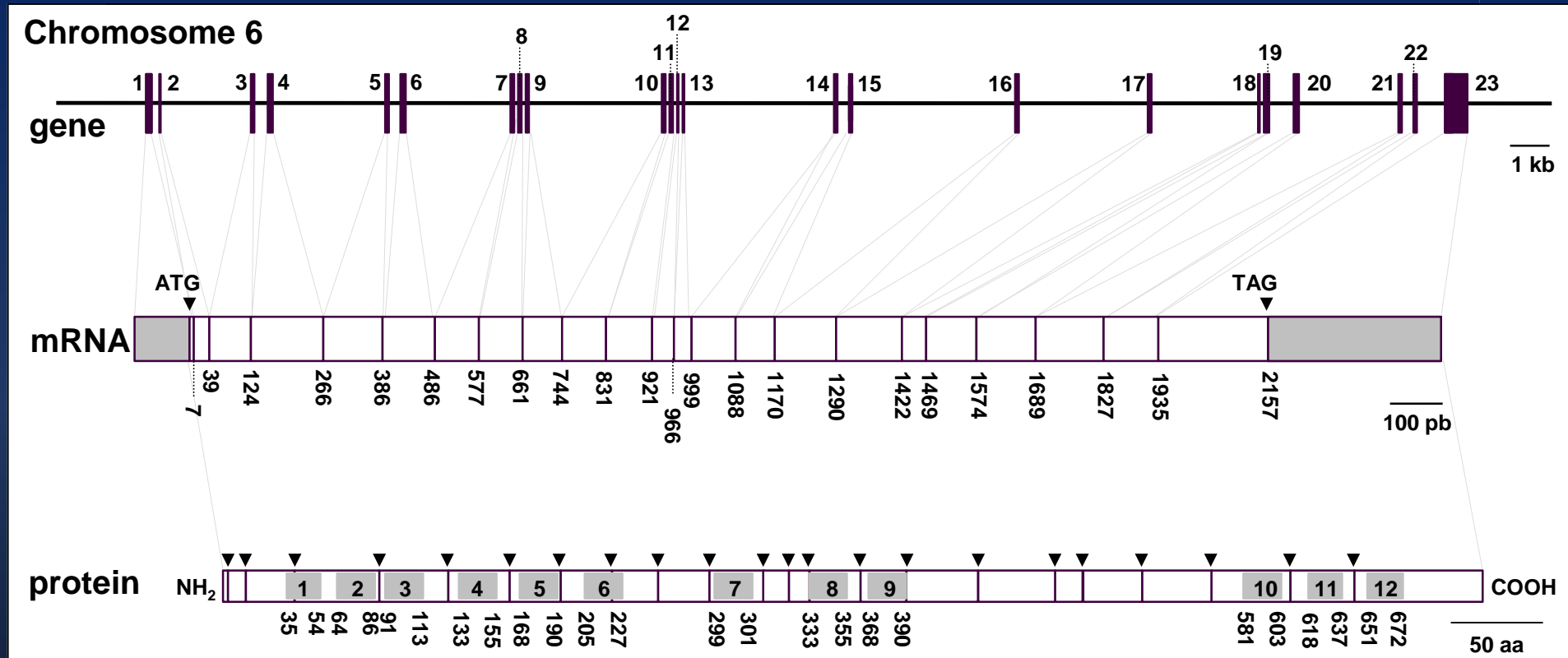


Zebrfish *slc15a1*
(*pept1*)

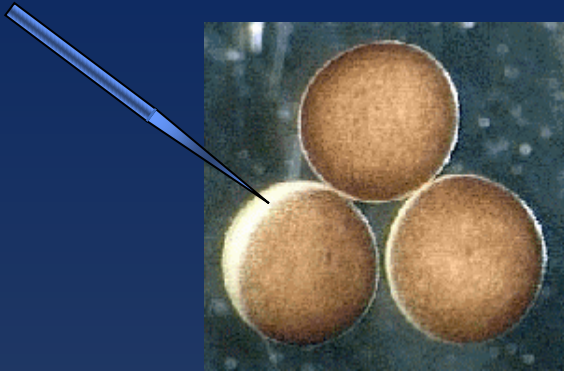
Zebrafish *slc15a1* (*pept1*): gene, mRNA and predicted protein structure



