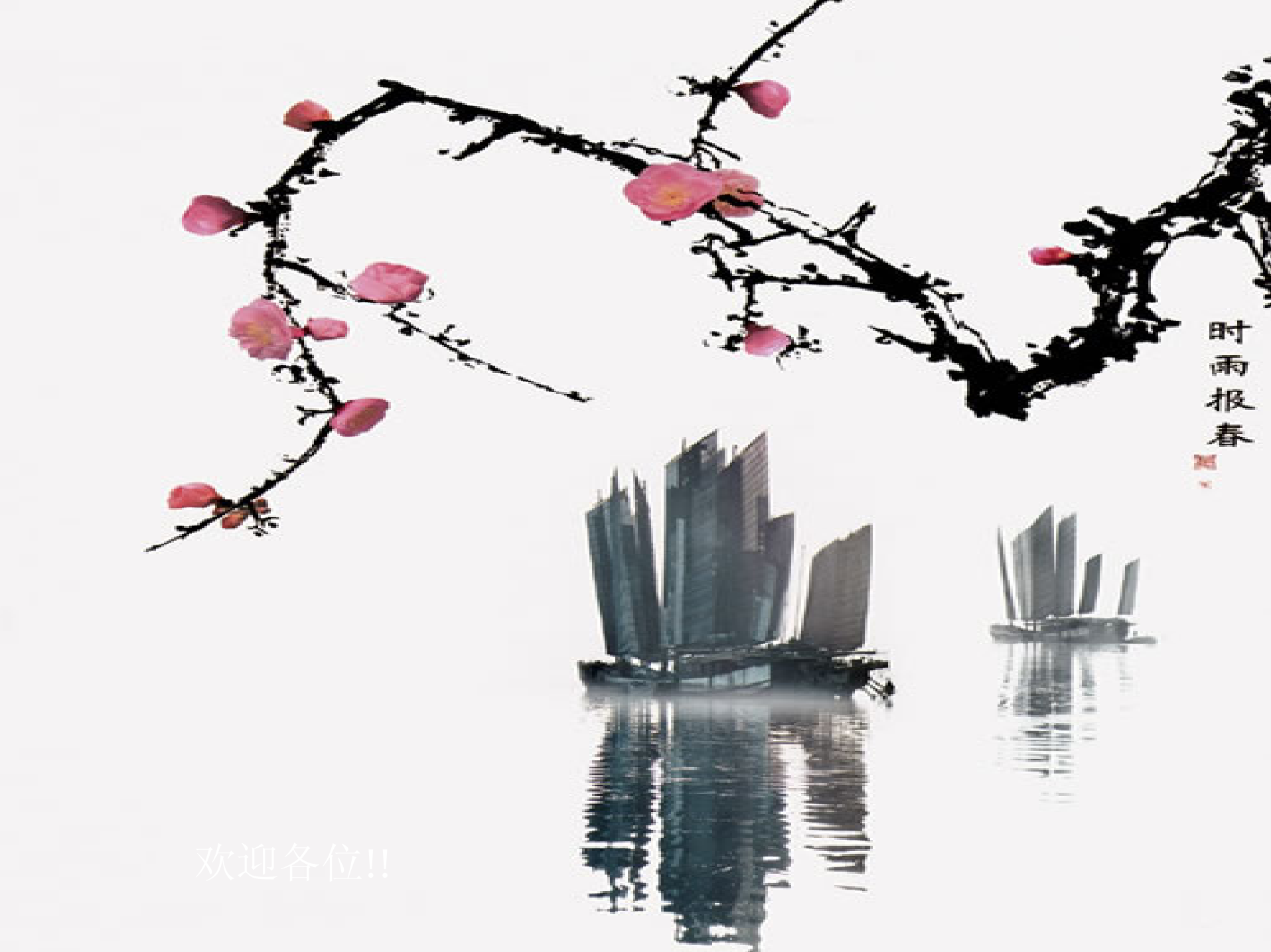

欢迎各位!!

