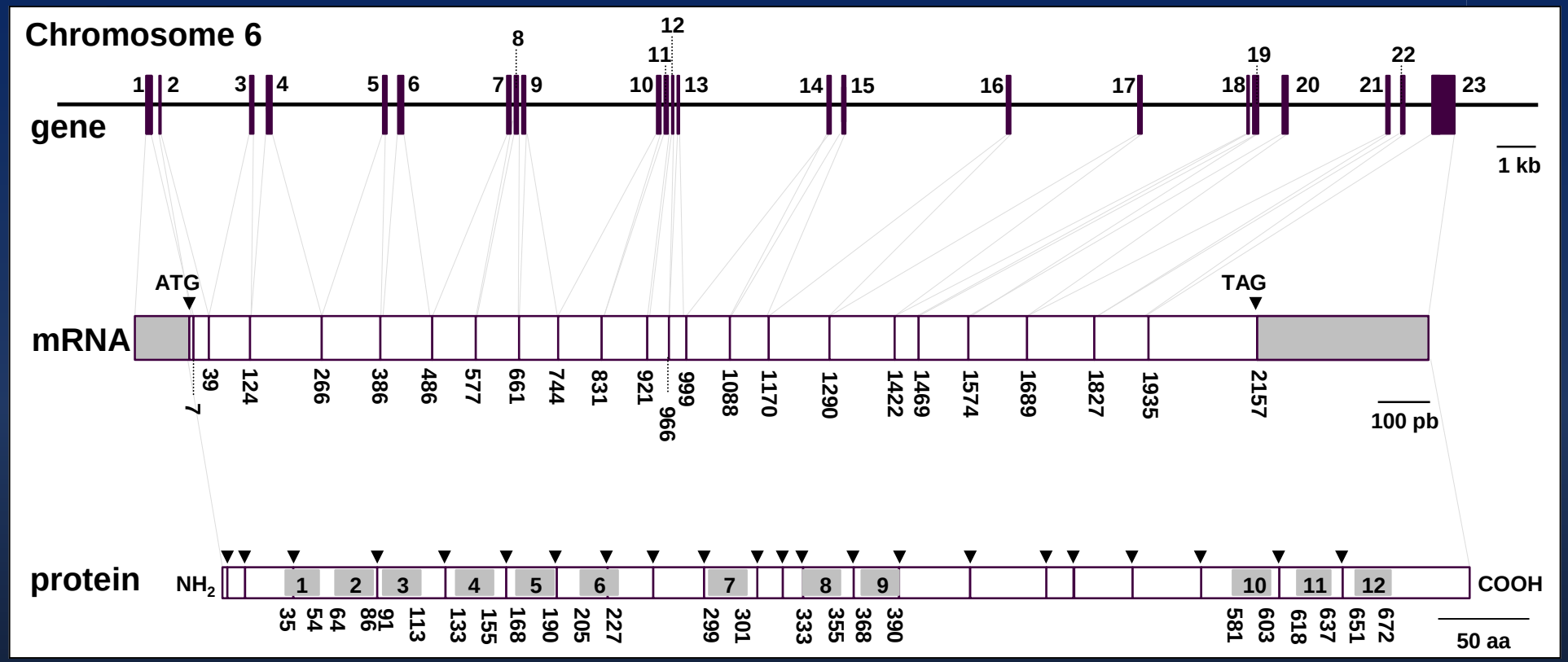
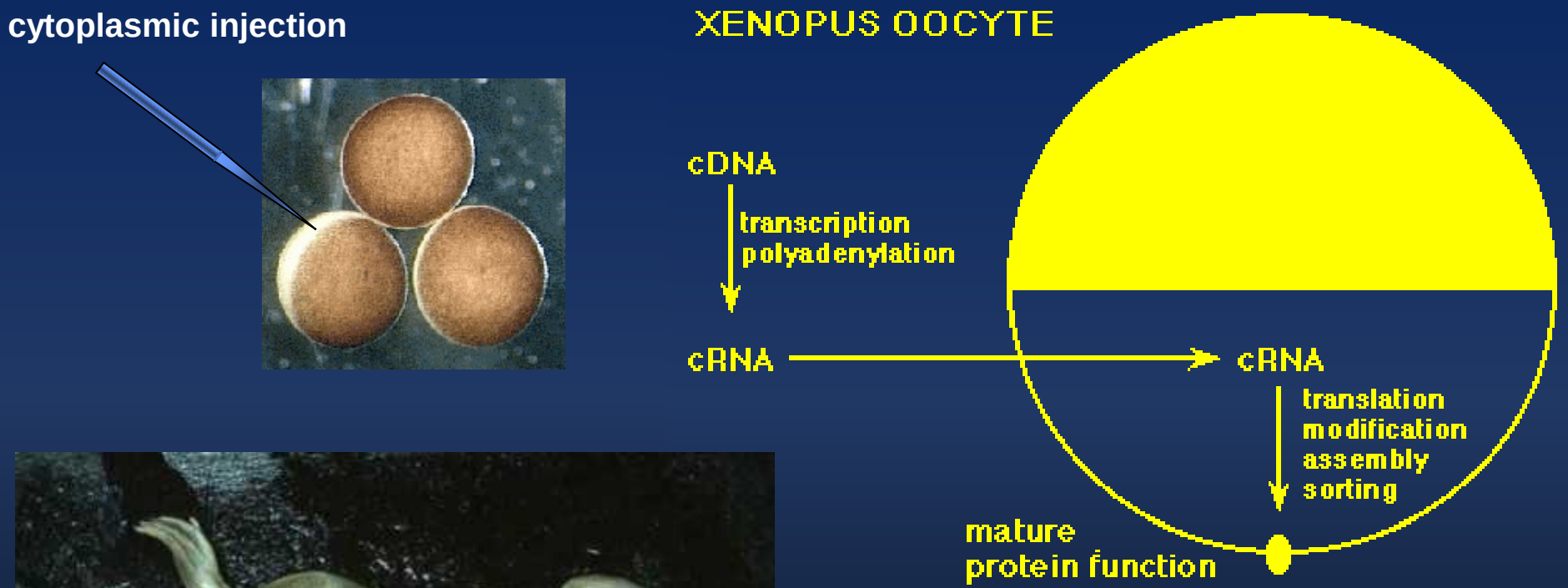
Zebrafish *slc15a1* (*pept1*)

Zebrafish *slc15a1* (*pept1*): gene, mRNA and predicted protein structure



- *slc15a1* (*pept1*) gene: 37,702 bp
- *slc15a1* (*pept1*) mRNA: 2,746 bp (open reading frame: 2,157 bp)
- Slc15a1 (Pept1) protein: 718 aa (12 transmembrane domains, 6 Asn glycosylation sites, 1 PKA site, 2 PKC sites)