- *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

cytoplasmic injection



XENOPUS OOCYTE

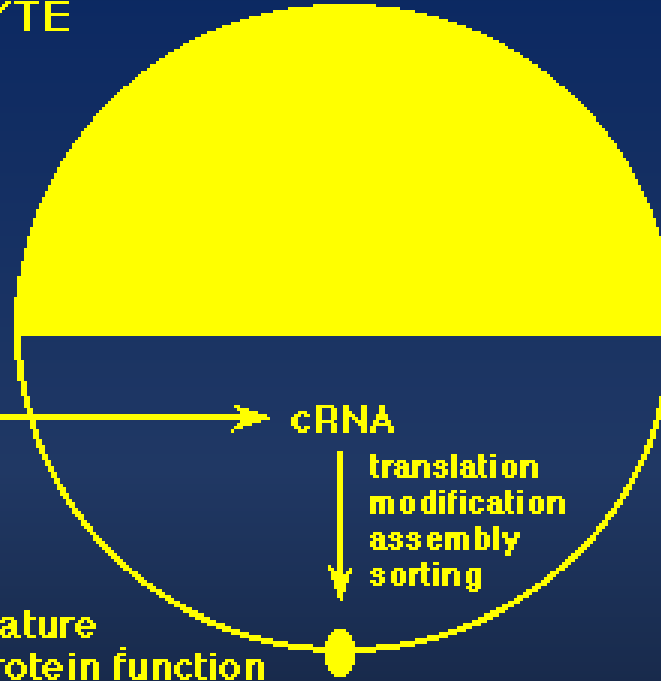
cDNA
↓ transcription
polyadenylation

crRNA

→ crRNA

↓ translation
modification
assembly
sorting

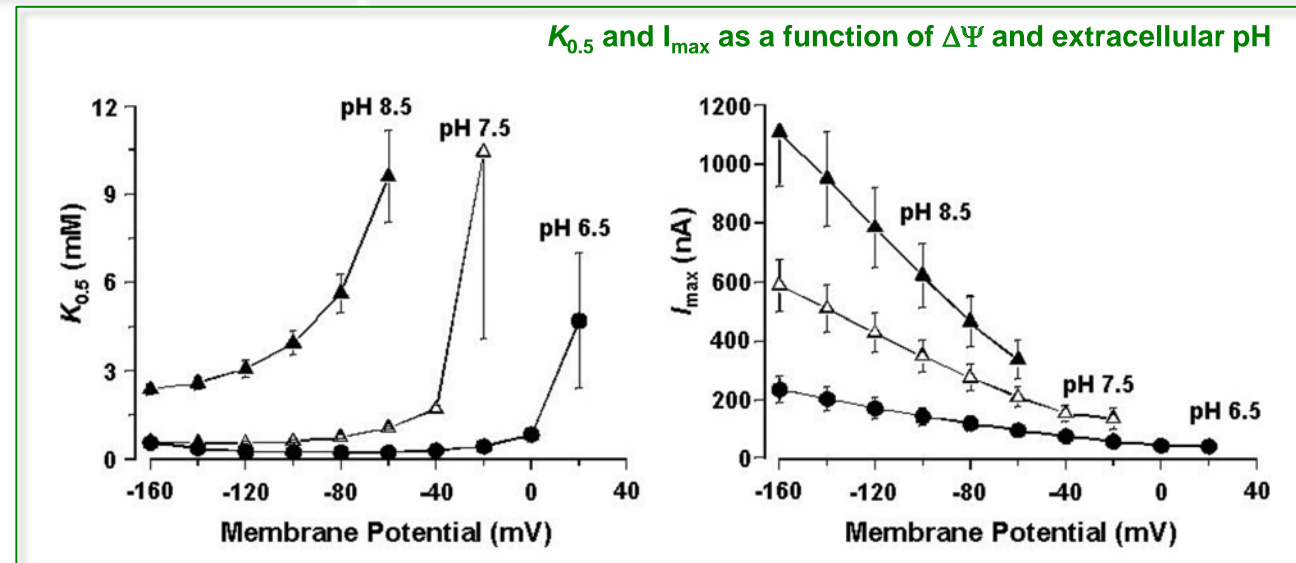
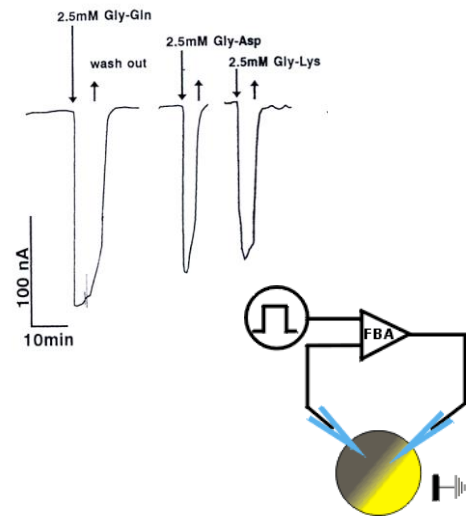
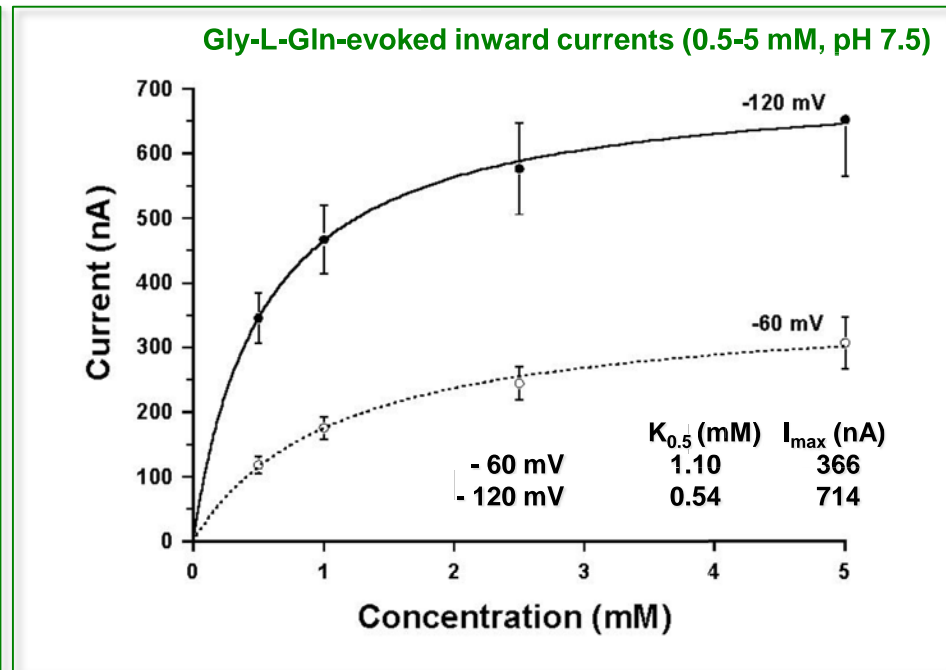
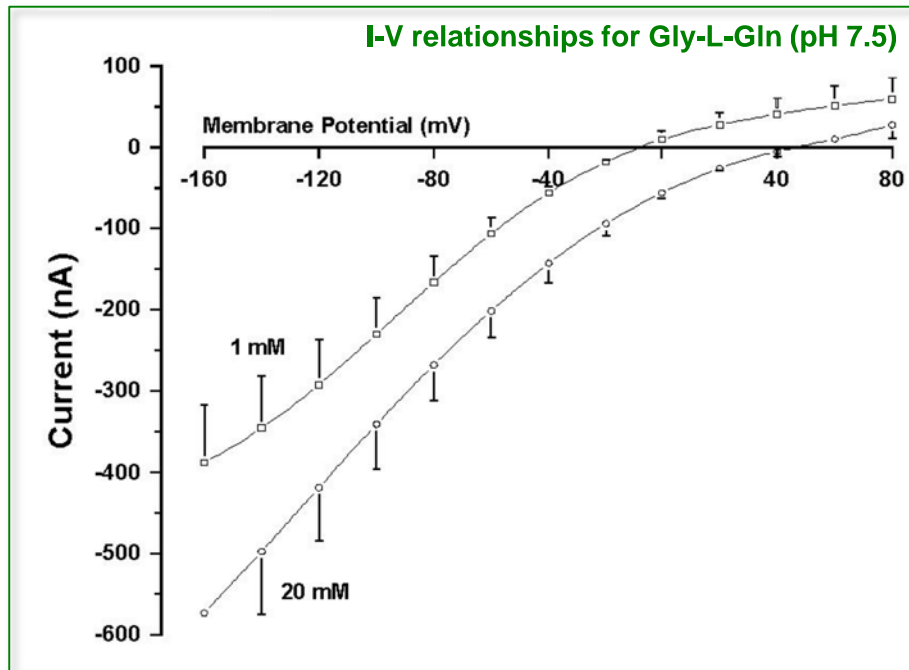
mature
protein function



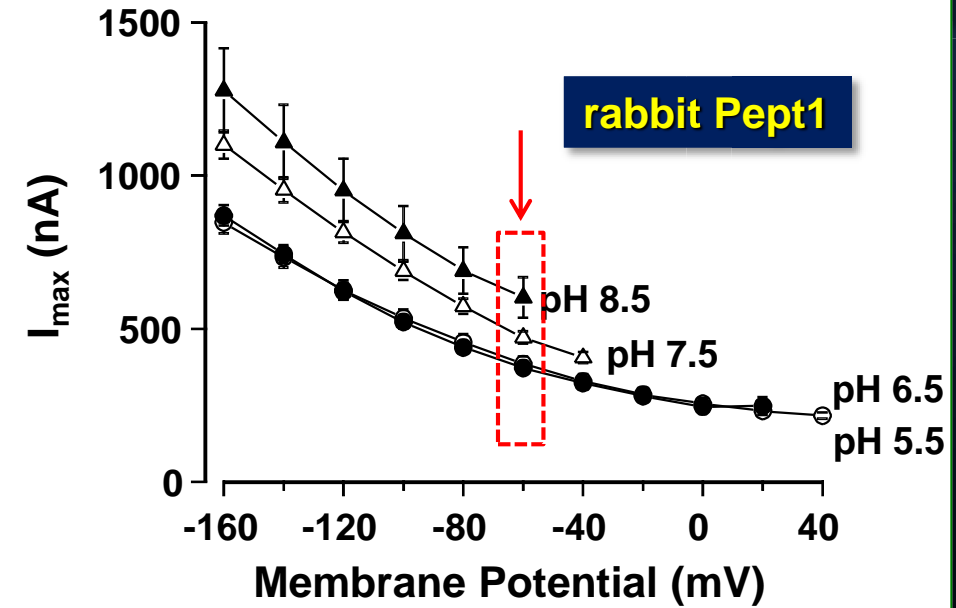
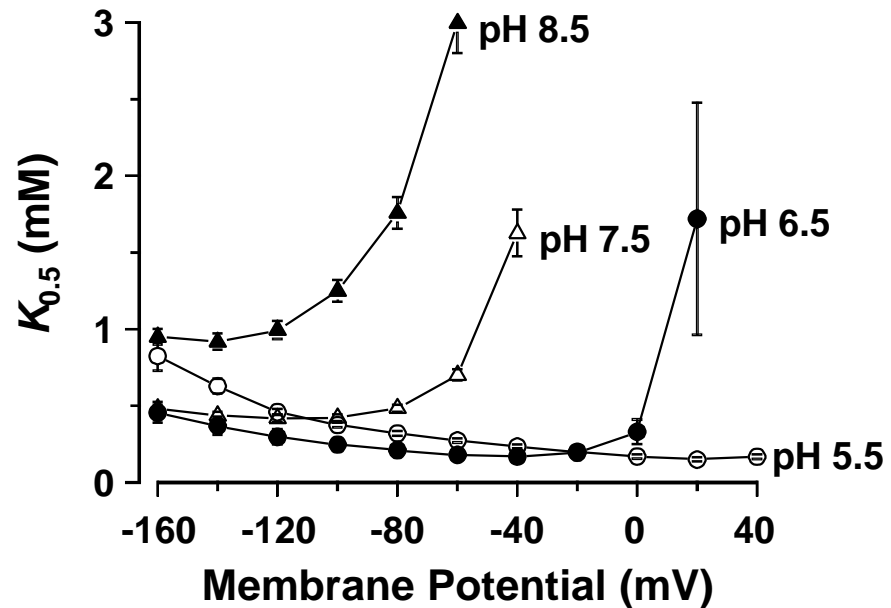
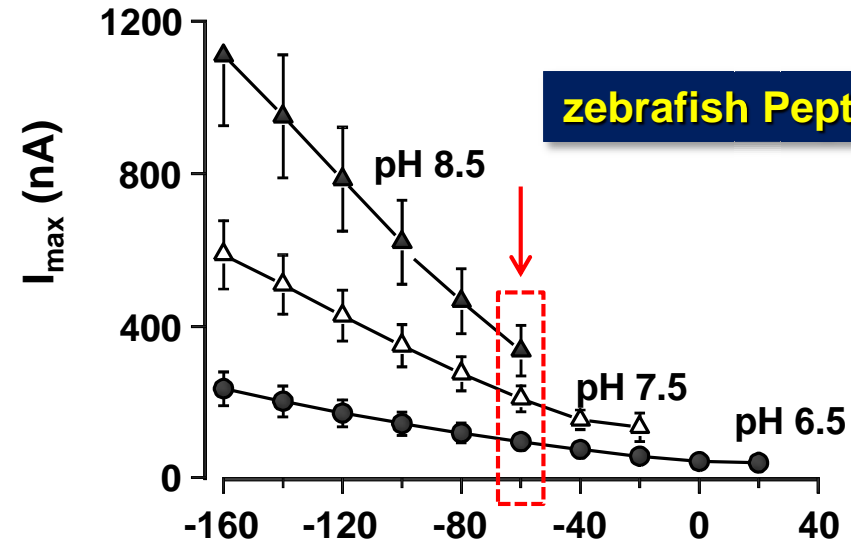
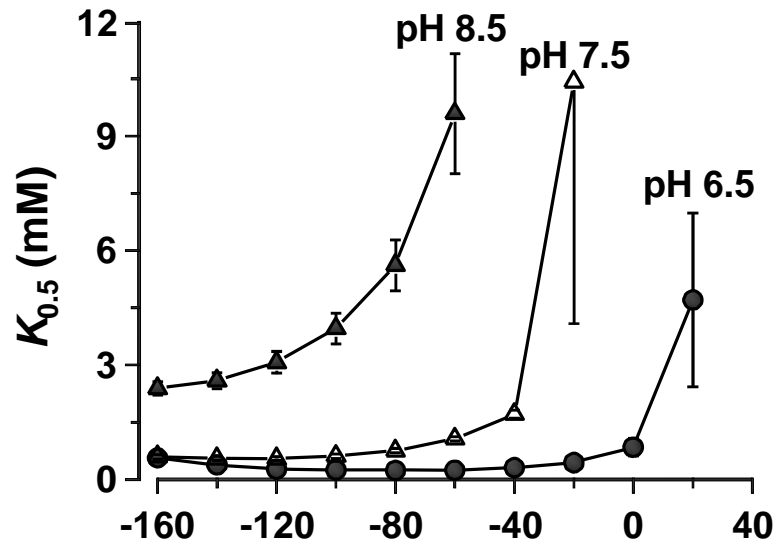
Analysis of the expressed protein