Amphioxus, a model organism for comparative biology, is an endangered species demanding responsible conservation

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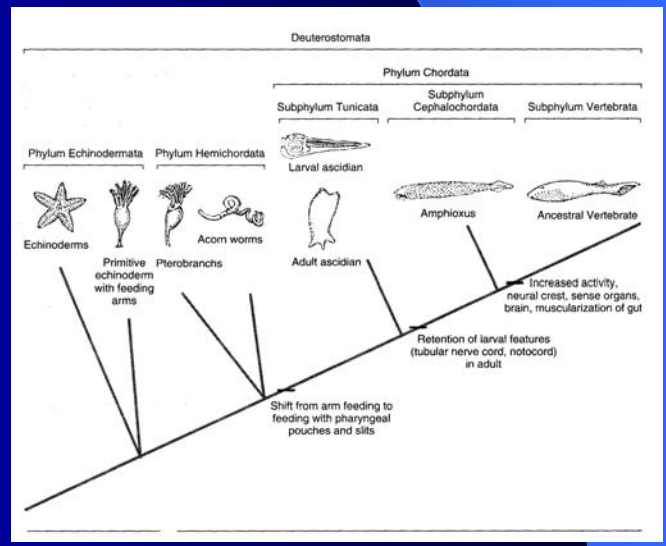
Everyone has his own pet!

It’s a long way from Amphioxus，
Its’ a long way to us;
It’s a long way from Amphioxus,
To the meanest human cuss;
Goodbye fins and gill slits,
Welcome teeth and hairs;
It’s a long way from Amphioxus
But we come from there!

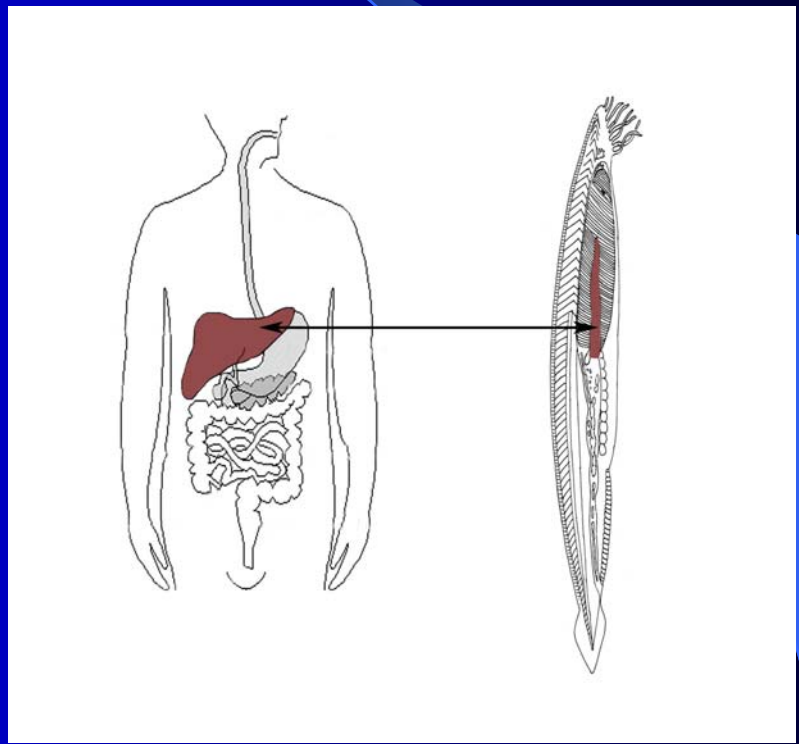
Phylogeny

- Anatomically and developmentally it appears closely related to the proximate ancestor of all vertebrates, possessing a notochord, a hollow dorsal neural tube, segmented muscle blocks, and gill slits.
- Amphioxus also retains a genome (17% that of the human genome) uncomplicated by extensive genomic duplication.
- All these make it an excellent model organism for gaining understanding the origin and evolution of vertebrates.



A model organism for insights into the origin of vertebrate organs and a food resource

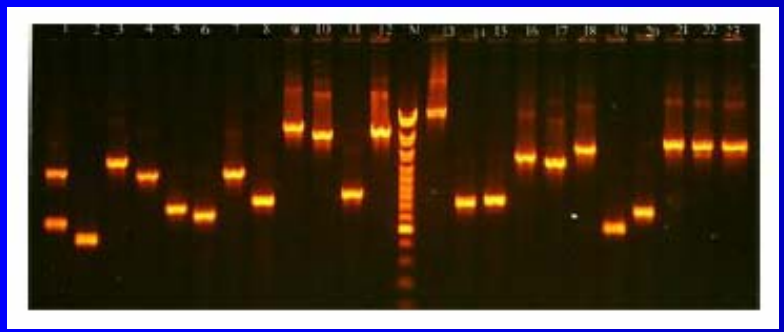
Liver, the largest organ of the body, plays a crucial role in keeping metabolic homeostasis. Amphioxus has a hepatic caecum, which has long been considered to be the precursor of vertebrate liver. However, the molecular evidences remain fragmentary.