Expression of membrane transporters in *Xenopus laevis* oocytes



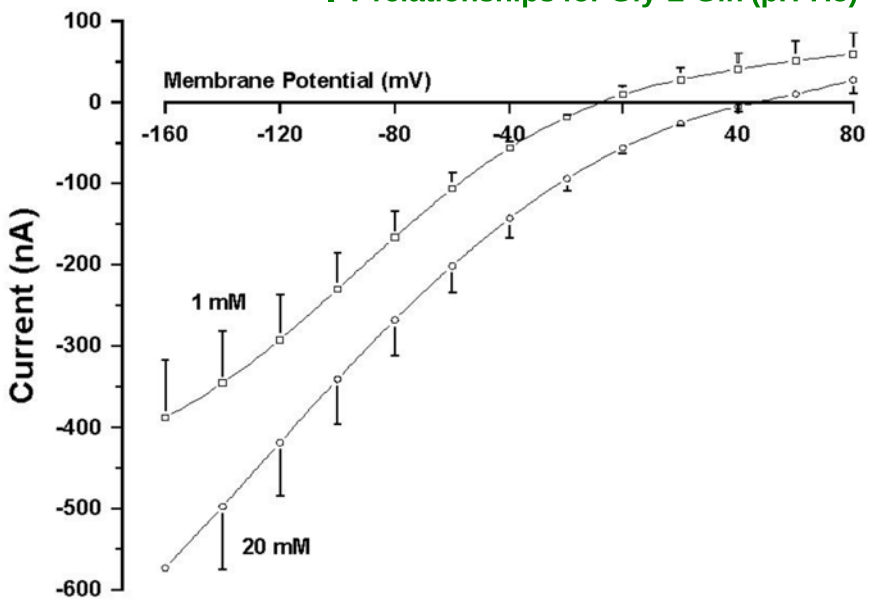
Analysis of the expressed protein

- Radioactive tracers
- Two-Electrode Voltage Clamp
- Others

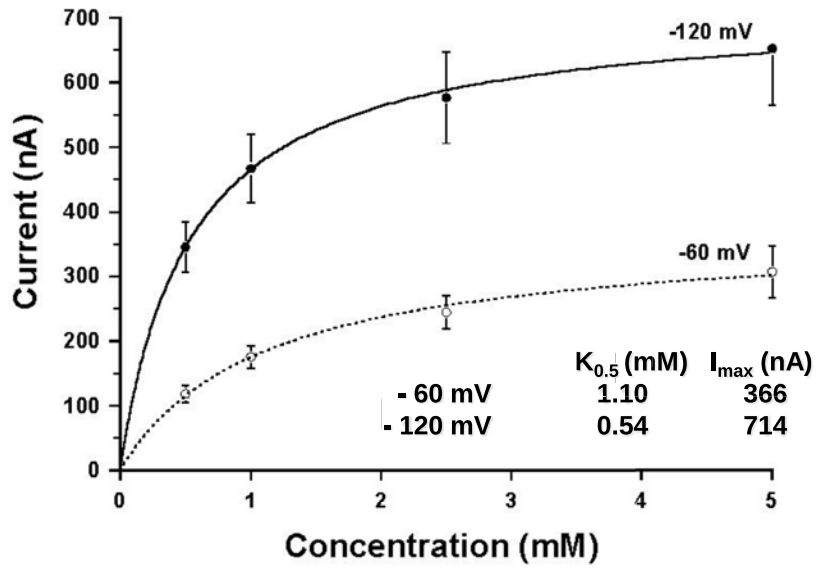
Romero et al., Methods Enzymol. 296:17 (1998)

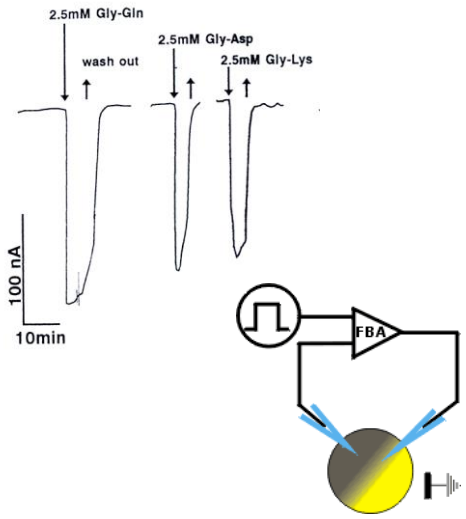
Kinetic analysis (by Two-Electrode Voltage Clamp)

I-V relationships for Gly-L-Gln (pH 7.5)

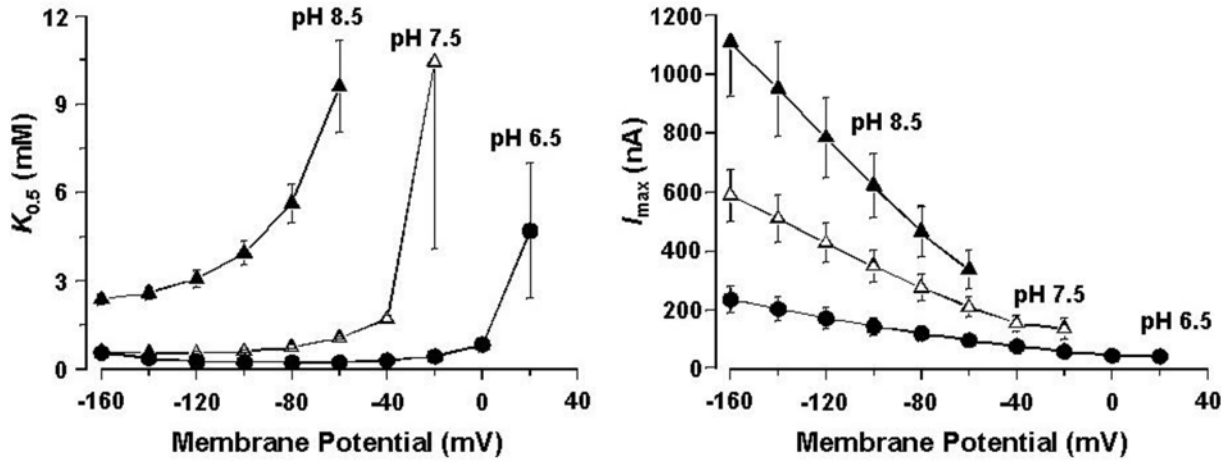


Gly-L-Gln-evoked inward currents (0.5-5 mM, pH 7.5)