- Radioactive tracers
- Two-Electrode Voltage Clamp
- Others

Kinetic analysis (by Two-Electrode Voltage Clamp)

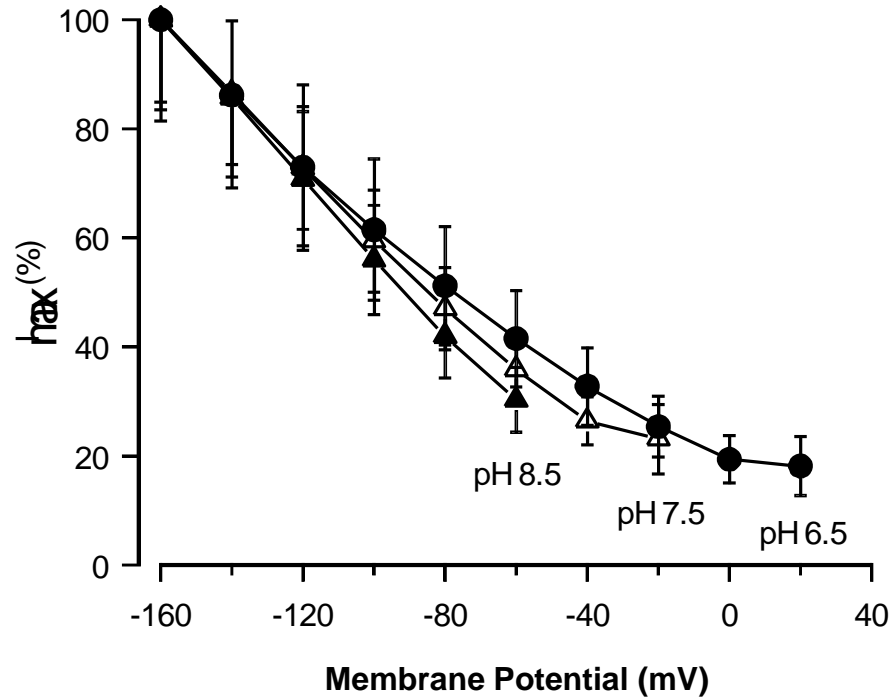


$K_{0.5}$ and I_{max} as a function of $\Delta\Psi$ and extracellular pH



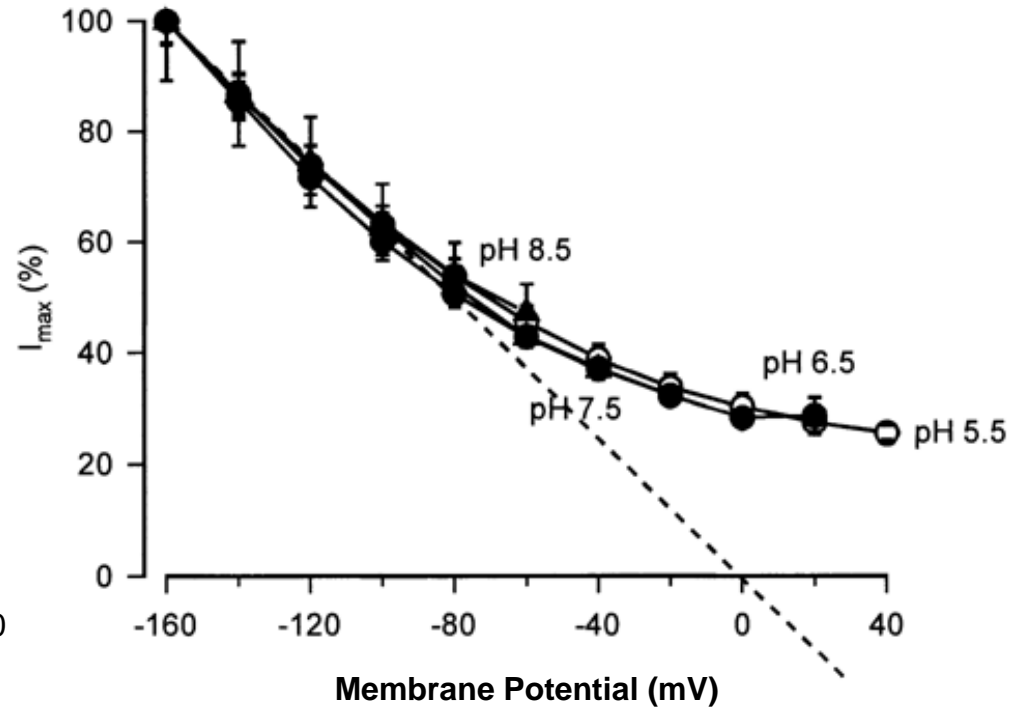
$K_{0.5}$ and I_{max} as a function of $\Delta\Psi$ and extracellular pH