Main Research Interests

- Gene and Development (EvoDevo)
- Gene and Immunity (Comparative Immunology)
- Identification of Hypothetical Genes

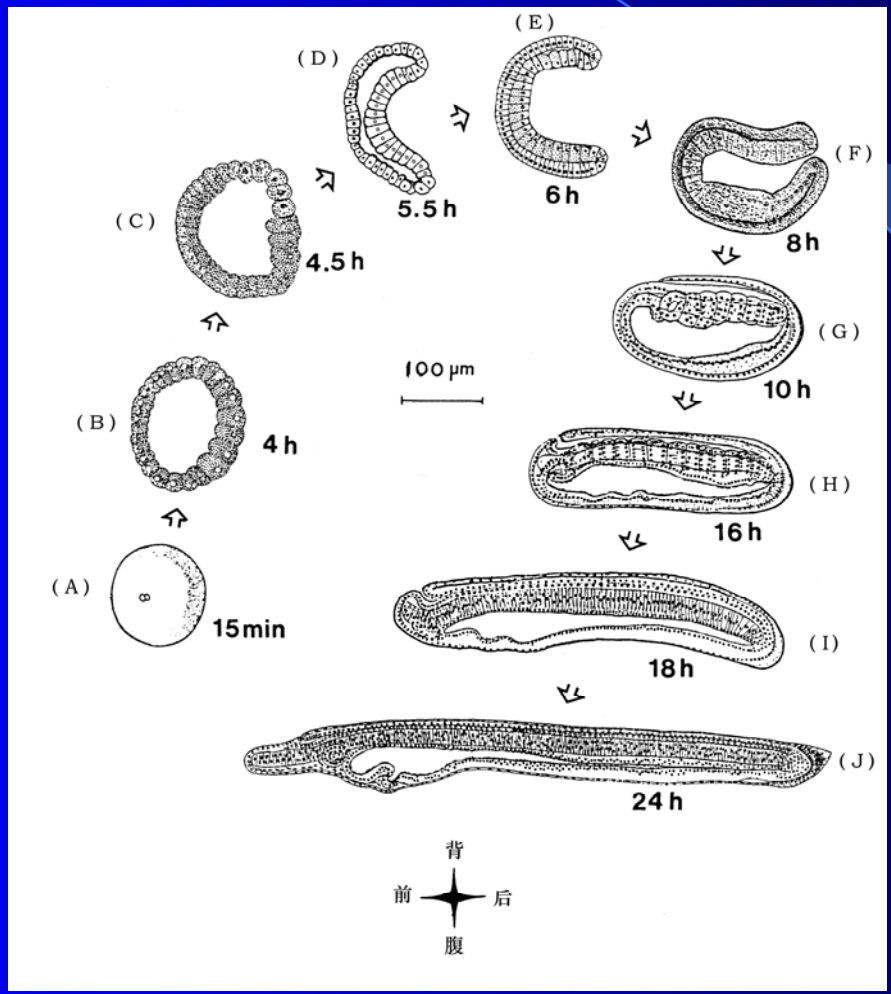
EST generation



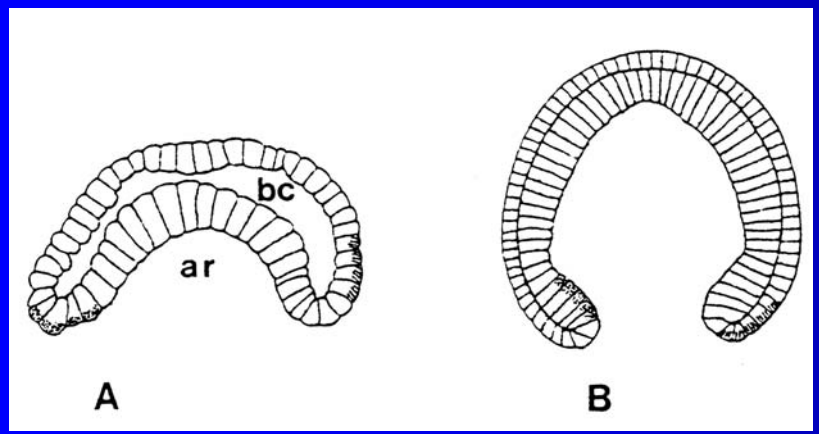
Size: ~500 bp

- **Construction of cDNA libraries: neural stage embryo cDNA library; gut cDNA library and whole animal cDNA library.**
- **Large scale sequencing.**
- **~5000 ESTs.**
- **~100 full-length cDNAs.**