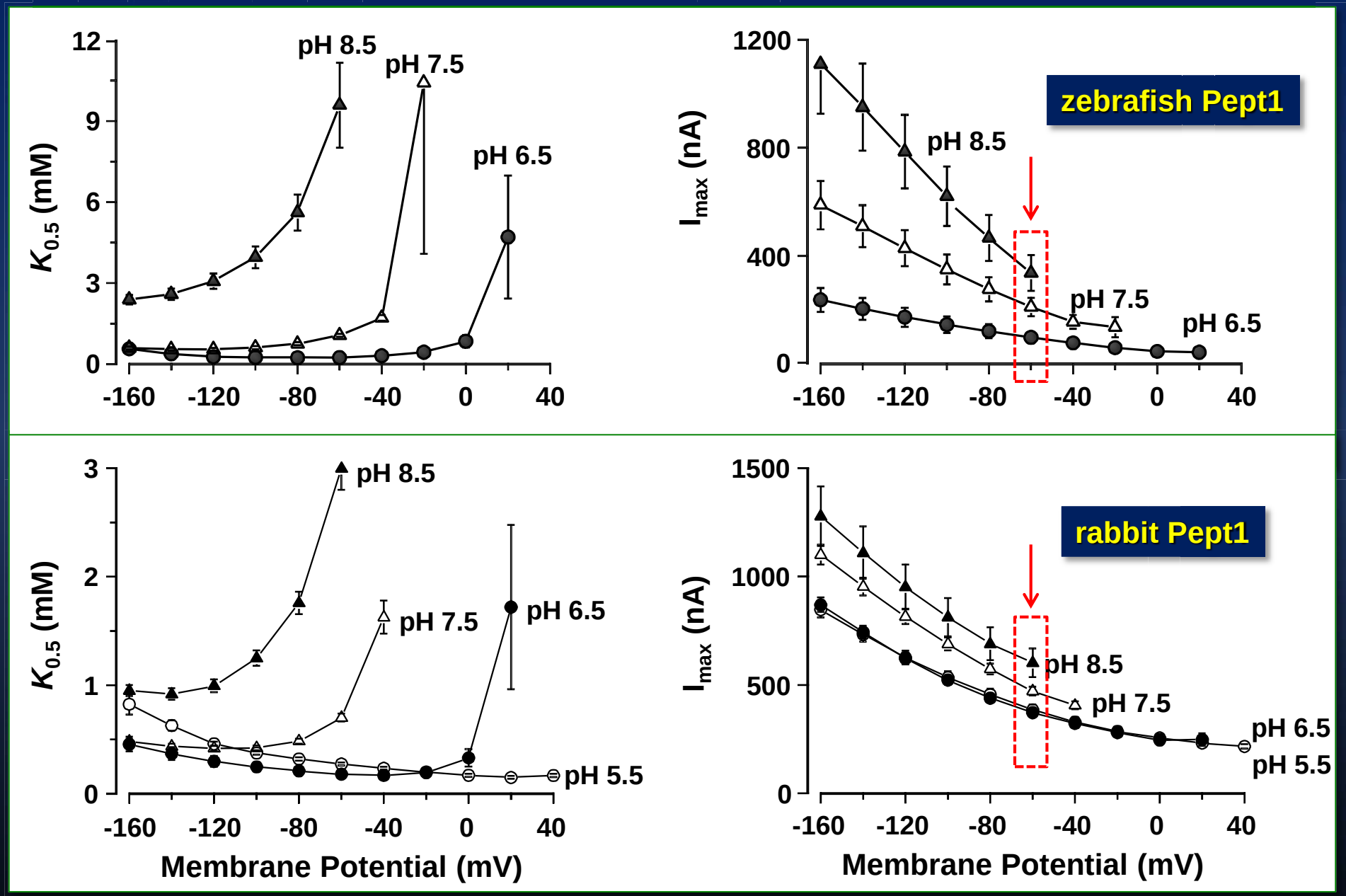




$K_{0.5}$ and I_{max} as a function of $\Delta\Psi$ and extracellular pH

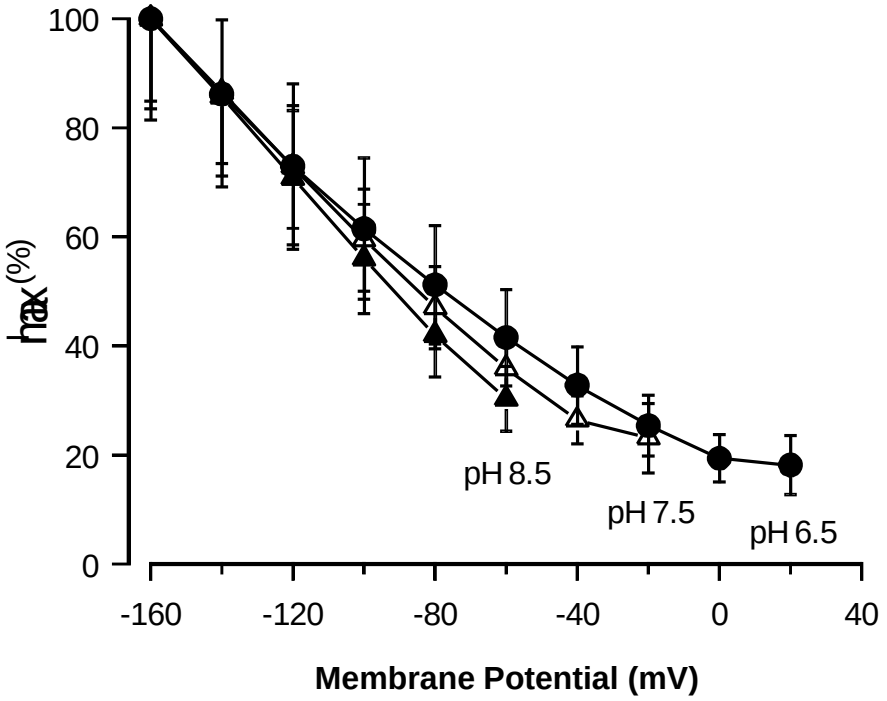


$K_{0.5}$ and I_{max} as a function of $\Delta\Psi$ and extracellular pH

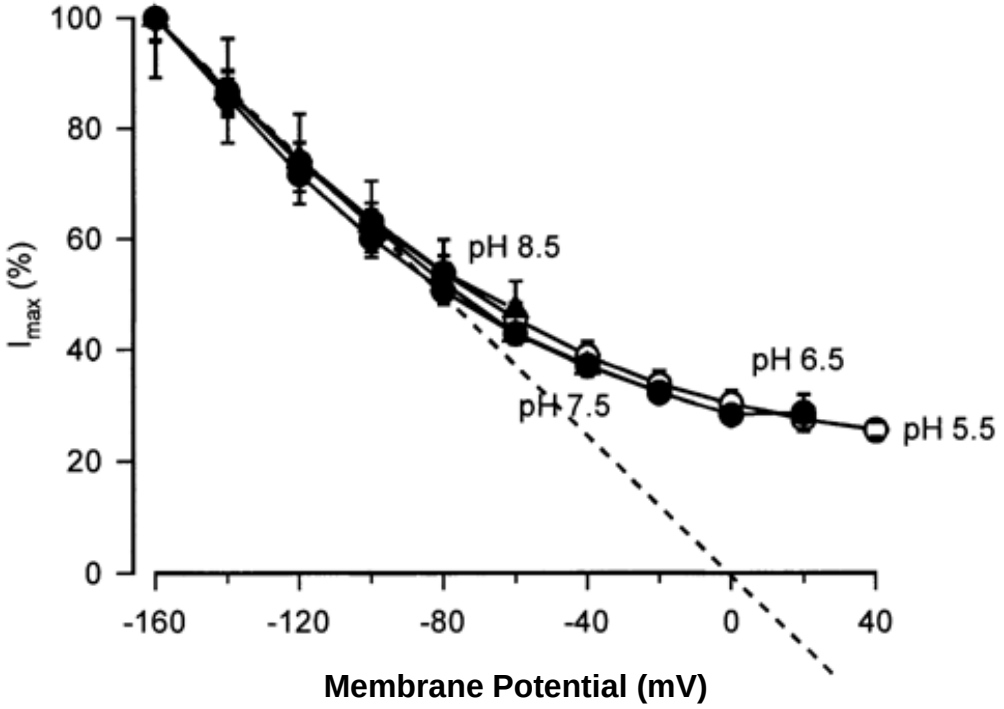


$K_{0.5}$ and I_{max} as a function of $\Delta\Psi$ and extracellular pH

zebrafish Pept1



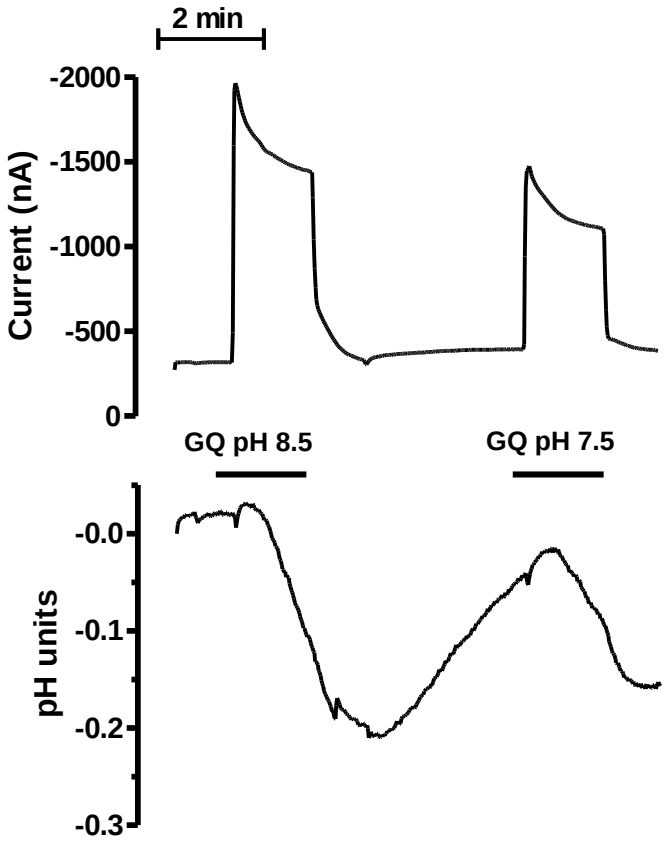
rabbit Pept1



	zfPept1	
pH	$K_{0.5}$ (mM)	I_{max} (%)
6.5	0.24 ± 0.06	43.4 ± 3.4
7.5	1.06 ± 0.07	100
8.5	9.58 ± 1.57	161.8 ± 10.0

	rPept1	
pH	$K_{0.5}$ (mM)	I_{max} (%)
6.5	0.18 ± 0.03	79 ± 4
7.5	0.70 ± 0.04	100
8.5	3.00 ± 0.20	128 ± 4