zebrafish Pept1



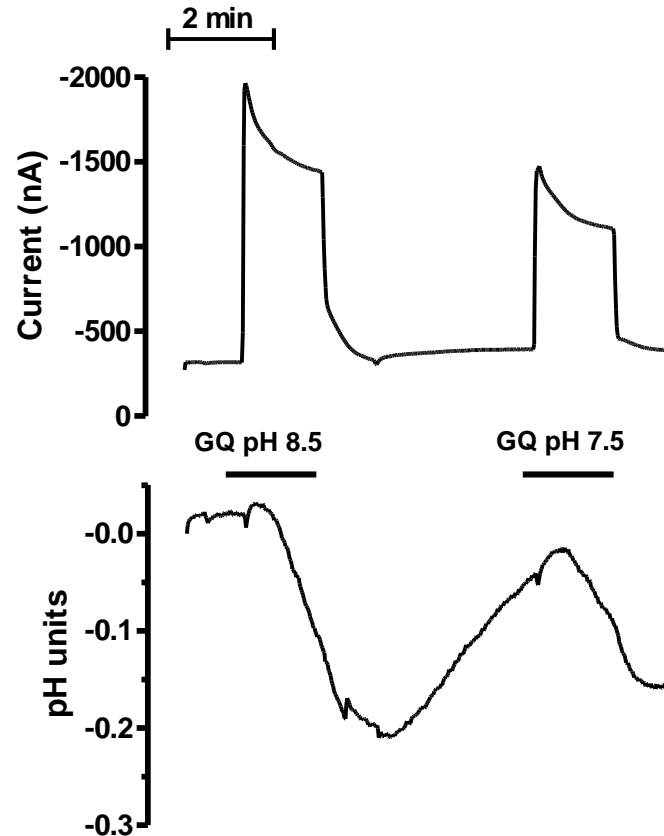
	zfPept1	
pH	$K_{0.5}$ (mM)	I_{max} (%)
6.5	0.24 ± 0.06	43.4 ± 3.4
7.5	1.06 ± 0.07	100
8.5	9.58 ± 1.57	161.8 ± 10.0

rabbit Pept1



	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



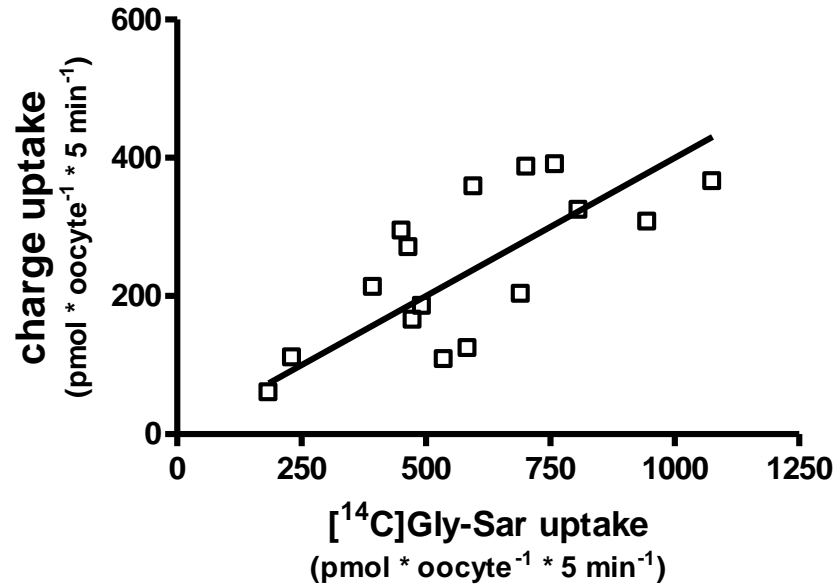
Combined TEVC and
pH measurements

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)

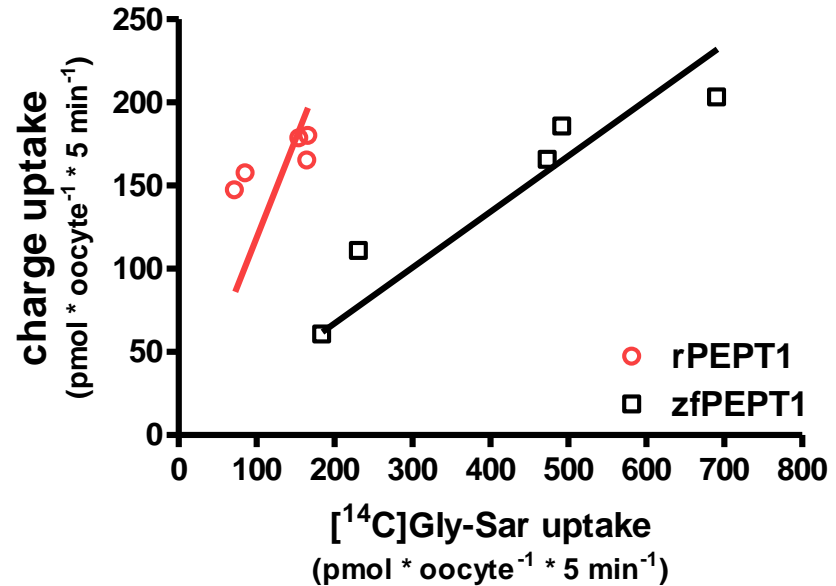
GQ was applied at 20 mM concentration. All data are relative values calculated as quotient of the values measured with GQ at pH 8.5 divided by the corresponding value measured with GQ at pH 7.5 in the same oocyte.

Substrate/charge ratio for zebrafish pept1

Zebrafish Pept1: flux coupling
(0.1 mM Gly-Sar)