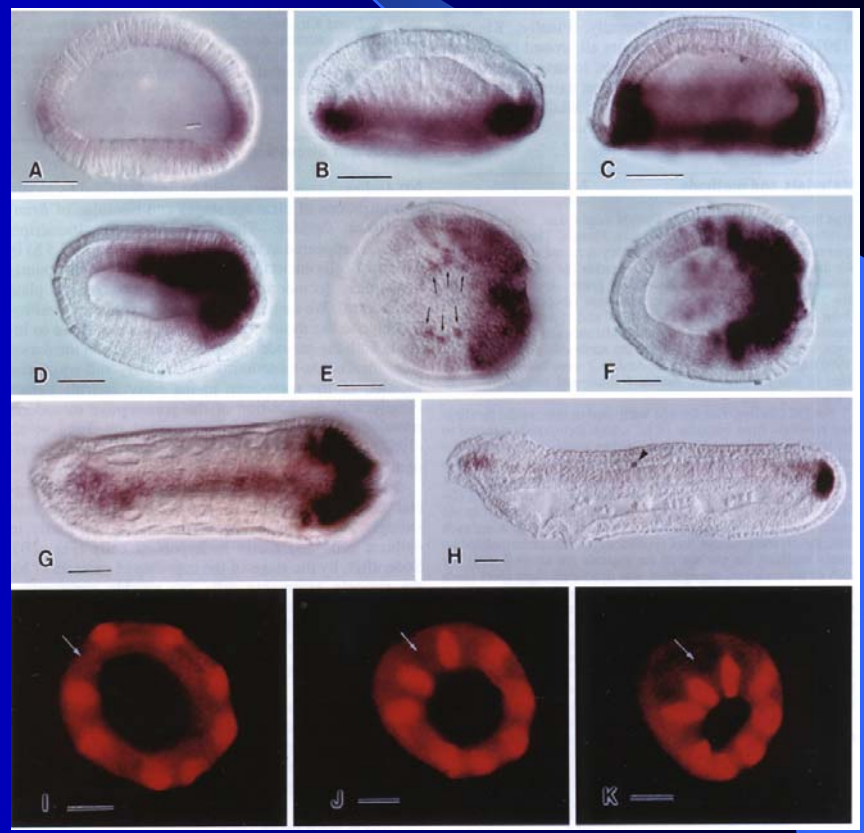
Early Development



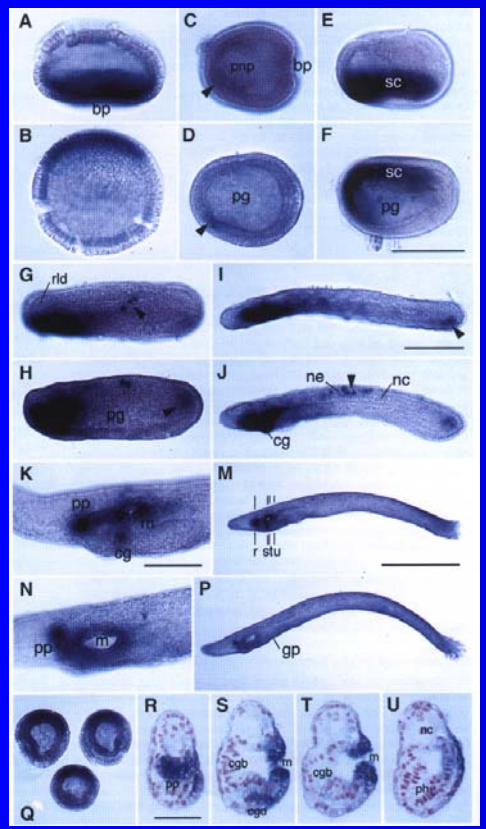
Brachyury **gene was expressed in nascent and early mesoderm. The entire mesoderm expressing *Brachyury* is internalized by a slight involution. The internalized mesoderm undergoes anterior extension mid-dorsally and dorsolaterally, which is similar to that in frog embryos.**