Intracellular pH/current ratio for zebrafish Pept1

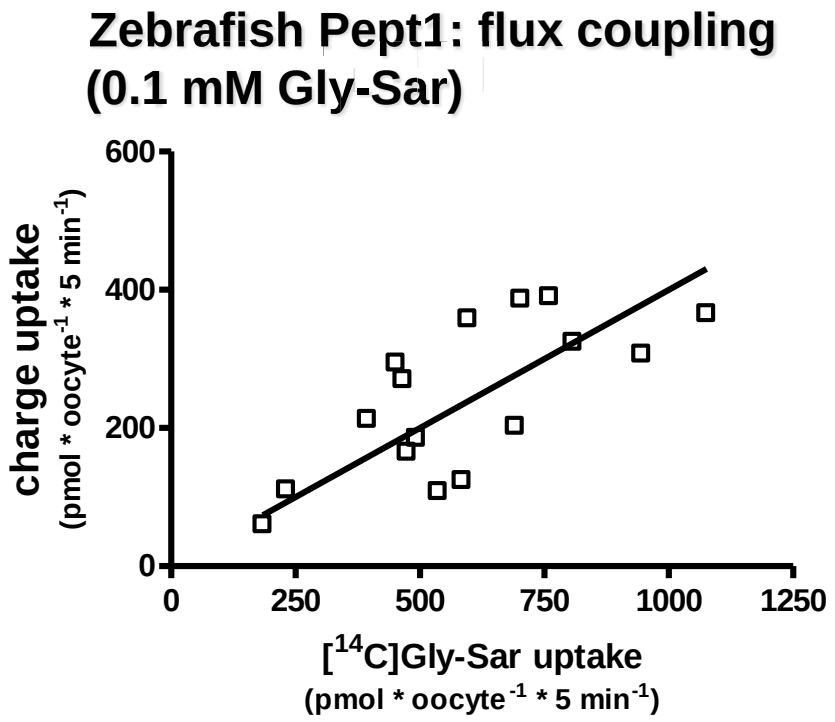


Combined TEVC and pH measurements

Substrate	Current ratio pH 8.5 / pH 7.5	pH change ratio pH 8.5 / pH 7.5	pH/current ratio pH 8.5 / pH 7.5
Gly-L-Gln	1.11 ± 0.09	1.35 ± 0.27	1. 10 ± 0.14 (n = 18)

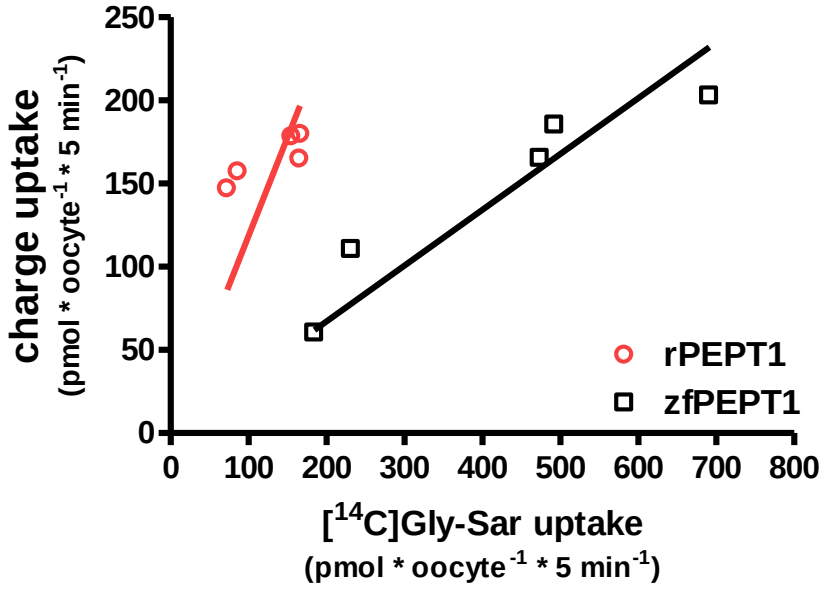
GQ was applied at 20 mM concentration. All data are relative values calculated as quotient of the values measured with GQ at pH 8.5 divided by the corresponding value measured with GQ at pH 7.5 in the same oocyte.

Substrate/charge ratio for zebrafish pept1

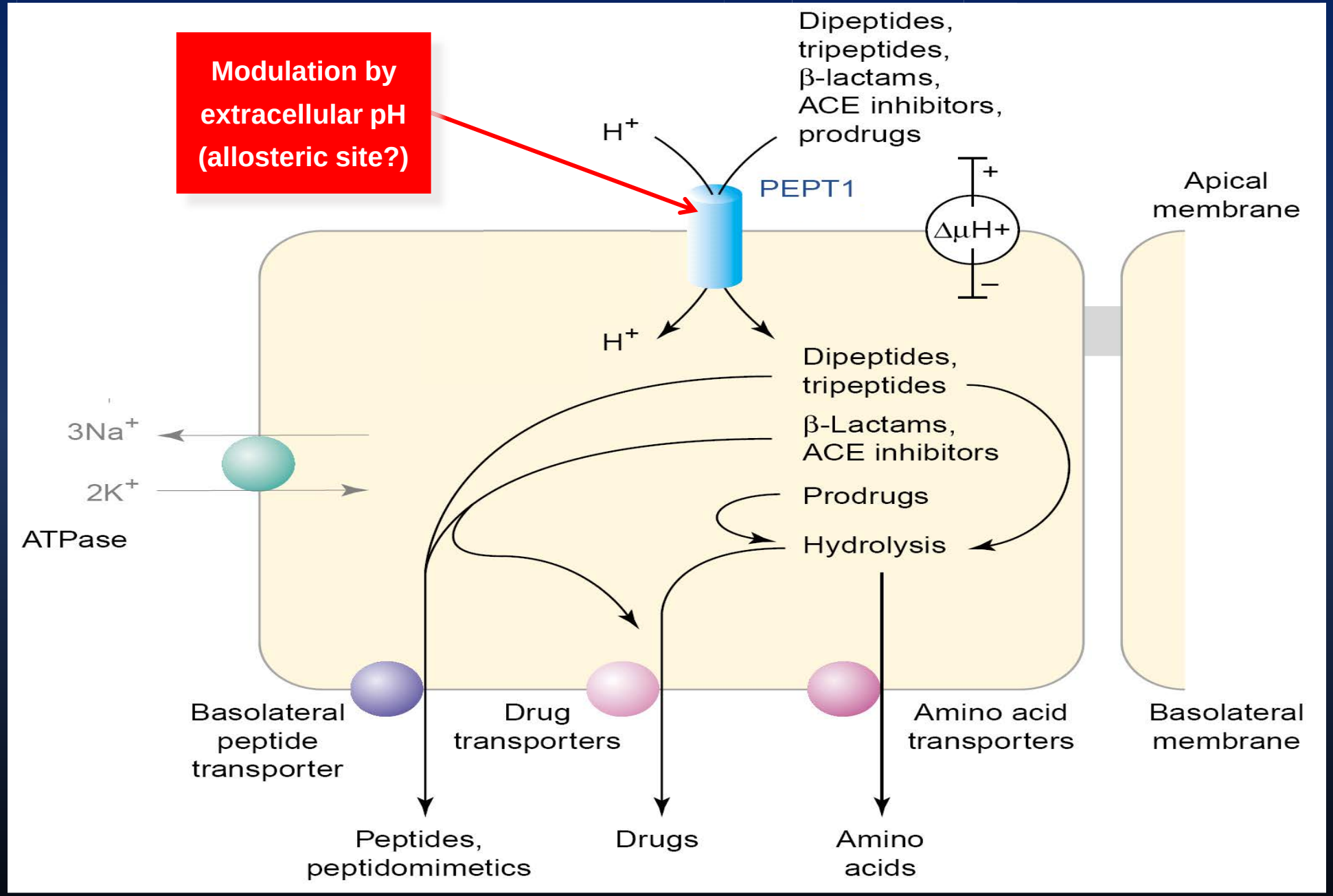


	$Q^{Gly\text{-}Sar}/[^{14}C]Gly\text{-}Sar$	N° oocytes
zfPEPT1	0.421 ± 0.035	16
rPEPT1	1.2 ± 0.19	5

