we can use the lateral line system to understand more about GABA in the inner ear. We have used RNA extraction and RT-PCR to detect the expression of 27 GABA-related genes in zebrafish. We have also identified a novel alternative exon in one isoform. Overall, our results suggest that the genes expressed in the lateral line are orthologs of genes expressed in the mammalian inner ear, and thus zebrafish appear to be an appropriate model organism with which to further study GABA function in the inner ear.

Introduction

The Neurotransmitter GABA

- GABA is released by neurons and binds to either presynaptic or postsynaptic **GABA Receptor** (GABAR) proteins.
- GABA_ARs**: Ion channels formed from 20 possible subunit isoforms.
- GABA_BRs**: G protein-coupled receptors formed from 3 possible subunit isoforms. Accessory KCTD proteins associate with GABA_BRs.



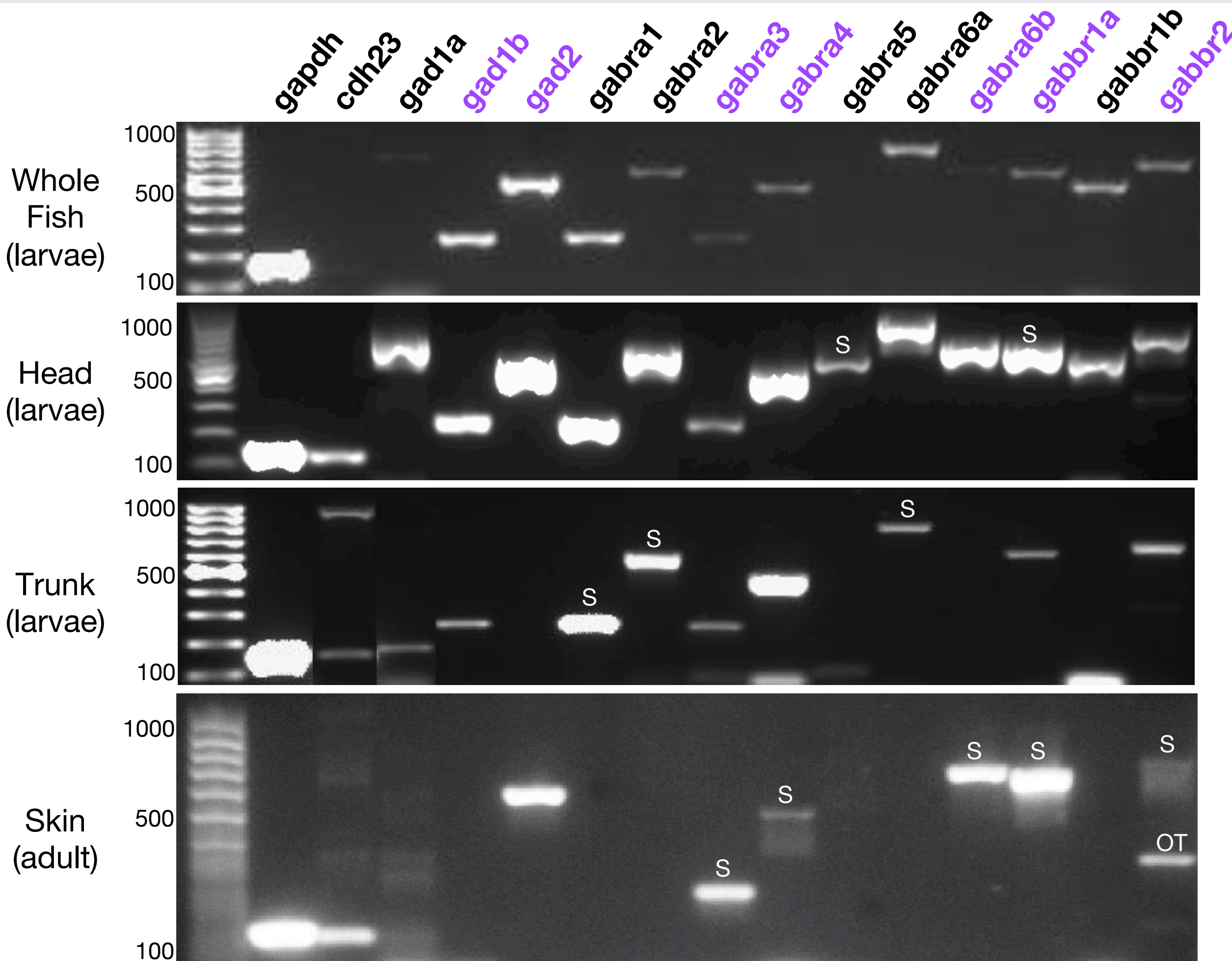
- GABA**, and **multiple isoforms** of **GABA_ARs** and **GABA_BRs**, have been detected in the mammalian inner ear (1, 2).
- The first physiological role for GABARs was recently determined: researchers showed that GABA acts as an autoinhibitory signal on presynaptic efferent terminals via GABA_BRs, and not GABA_ARs (3).
- The physiological role of GABA and GABA_ARs in the inner ear thus remains poorly understood.