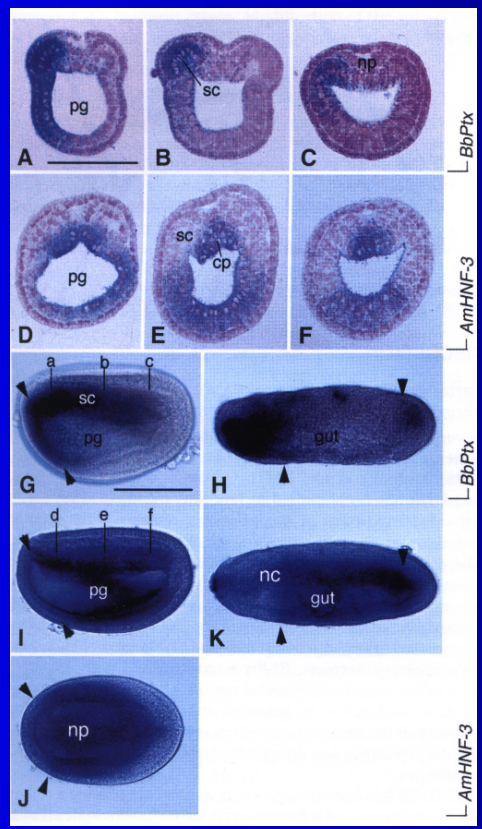


Combination of vital dye DiI labeling and *Brachyury* gene expression



Pitx2 is expressed in tissues derived from coelom in vertebrates.
Pitx2 and HNF-3 are expressed in a complementary fashion on the left side of embryo in vertebrates.
Ptx (Pitx2-like gene) and HNF-3 are similarly expressed in the amphioxus embryo.





Expression of both amphioxus Ptx and vertebrate Pitx2 in tissues derived from coelom suggests that the left-right assymetric development has a common origin between amphioxus and vertebrates.

Characterization and tissue-specific expression of phosphatidylcholine transfer protein gene from amphioxus *Branchiostoma belcheri*