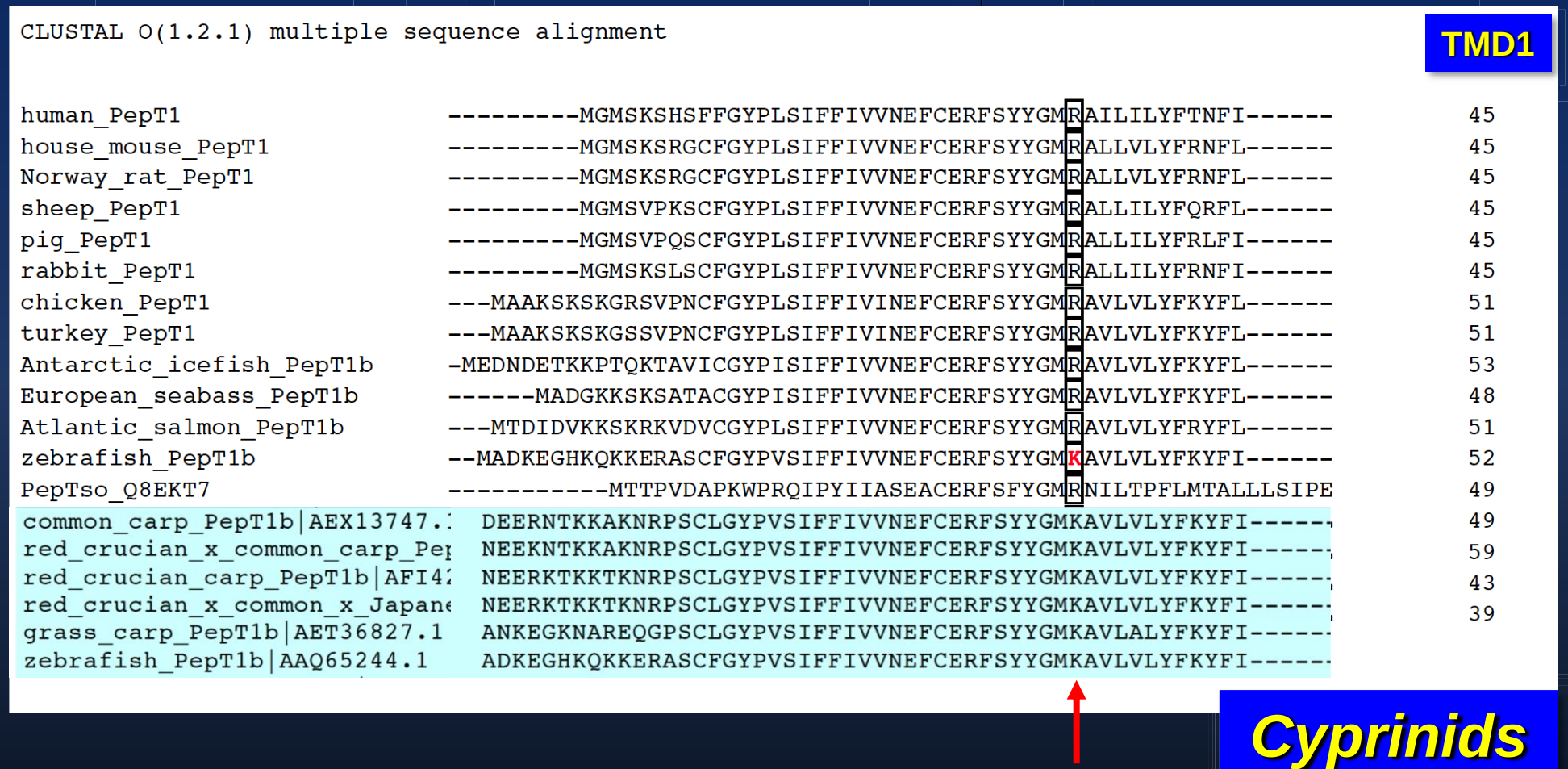
Combined TEVC and radiolabeled Gly-Sar measurements



Mode of peptide transport in zebrafish enterocytes



Pept1 - Domains 1-to-3 (pH 6.5)



- R-to-K change occurs in zebrafish TMD1

Conclusions (1)

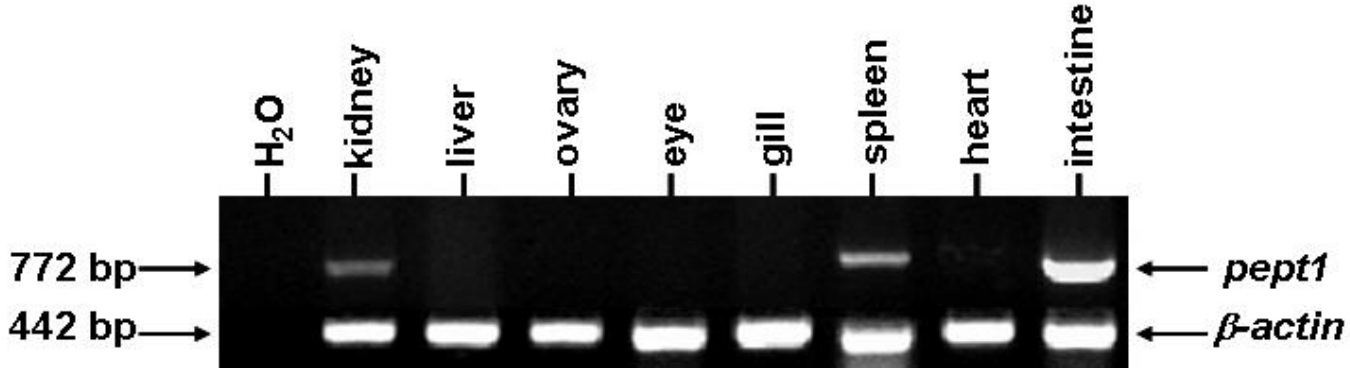
- zebrafish Slc15a1 (Pept1) is a low-affinity/high-capacity system, which operates in the 0.1-10 mM range

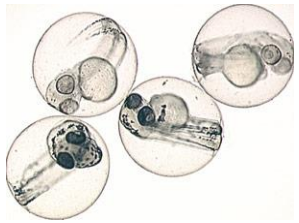
- zebrafish Slc15a1 (Pept1) is strongly affected by transmembrane potential and extracellular pH (acidic pH inhibits and alkaline pH increases I_{max}) due to a R-to-K substitution

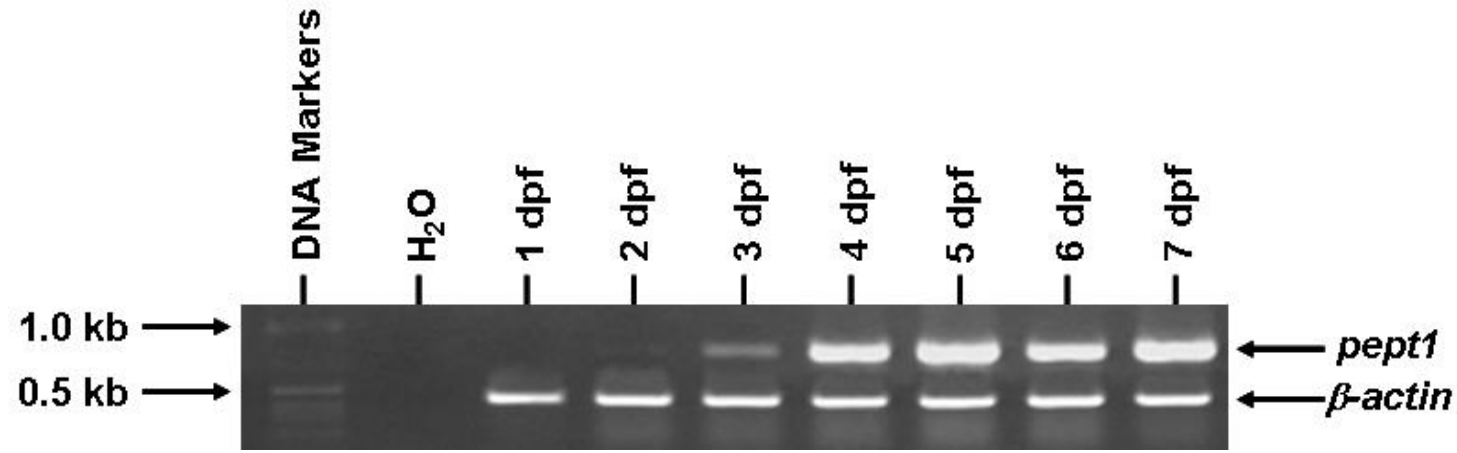
Tissue distribution …and spatial distribution along the gut

Tissue distribution of zebrafish *slc15a1* (*pept1*) in adult fish and embryos (RT-PCR)

slc15a1
(*pept1*)