The Zebrafish Lateral Line

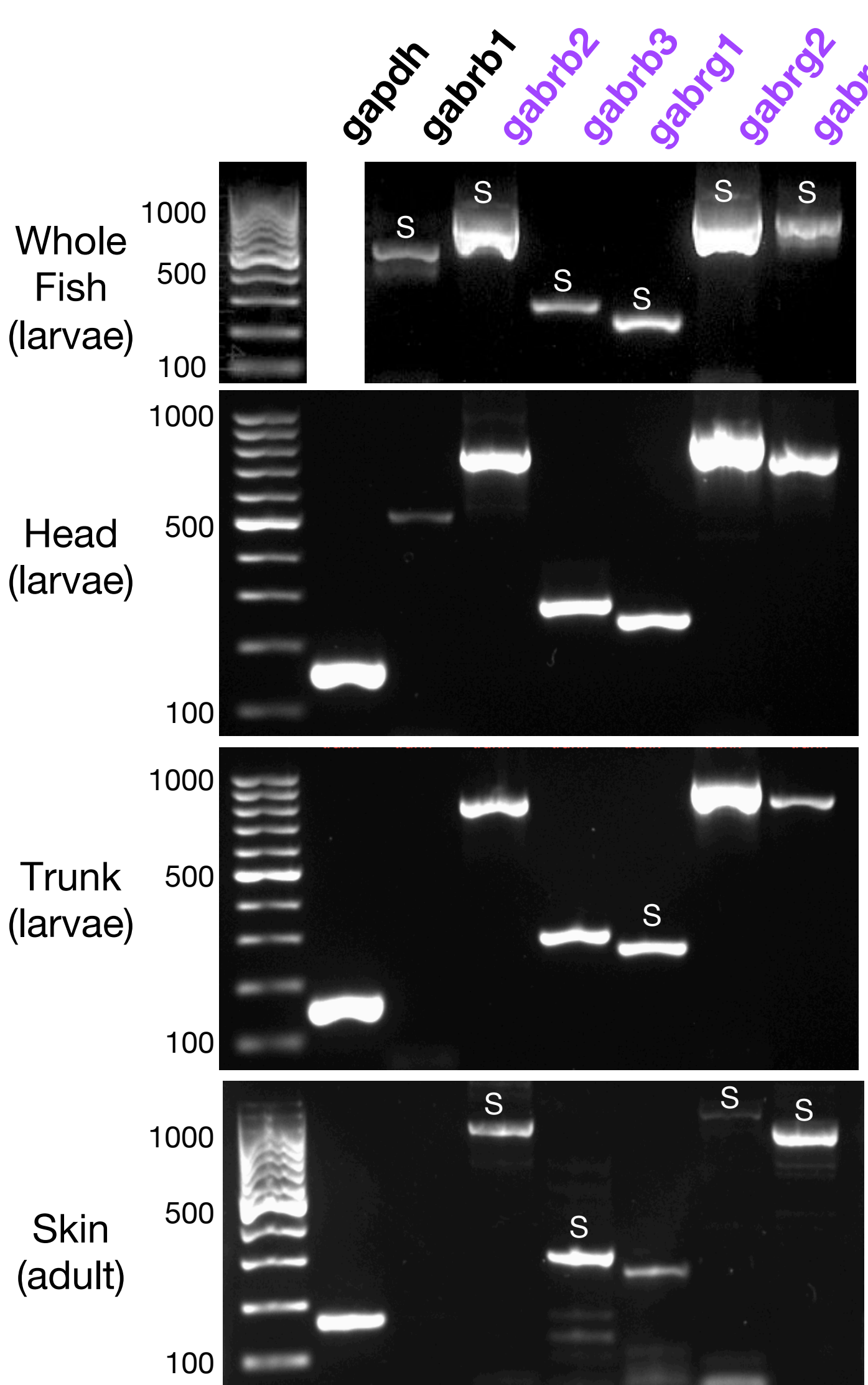
- The lateral line is used by aquatic vertebrates to detect water movement.
- It is closely related to the inner ear, and is externally located and therefore more easily accessible for study.
- We seek to use the zebrafish lateral line as a model to study the function of GABA in the mammalian inner ear.**
- Here, we have investigated whether the GABAR isoforms expressed in zebrafish correspond to the mammalian orthologs that have been detected by others (1, 2, 3).
- We have isolated zebrafish tissues, including larval head, trunk and adult skin. Lateral line tissue remains associated with dissected skin, and we therefore hypothesized that the skin sample would serve as a proxy for isolated lateral line.
- We extracted RNAs, synthesized cDNAs, and amplified regions of all 20 GABA_AR, 3 GABA_BR, and 6 KCTD accessory protein isoforms, as well as 3 isoforms of the enzyme necessary for GABA synthesis, GAD.