- ***PCTP*** **is expressed mainly in liver in vertebrates.**
- ***BbPCTP*** **has an exon-intron organization similar to that of human and rat PCTP genes.**

```
GATAGCGGCCTAGCTACGCCGGTTACAACAACGGAAATACCTCCAGCCACCCAGTCTCGA   60
CAGATGGCAGTGGTCACATTTGAAGACGAGGAGTTTGAAAAGGTGTGTAAGGAGCTCCAG  120
   M  A  V  V  T  F  E  D  E  E  F  E  K  V  C  K  E  L  Q     19
AGTCCTGAACTAGACGGAGGATGGGAGTTCTTCACAGAGTCACACGATGTGCAGATCCAC  180
S  P  E  L  D  G  G  W  E  F  F  T  E  S  H  D  V  Q  I  H     39
AGATTATACAGGGAGGAGTCGGGTCTGTATGAGTACAAAGTCCTGGGAATGTTAAGTGAC  240
R  L  Y  R  E  E  S  G  L  Y  E  Y  K  V  L  G  M  L  S  D     59
GTCTCTCCGTCAGTCTGTGCTGATGTCTACATGGACCTGGAGTACAGGAAAAAATGGGAC  300
V  S  P  S  V  C  A  D  V  Y  M  D  L  E  Y  R  K  K  W  D     79
AGCTATGTCAATGAGCTGTATGAGAGAGAGGTGGAAGGCAAGAAGGTCATCTACTGGAAC  360
S  Y  V  N  E  L  Y  E  R  E  V  E  G  K  K  V  I  Y  W  N     99
GTCAACTTCCCCATGTTTATGTCCAACAGGGATTATGTGTACATGCGTGAACTACGTGAG  420
V  N  F  P  M  F  M  S  N  R  D  Y  V  Y  M  R  E  L  R  E    119
TTTGATGTAGAGTATGGTCATGTGTGGGCTGTGTTGGCCCAGAGTACTACGTTAGGAAAT  480
F  D  V  E  Y  G  H  V  W  A  V  L  A  Q  S  T  T  L  G  N    139