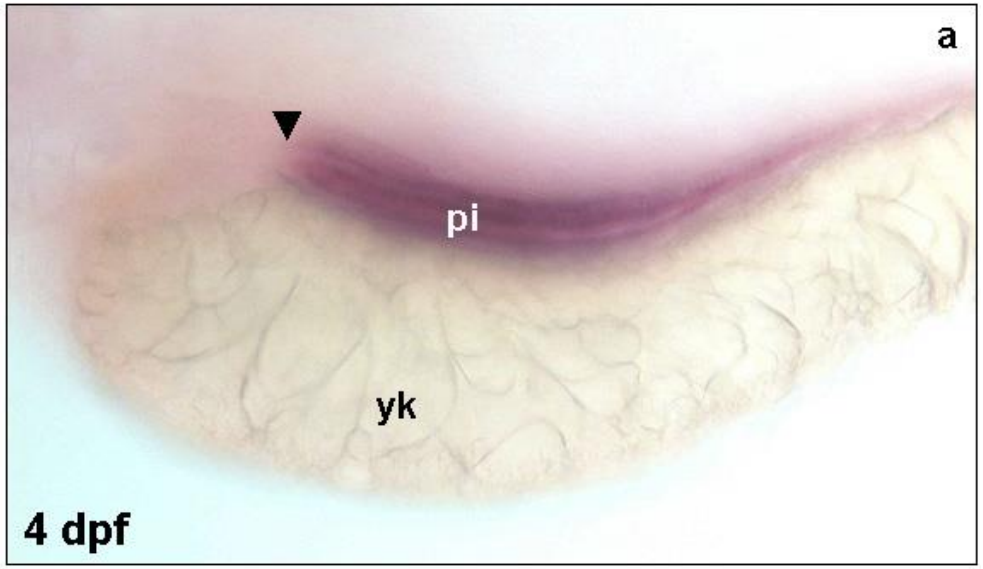


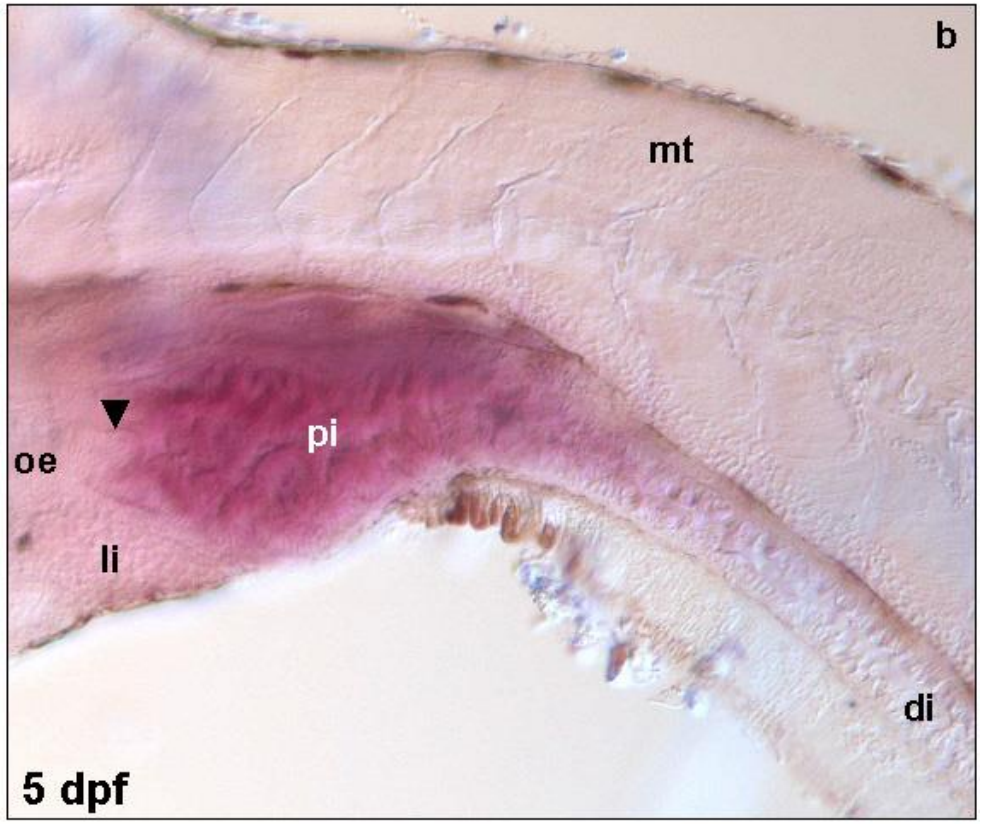


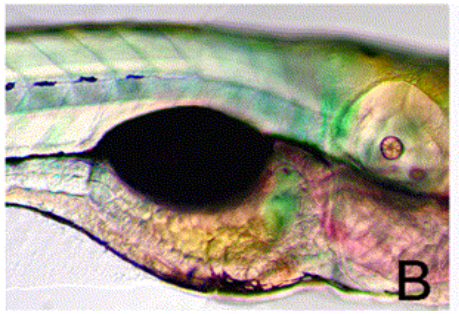


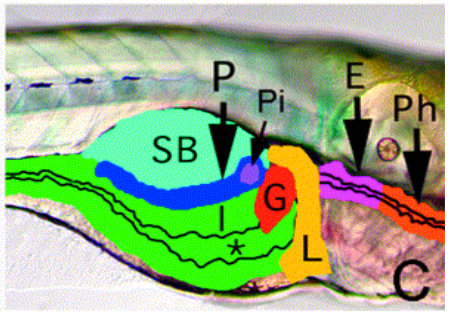
Verri et al., FEBS Lett. 549:115 (2003)

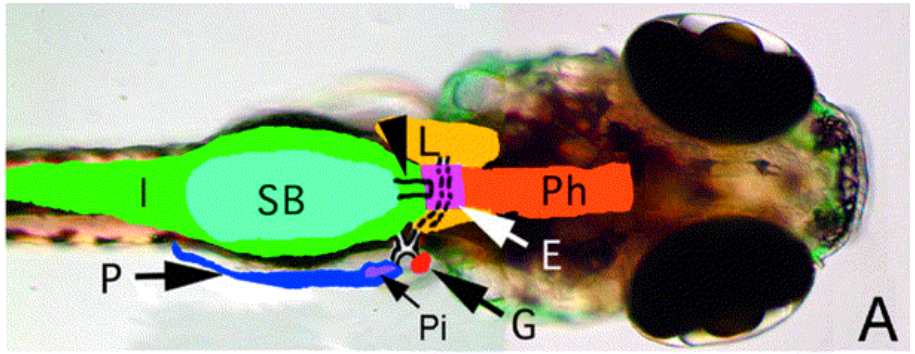
Zebrafish *slc15a1* (*pept1*) (whole mount in situ hybridization)