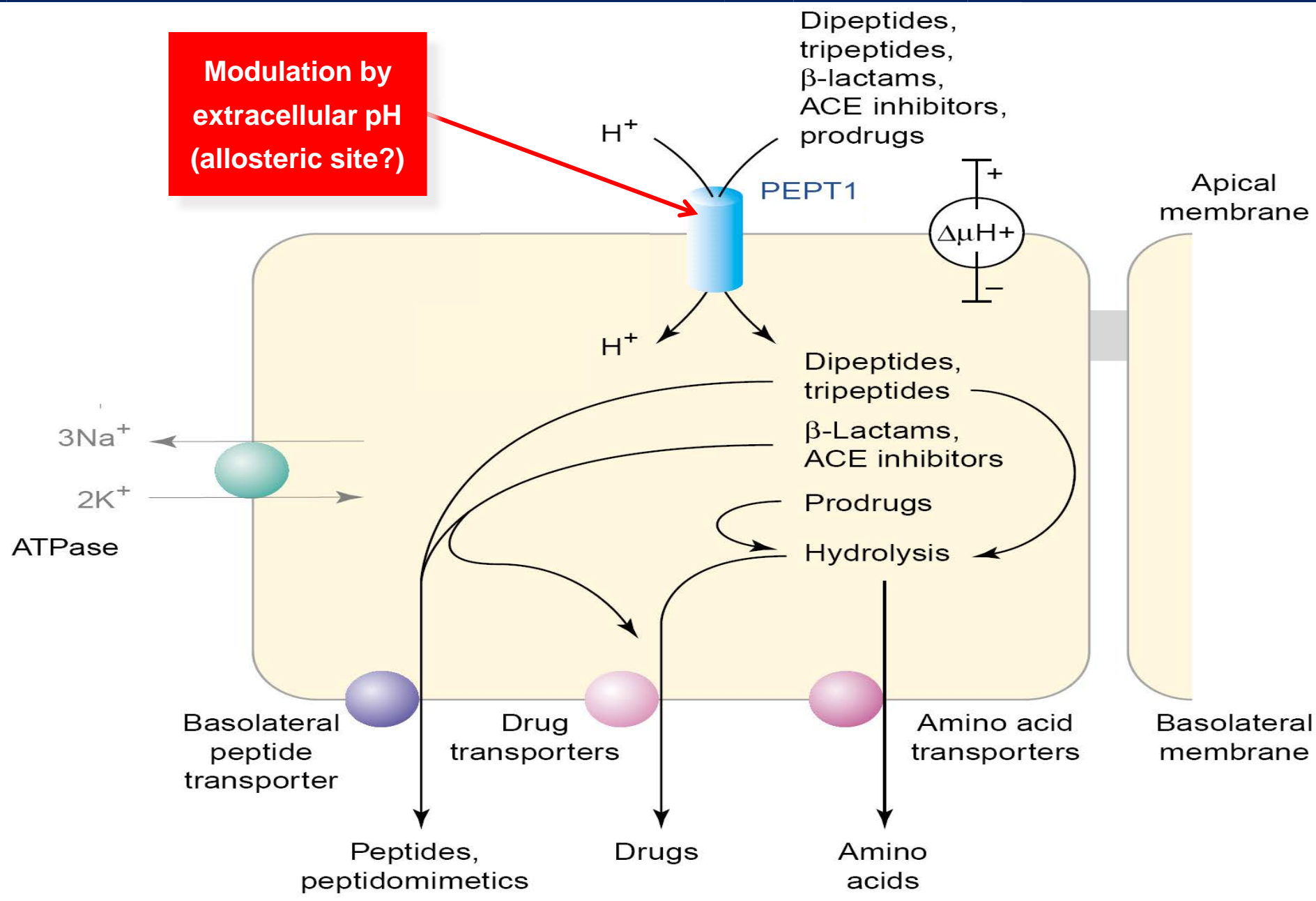


Combined TEVC and
radiolabeled Gly-Sar
measurements



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

Mode of peptide transport in zebrafish enterocytes



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

CLUSTAL O(1.2.1) multiple sequence alignment

TMD1

```
human_PepT1          -----MGMSKSHSFFGYPLSIFFFIVVNEFCERFSYYGMRRAILILYFTNFI----- 45
house_mouse_PepT1    -----MGMSKSRGCFGYPLSIFFFIVVNEFCERFSYYGMRRAALLVLYFRNFL----- 45
Norway_rat_PepT1     -----MGMSKSRGCFGYPLSIFFFIVVNEFCERFSYYGMRRAALLVLYFRNFL----- 45
sheep_PepT1          -----MGMSVPKSCFGYPLSIFFFIVVNEFCERFSYYGMRRAALLILYFQRF----- 45
pig_PepT1            -----MGMSVPQSCFGYPLSIFFFIVVNEFCERFSYYGMRRAALLILYFRLF----- 45
rabbit_PepT1         -----MGMSKSLSCFGYPLSIFFFIVVNEFCERFSYYGMRRAALLILYFRNFI----- 45
chicken_PepT1        ---MAAKSKSKGRSVPNCFGYPLSIFFFIVINEFCERFSYYGMRRAVLVLYFKYFL----- 51
turkey_PepT1         ---MAAKSKSKGSSVPNCFGYPLSIFFFIVINEFCERFSYYGMRRAVLVLYFKYFL----- 51
Antarctic_icefish_PepT1b -MEDNDETKKPTQKTAVICGYPISIFFFIVVNEFCERFSYYGMRRAVLVLYFKYFL----- 53
European_seabass_PepT1b -----MADGKKSATSACGYPISIFFFIVVNEFCERFSYYGMRRAVLVLYFKYFL----- 48
Atlantic_salmon_PepT1b ---MTDIDVKKSKRKVDVCGYPLSIFFFIVVNEFCERFSYYGMRRAVLVLYFRYFL----- 51
zebrafish_PepT1b     --MADKEGHKQKKERASCFGYPVSIFFFIVVNEFCERFSYYGMRRAVLVLYFKYFI----- 52
PepTso_Q8EKT7        -----MTTPVDAPKWPRQIPYIIASEACERFSFYGMRRNILTPFLMTALLLSIPE 49
common_carp_PepT1b|AEX13747.1 DEERNNTKKAKNRPSCLGYPVSIFFFIVVNEFCERFSYYGMRKAVLVLYFKYFI----- 49
red_crucian_x_common_carp_PepT1b NEEKNTKKAKNRPSCLGYPVSIFFFIVVNEFCERFSYYGMRKAVLVLYFKYFI----- 59
red_crucian_carp_PepT1b|AFI42 NEERKTKKTKNRPSCLGYPVSIFFFIVVNEFCERFSYYGMRKAVLVLYFKYFI----- 43
red_crucian_x_common_x_Japanese NEERKTKKTKNRPSCLGYPVSIFFFIVVNEFCERFSYYGMRKAVLVLYFKYFI----- 39
grass_carp_PepT1b|AET36827.1 ANKEGKNAREQGPSCLGYPVSIFFFIVVNEFCERFSYYGMRKAVLALYFKYFI-----
zebrafish_PepT1b|AAQ65244.1 ADKEGHKQKKERASCFGYPVSIFFFIVVNEFCERFSYYGMRKAVLVLYFKYFI-----
```

Cyprinids

- R-to-K change occurs in zebrafish TMD1

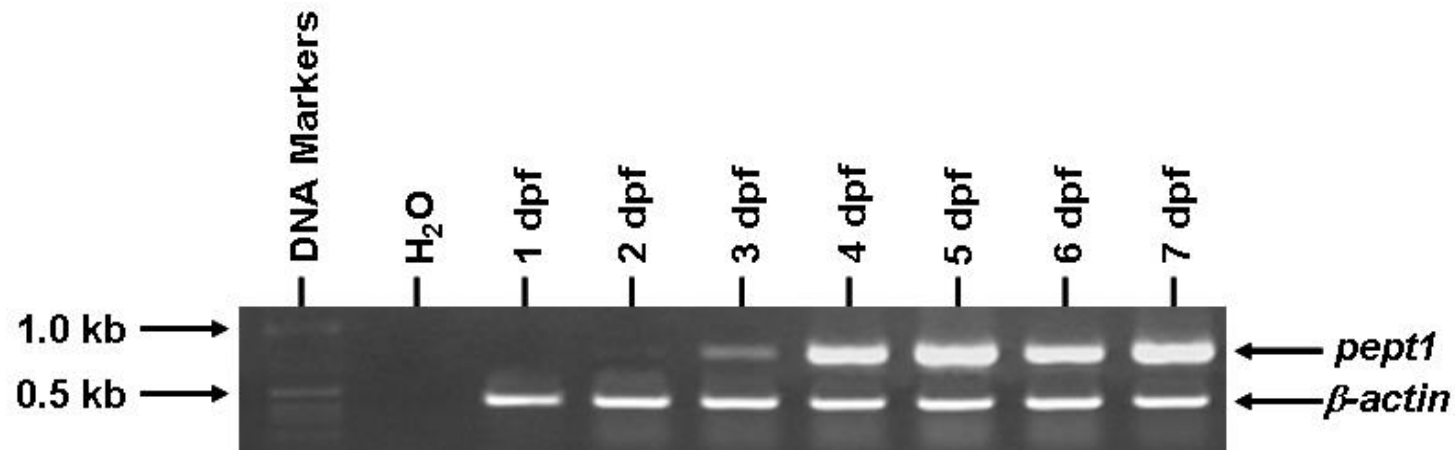
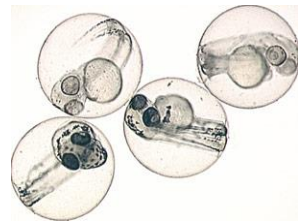
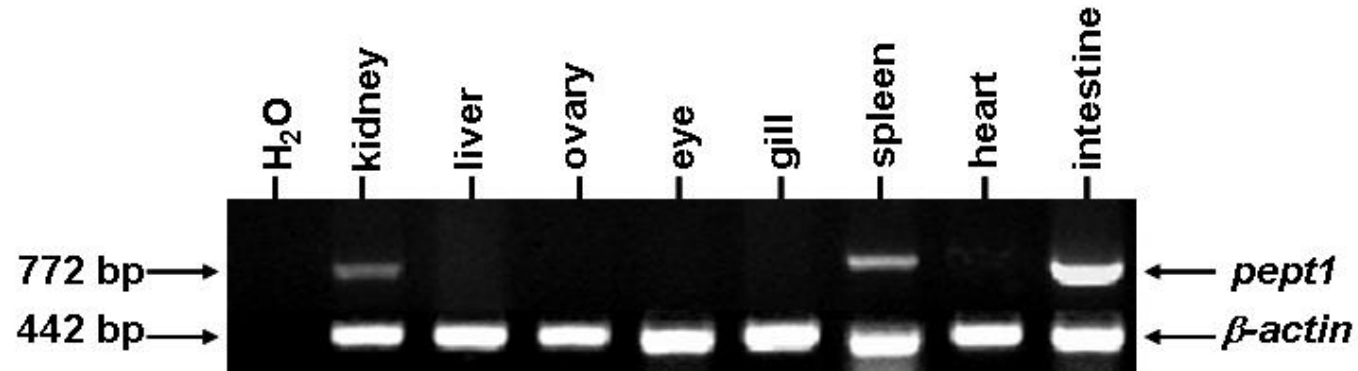
Conclusions (1)