GTTCCTGAAAAAGATGGCGTCATCCGCGTGGATGACTACAAGTGTTCGCTTGTCTTCGCT  540
V  P  E  K  D  G  V  I  R  V  D  D  Y  K  C  S  L  V  F  A    159
TCAGATGGAAAACAAGGAACCAAAGCCTTCATGTATTACTATGACAACCCAAAAGGGATG  600
S  D  G  K  Q  G  T  K  A  F  M  Y  Y  Y  D  N  P  K  G  M    179
ATTCCAACCTTTATTATCAACTGGGCAGCCAAGACCGGTGTTCCTGGCTTCCTGACTTCT  660
I  P  T  F  I  I  N  W  A  A  K  T  G  V  P  G  F  L  T  S    199
ATGCAGAAAGCCTGCAGGAAGTACCCGGAGTTCTGCAGCAAGAGGAAATCTGCCGCATCA  720
M  Q  K  A  C  R  K  Y  P  E  F  C  S  K  R  K  S  A  A  S    219
TAGCACATAGGAACTCAAACATGTCACAAACTACTCTTACATATAGGAAAGATTGTAGCA  780
*
AAATTGTGGCTTGAAAGAGGGAAAAATTGAAGGTAGCAAAATTGTTGTTAGATAGAGGAA  840
AAATTAAATGTGTAAAATTGTAGCTAGATAGAGGAGAAAATTGGATGTAGCAAAATTGTT  900
GTTAGAGACAAAATTGGATGTAAAATTGTGGTGATGTAGTGAAAATGTGGTGAAATTGTG  960
TTCACATGCTAGAAAAAAATGAGAGCACTAAAATTGTGCCTACATAATGGGAAATTTTATG 1020
TAGCAGGAATTTCCCTACACATTGAAAAAAATTAAATGTAGCCAAATATGCCCTACTTGG 1080
AGAAAGTAGCATAACTGTGTCTGTATATTGGAAAAATTAGATATAGCAAAATTGTGTCTG 1140
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AATTATTATGATGACGGCGTTTTTGGCAATTTTATCATATTACAAGAGGCACCAGTTTGA 1260