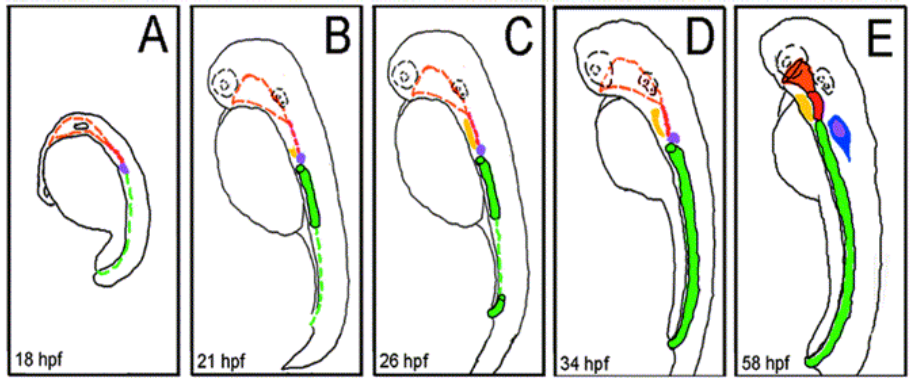




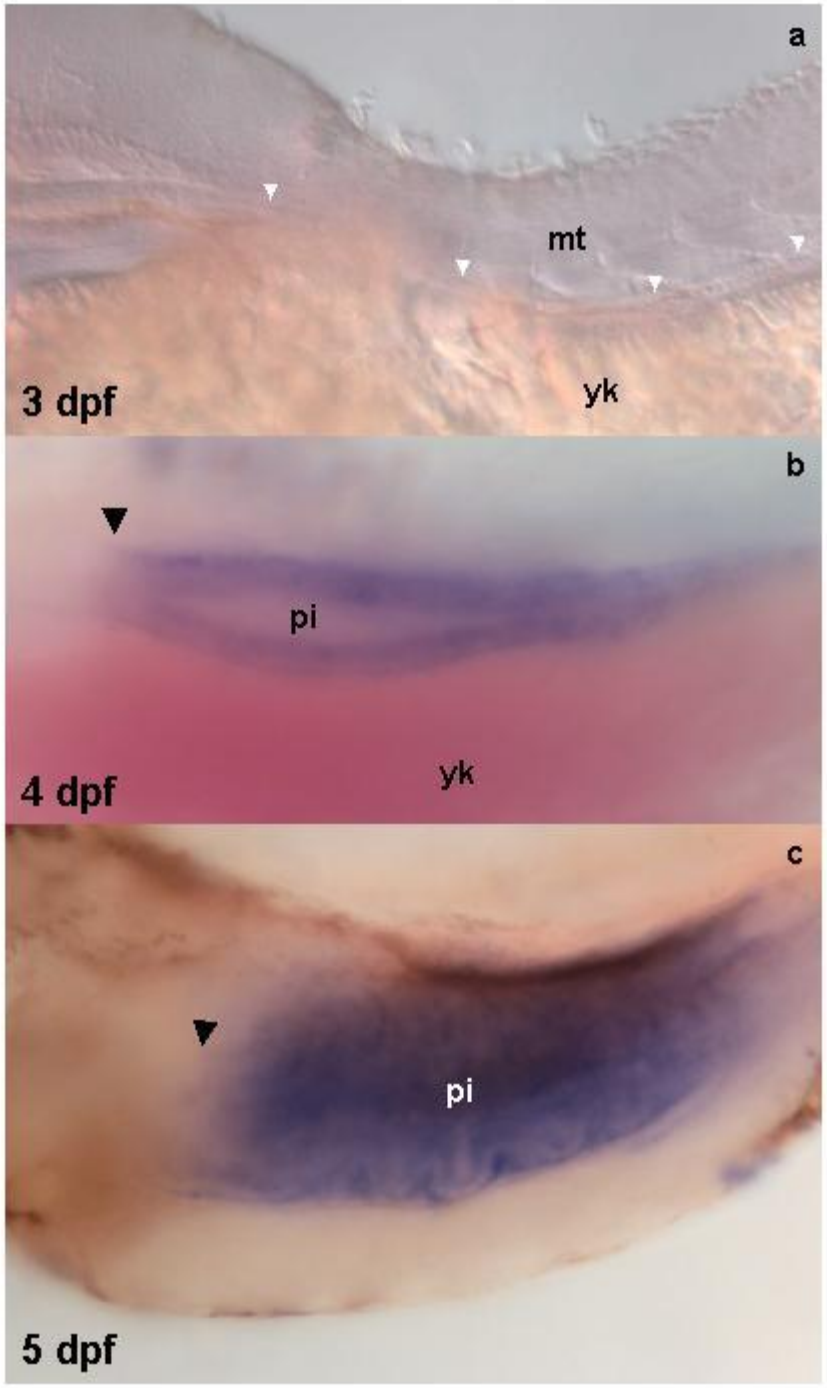


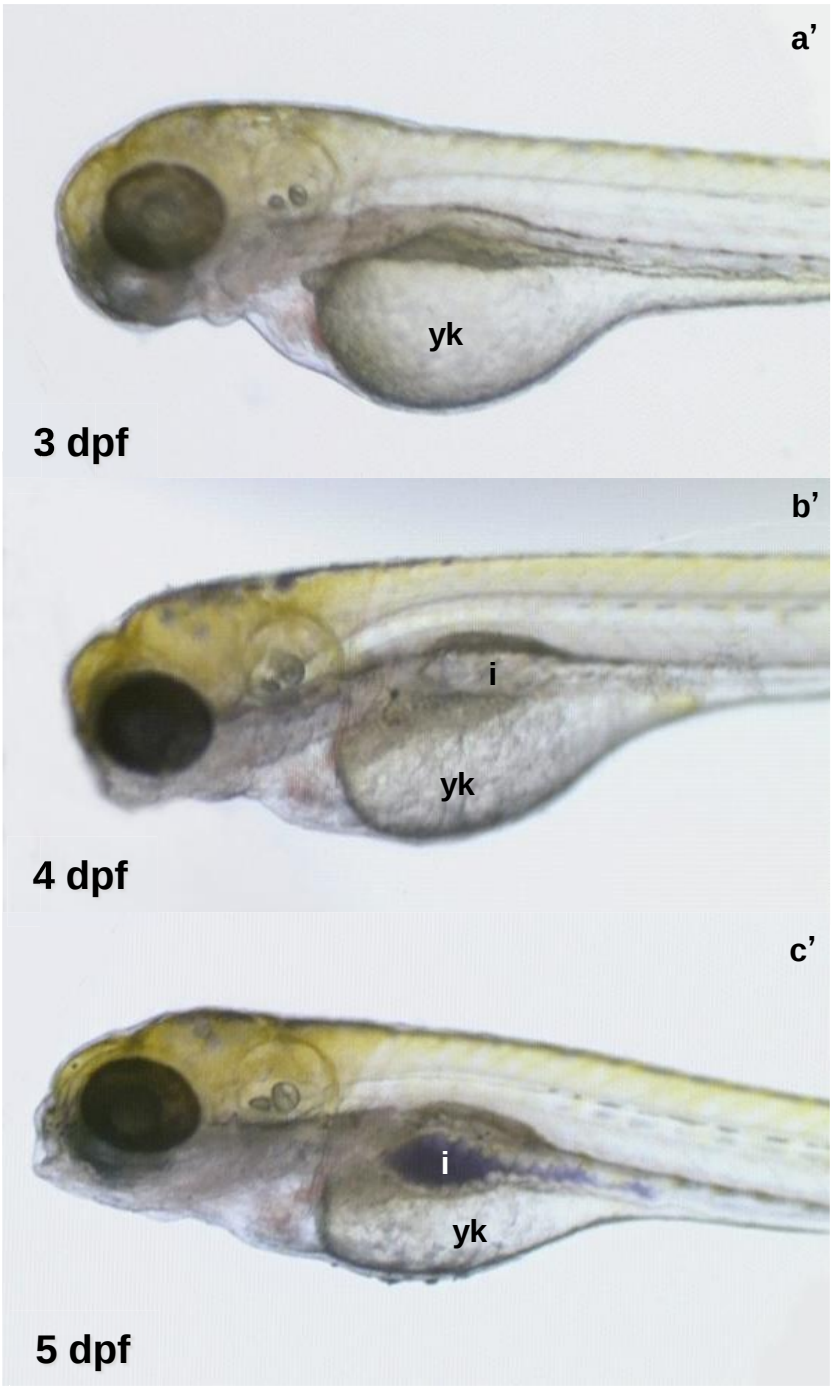




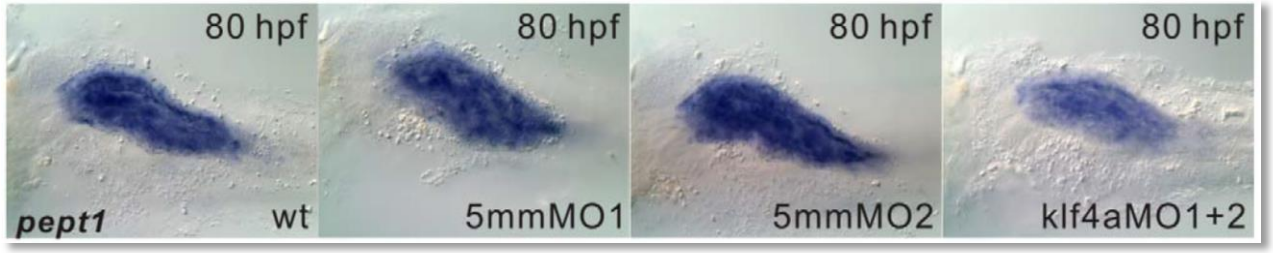


Timing of zebrafish *slc15a1* (*pept1*) expression



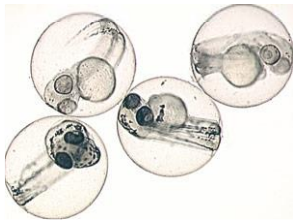


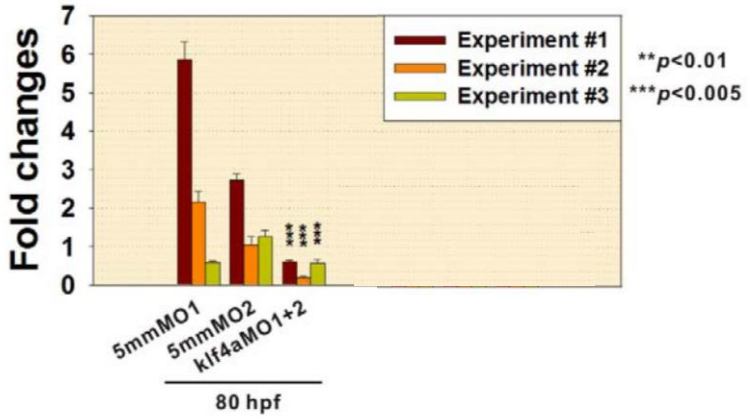
Spatial distribution of *slc15a1* (*pept1*) mRNA in the digestive tract (RT-PCR)