- 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

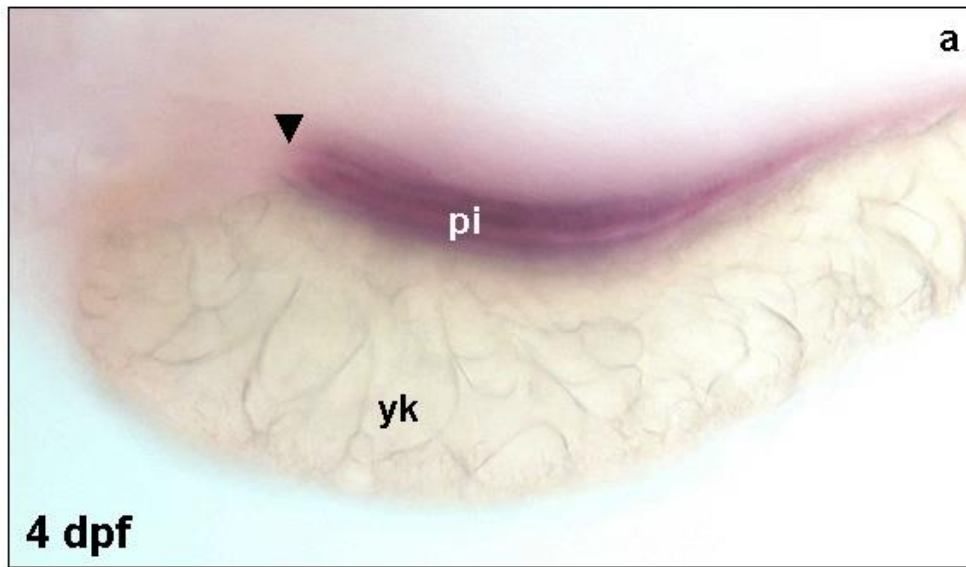
**Tissue distribution
...and spatial
distribution along
the gut**

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

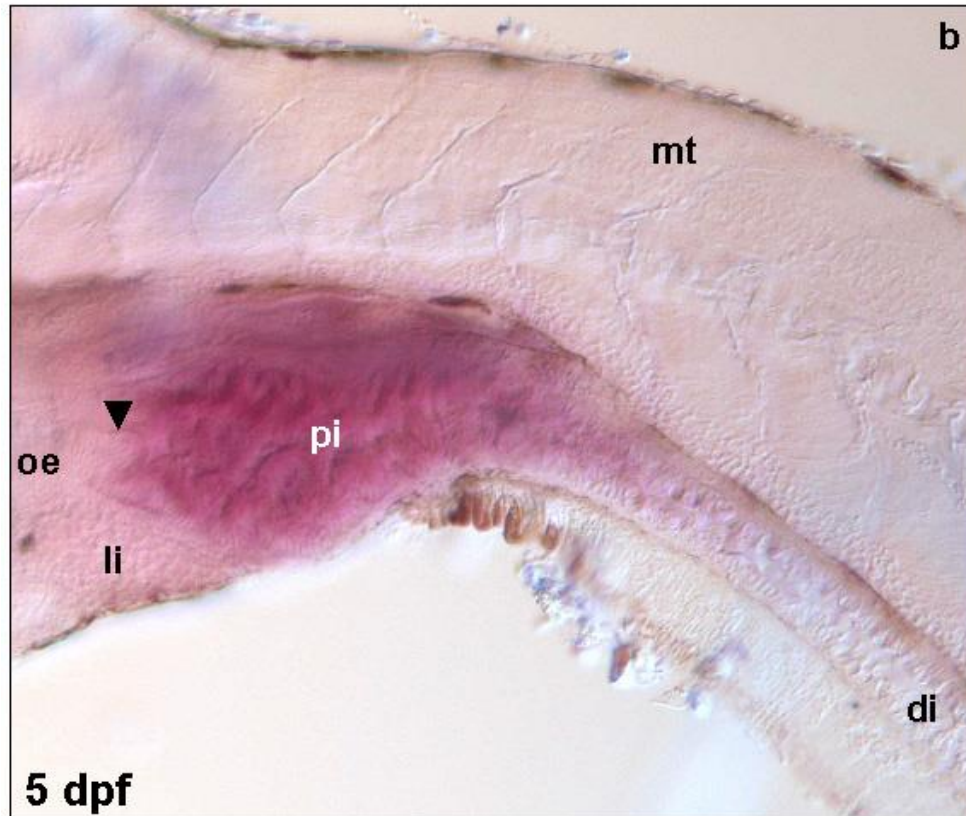
slc15a1
(*pept1*)



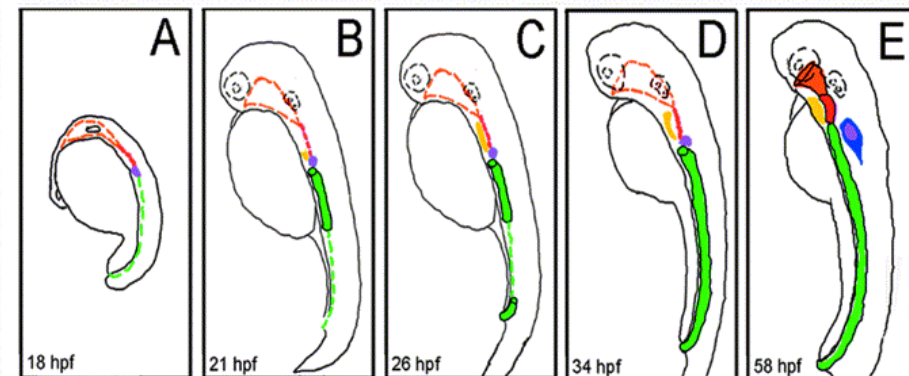
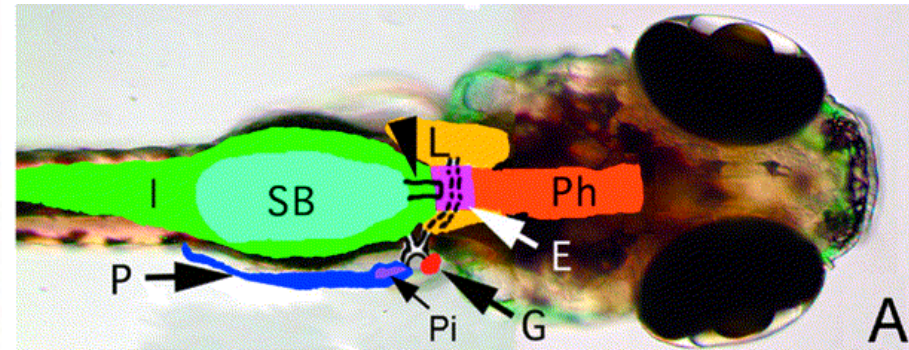
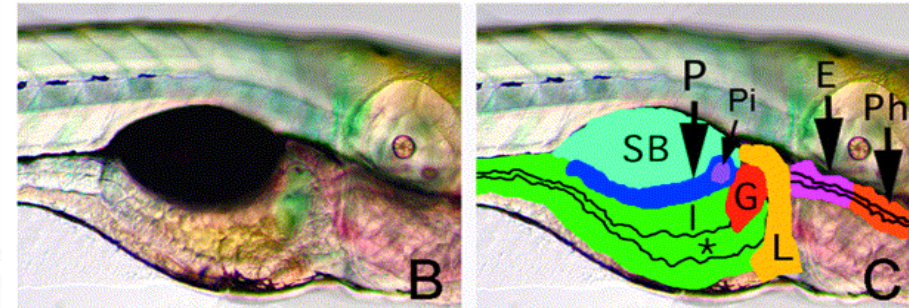
Zebrafish *slc15a1* (*pept1*) (whole mount in situ hybridization)