Results

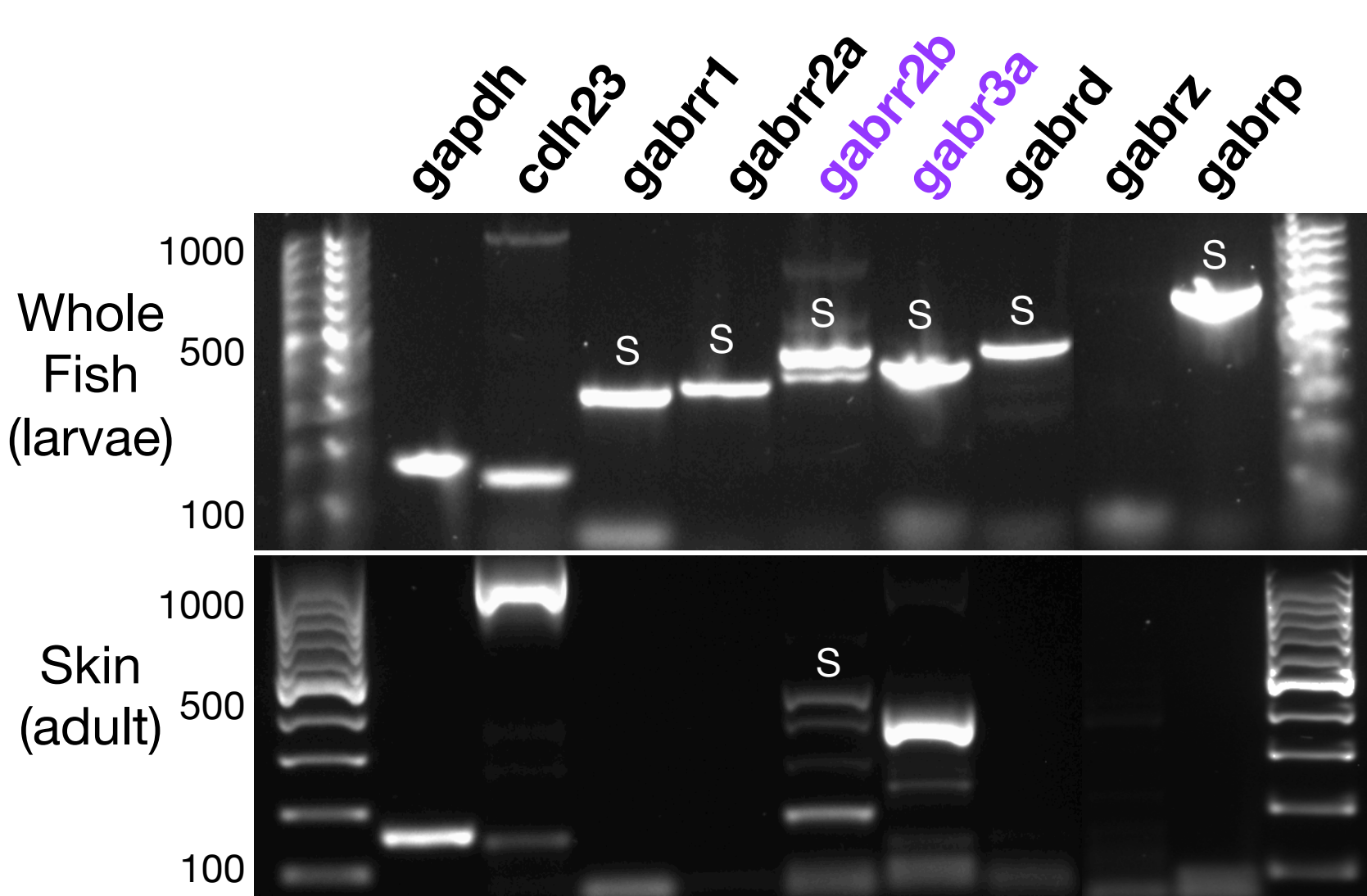
GAD, GABA_AR α , and GABA_BR isoforms



GABA_AR β and γ isoforms



GABA_AR ρ , δ , ζ and π isoforms



KCTD isoforms

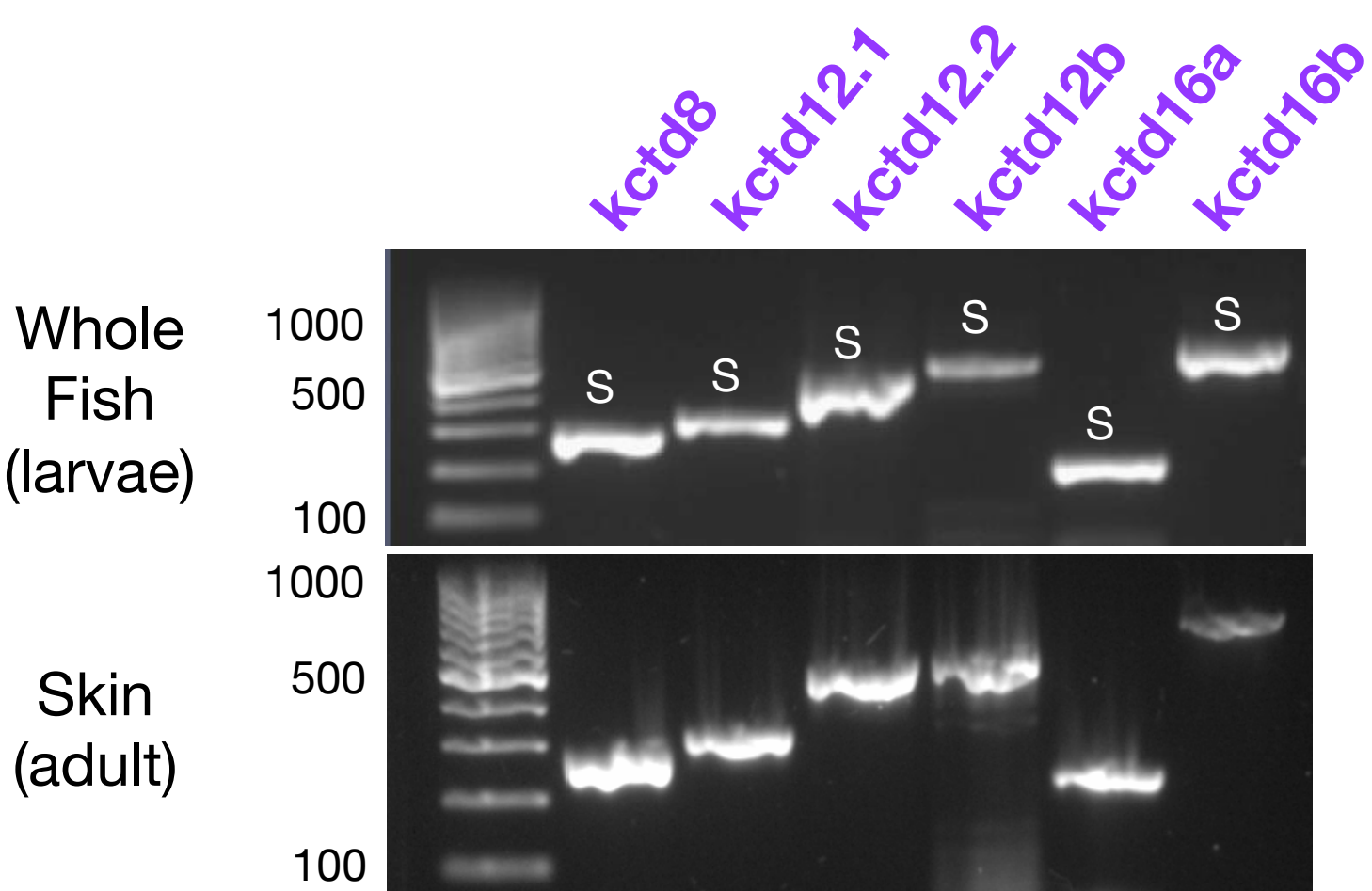


Figure 1. Tissue-specific expression of GABA-related genes in zebrafish. 1% agarose gels showing products from GABA-related gene amplifications. RNAs were extracted from zebrafish tissues as indicated at left of gels using RNeasy Mini Kit (Qiagen). RT-PCR was performed on genes identified with ZFIN and NCBI, using gene-specific primers designed with NCBI Primer BLAST, Superscript IV First-Strand Synthesis System (Invitrogen), and GoTaq Hot Start PCR Master Mix (Promega). Target genes are indicated above gels, and those that are candidate lateral line genes are highlighted in purple. S: products confirmed by sequencing; OT: off-target product discovered by sequencing. Select size markers labeled at left in basepairs. *gapdh* is an RT-PCR control gene, and *cdh23* is a hair-cell specific gene and is thus a control for hair-cell RNAs in tissue samples.