***pept1* as a marker of terminal enterocyte differentiation**

zebrafish



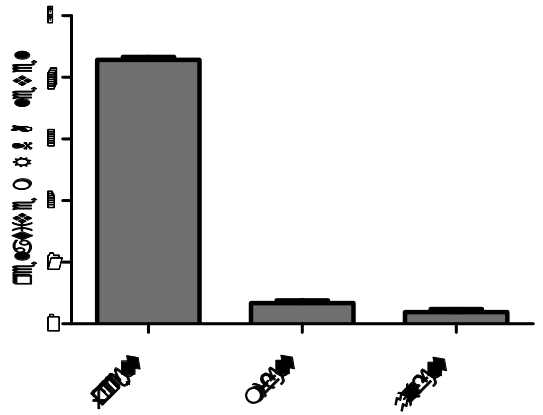


Kruppel-like factor 4a (klf4a) represses intestinal cell proliferation. It is required for the differentiation of goblet cells and the terminal differentiation of enterocytes

Data adapted from:
Li et al., PLOS One 6:e20974 (2011)

Liu et al., Comp. Biochem. Physiol B Biochem. Mol. Physiol. 164:194 (2013)

grass carp

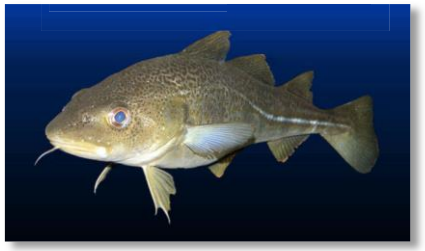


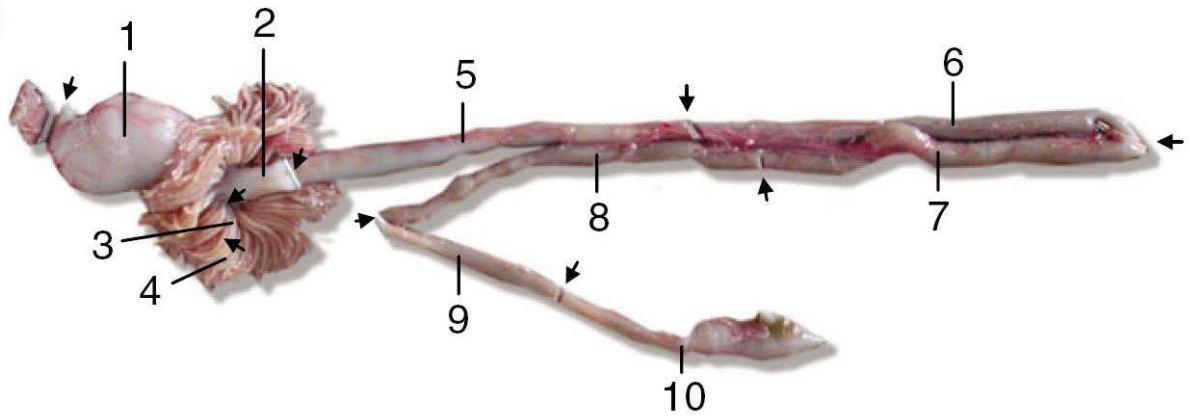
… translating findings in zebrafish to other species

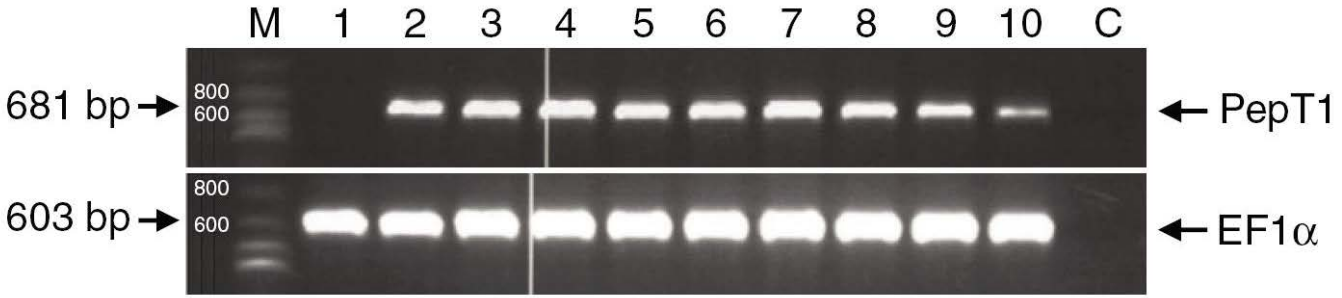
Spatial distribution of *slc15a1* (*pept1*) mRNA in the digestive tract (RT-PCR)

Rønnestad et al., J. Exp. Biol. 210:3883 (2007)

Atlantic cod



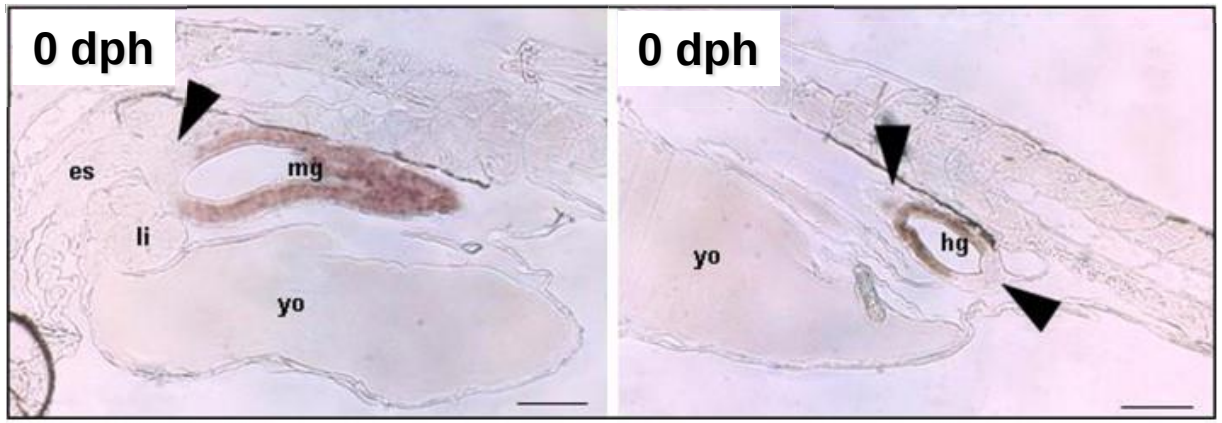




Amberg et al., Comp. Biochem. Physiol. B Biochem. Mol. Biol. 150:177 (2008)

(in situ hybridization)

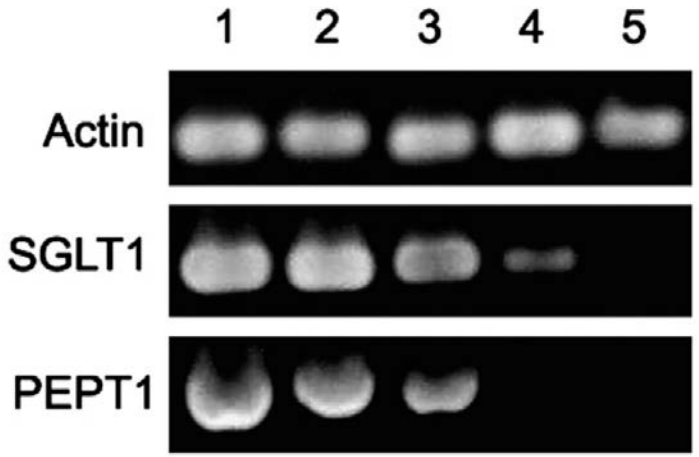
Atlantic cod



Spatial distribution of pept1 mRNA in the digestive tract (RT-PCR)

Gonçalves et al., Comp. Biochem. Physiol. Part D Genomics Proteomics 2:345 (2007)

Asian weatherloach



1. Anterior zone of the foregut
2. Posterior zone of the foregut
3. Midgut
4. Anterior zone of the hindgut
5. Posterior zone of the hindgut

Rønnestad et al., J. Nutr. 140:893 (2010)

Atlantic salmon