4 dpf



5 dpf



18 hpf

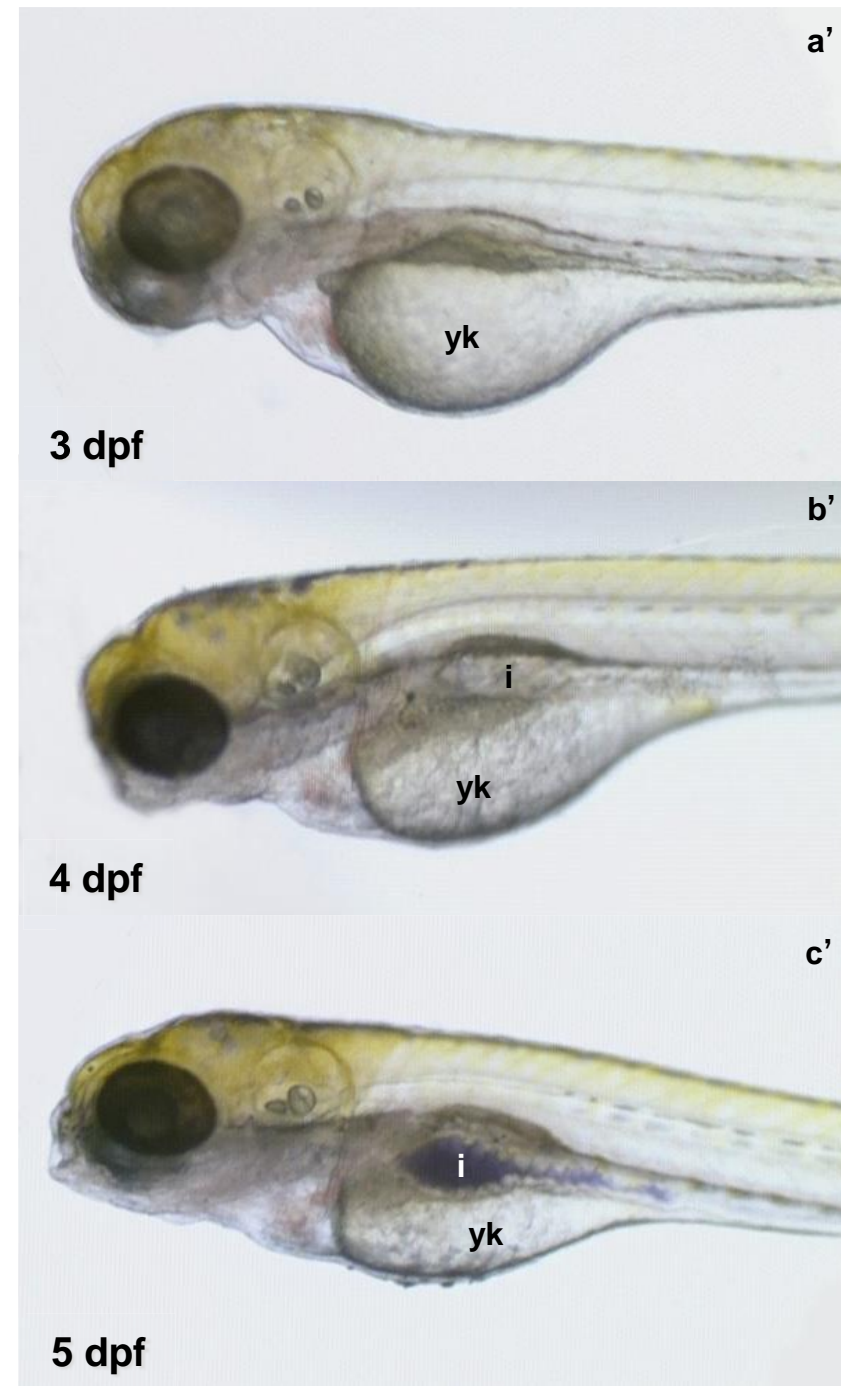
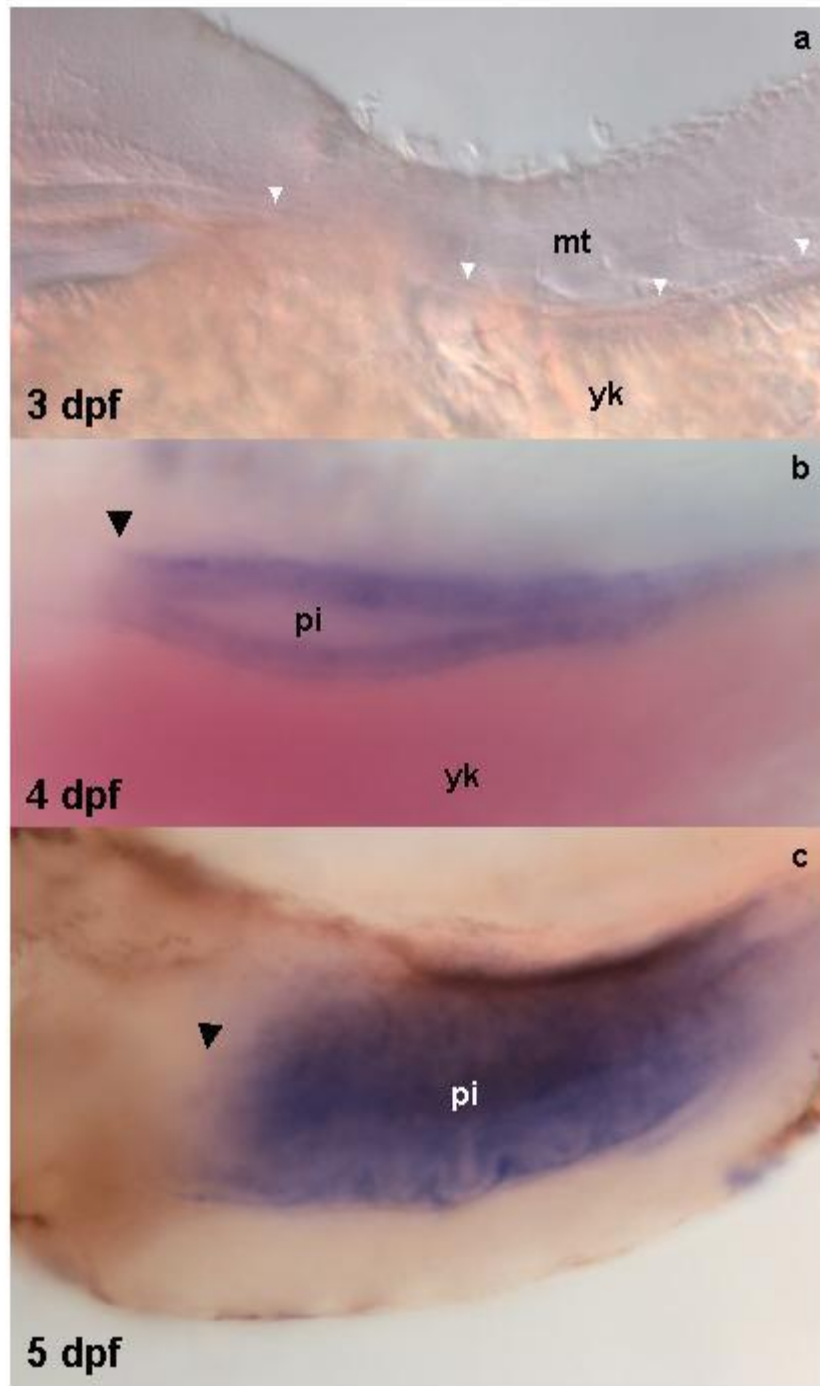
21 hpf

26 hpf

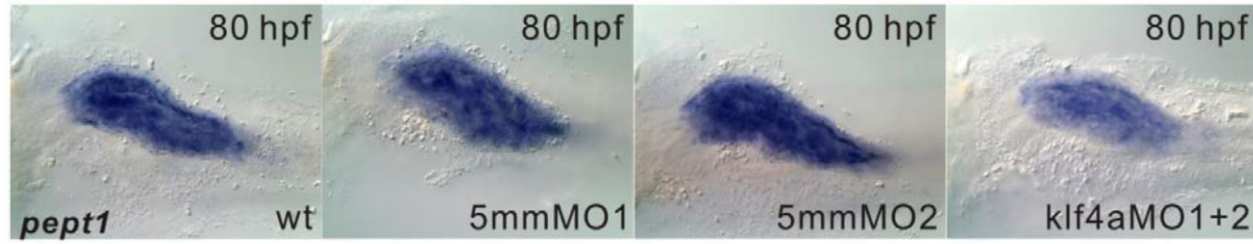
34 hpf

58 hpf

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

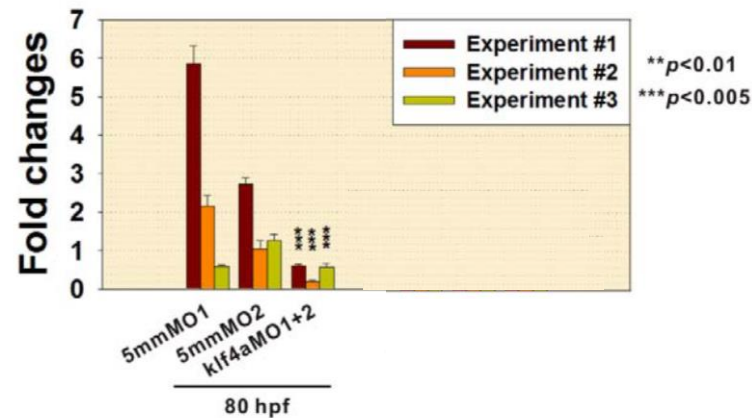
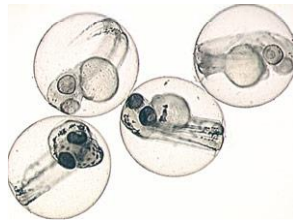