Novel Alternative Exon in GABA_AR p2b Gene

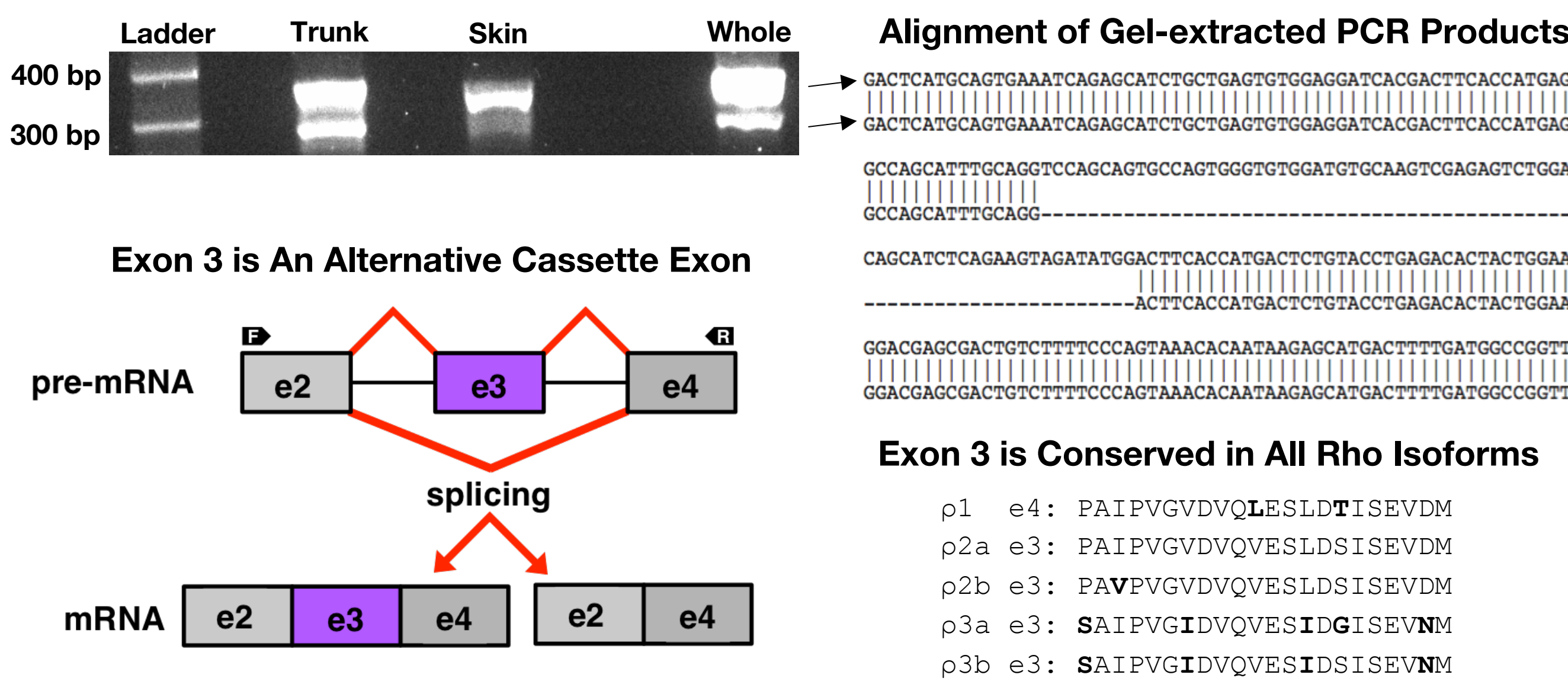


Figure 2. Novel alternative exon discovered in GABA_AR p2b gene. Two PCR products from amplified cDNAs were gel extracted and sequenced. Sequencing revealed a cassette exon, exon 3 (e3), which is included or not in final mRNAs. Amino acid sequence of closely related exons in all zebrafish p isoforms reveals a high degree of sequence conservation.

Conclusions + Future Directions

We detected 31/32 GABA-related genes in whole zebrafish larvae.

GABA_BR dimers expressed in zebrafish skin (GB1a + GB2) are the same as those found in mammalian inner ear (3), suggesting a presynaptic GABA_BR location.

GABA_AR isoforms detected in zebrafish skin include some isoforms detected in mammalian inner ear (1, 2), but not all.

Figure 3: Candidate genes: GABAR and GAD isoforms identified in zebrafish skin. GABAR and GAD subunit isoforms were, or were not, detected in zebrafish skin by RT-PCR, gel electrophoresis and sequencing. Isoforms labeled in red were not detected, and thus have been eliminated as candidate isoforms. Isoforms labeled in purple were found in adult skin, and thus remain candidate lateral line GABAR genes.

GABA _A R α Subunits				GABA _A R β Subunits		
$\alpha 1$	$\alpha 2$	$\alpha 3$	$\alpha 4$	$\beta 1$	$\beta 2$	$\beta 3$
$\alpha 5$	$\alpha 6a$	$\alpha 6b$		GABA _A R ρ Subunits		
GABA _A R γ Subunits				$\rho 1$	$\rho 2a$	$\rho 2b$
$\gamma 1$	$\gamma 2$	$\gamma 3$	δ	GABA _B R Subunits		
π				$1a$	$1b$	2
KCTD Isoforms				GAD Isoforms		
8	12.1	12.2	$12b$	$1a$	$1b$	2
$16a$	$16b$					

Our next steps are to determine the expression of candidate isoform mRNAs in intact larval tissue using *in situ* hybridization, and then to determine the subcellular localization of candidate receptor proteins using immunohistochemistry.

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References

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