Spatial distribution of *slc15a1* (*pept1*) mRNA in the digestive tract (RT-PCR)



pept1 as a marker of terminal enterocyte differentiation

zebrafish

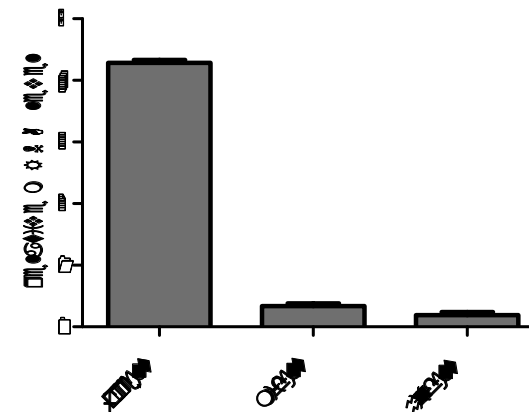


Kruppel-like factor 4a (*klf4a*) represses intestinal cell proliferation. It is required for the differentiation of goblet cells and the terminal differentiation of enterocytes

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

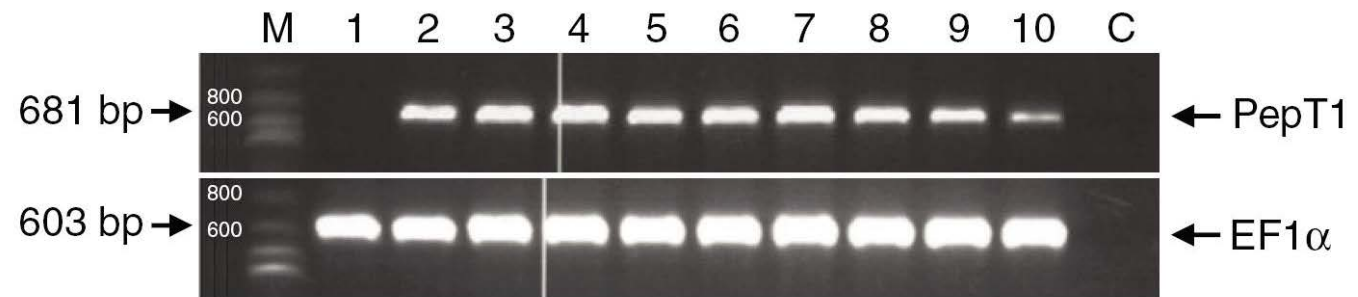
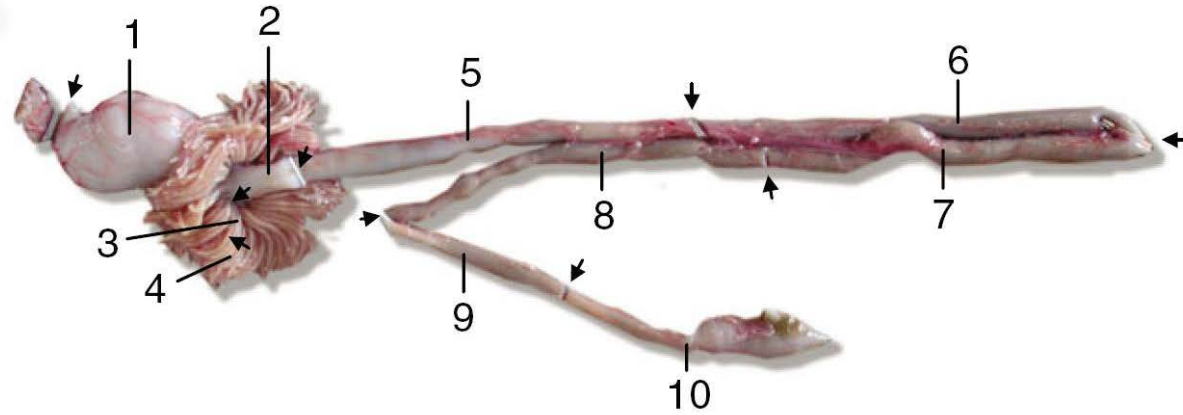
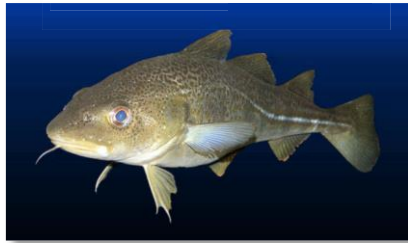


**... translating
findings in zebrafish
to other species**

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

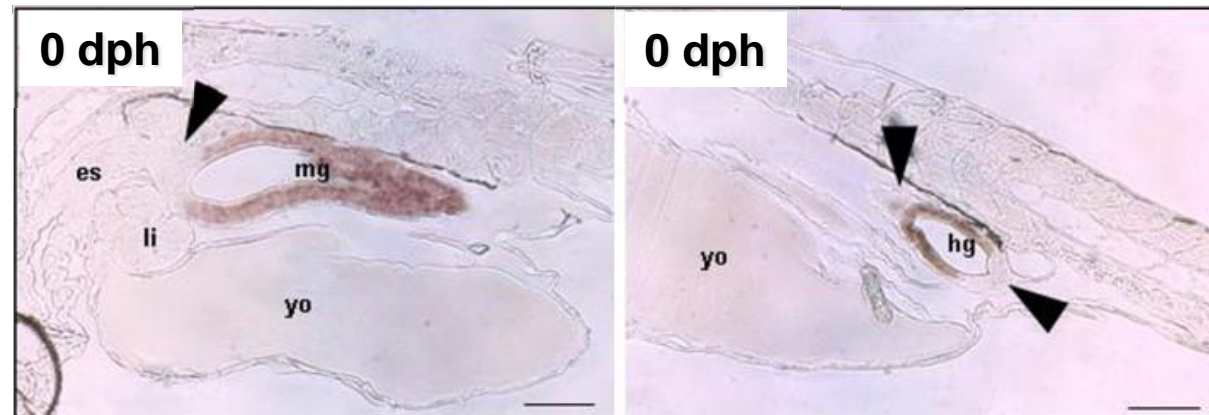
Rønnestad et al., J. Exp. Biol. 210:3883 (2007)

Atlantic cod



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

Atlantic cod

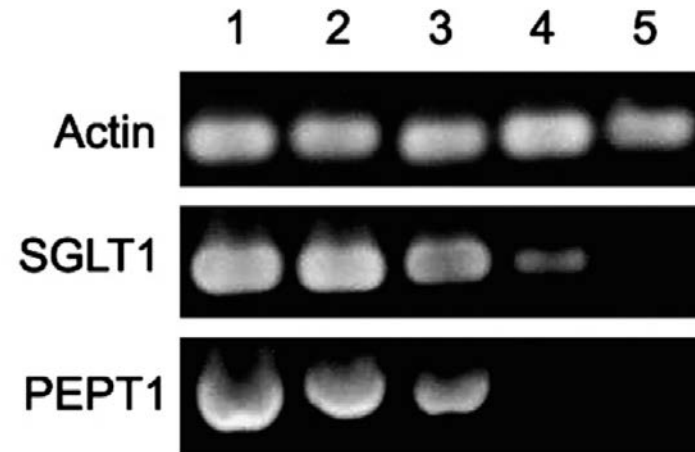


(in situ hybridization)

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