TTTTTATGACTAACAGCAACAAGAAGGTATTCAATGACGCACCAGTCAACTTACCTCAGC 1320
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GGGTGTGTTAGATGATGTTCTTTCTATGATTAAATTCTATTTTCCGGAAAAAAAAAAAAA 1440
AAAAAAAAAAAAAAAA                                               1457
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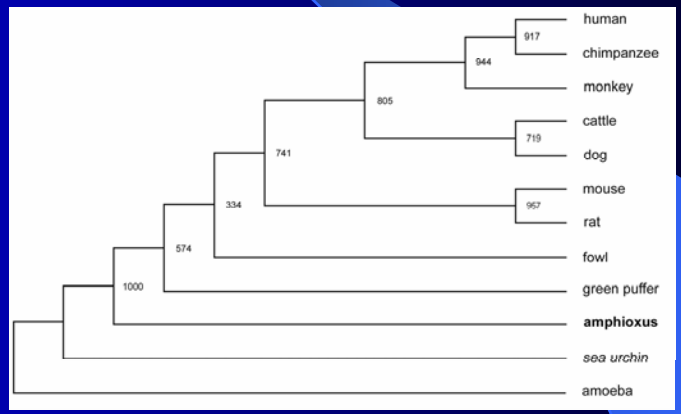
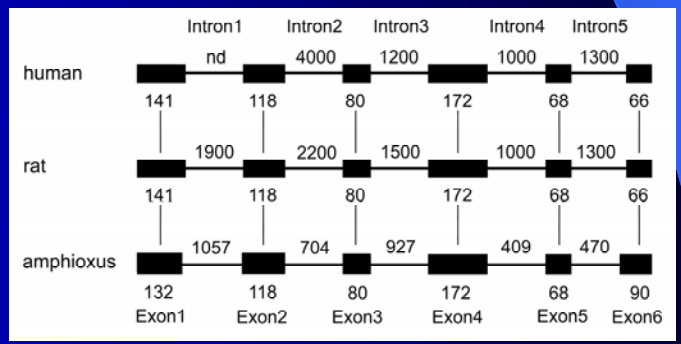
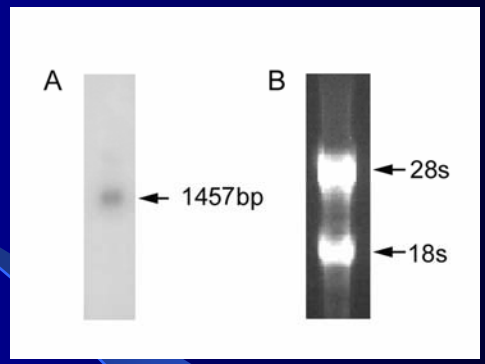
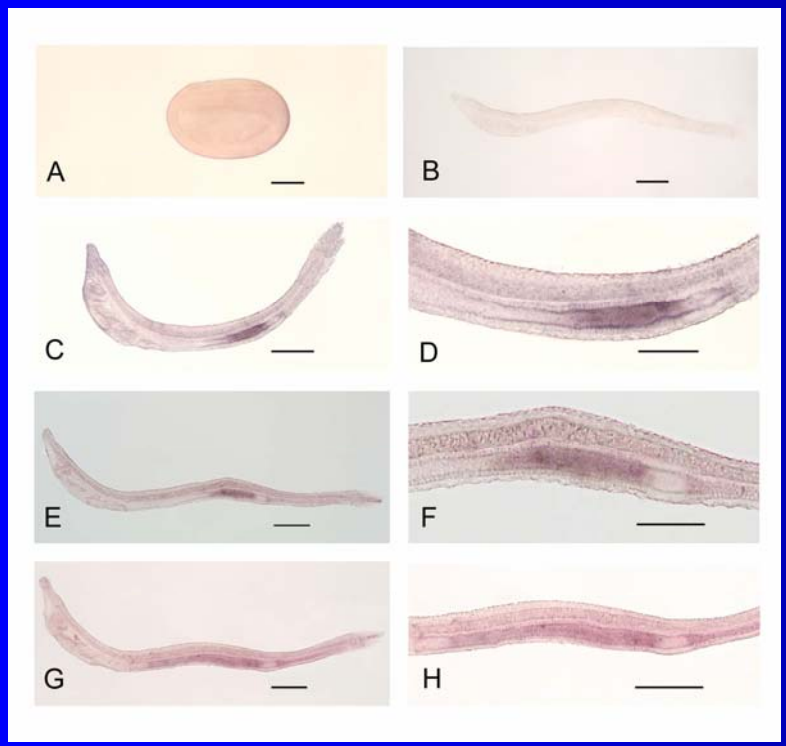
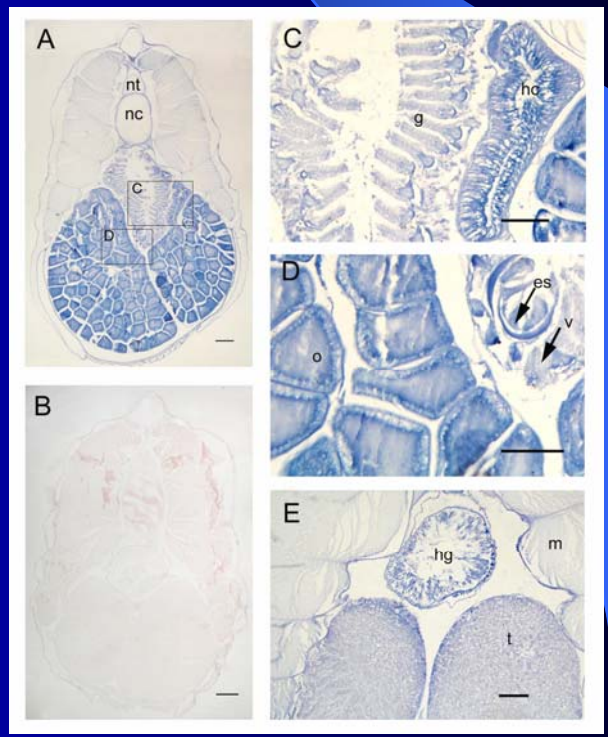




Both *in situ* hybridization histochemistry and whole-mount *in situ* hybridization revealed a tissue-specific expression pattern of *BbPCTP* with the high levels in the hepatic caecum and primitive gut including the region where the hepatic caecum will form later during development, suggesting that amphioxus hepatic caecum is similar to vertebrate liver in respect to *PCTP* expression.

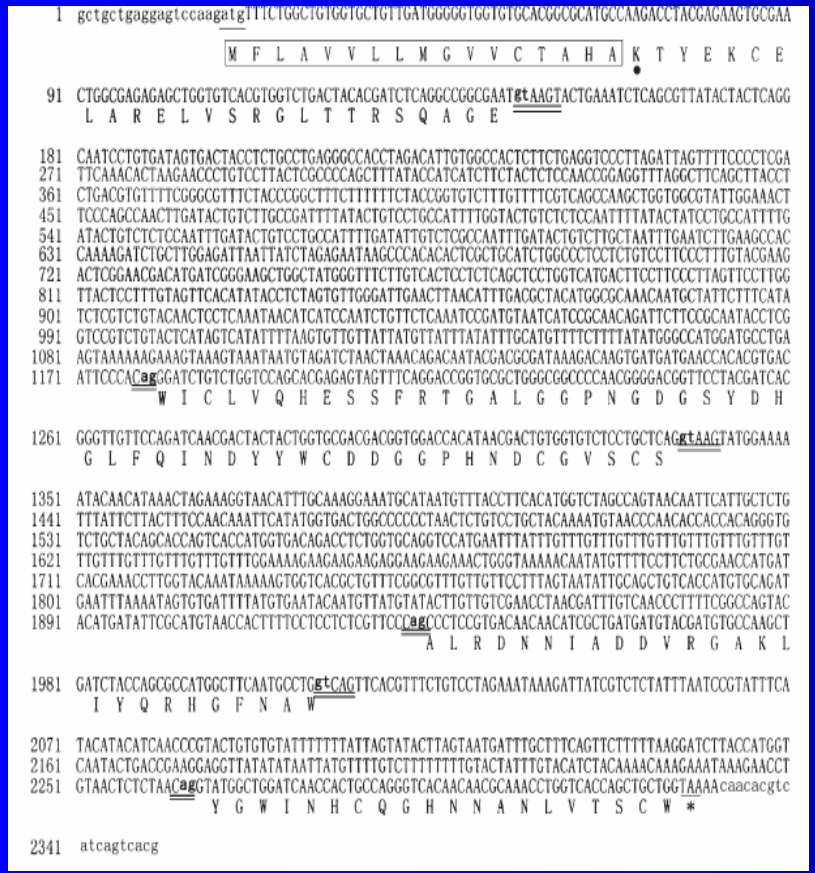


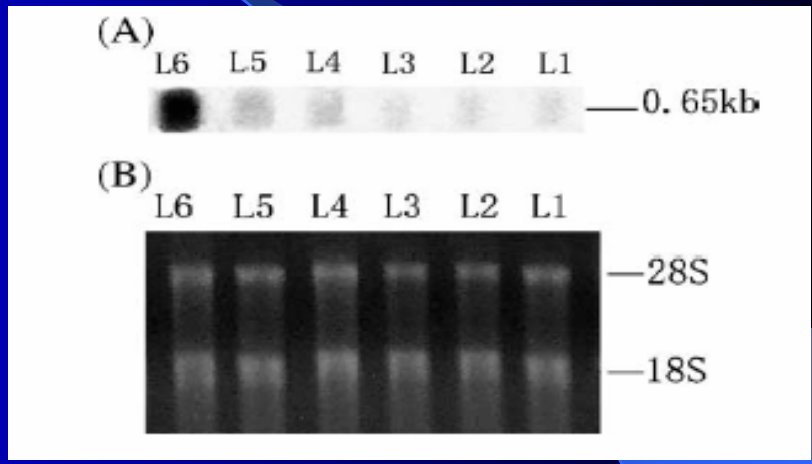




Characterization and expression of *BbLysC*

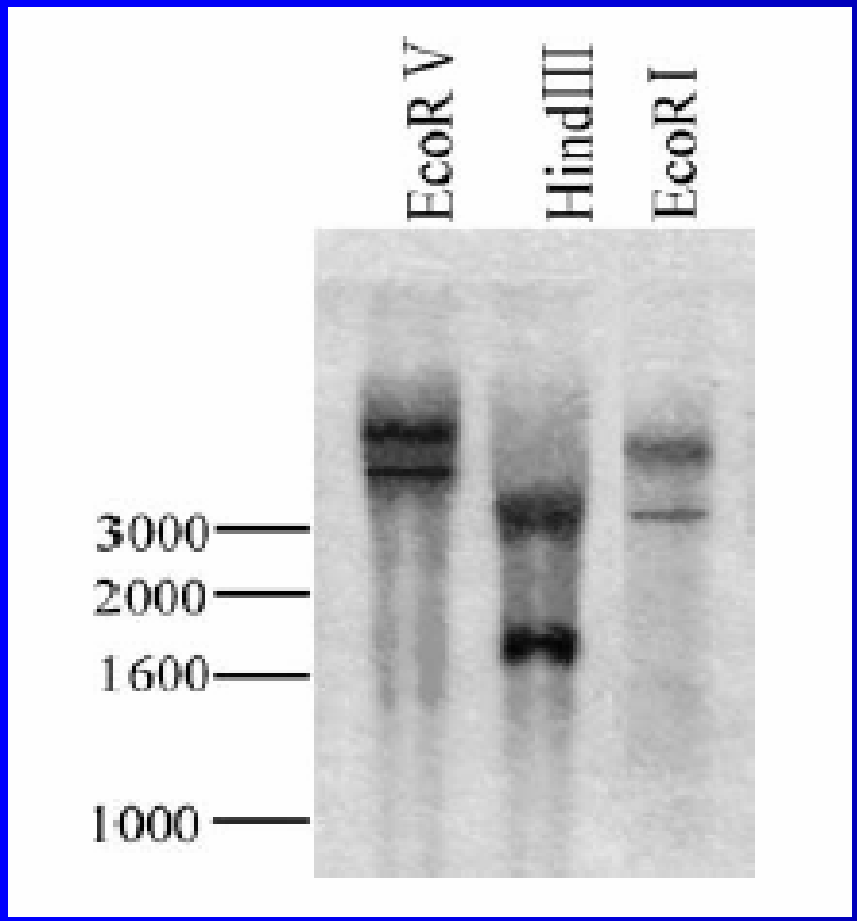
-C-type lysozyme comprises two subtypes, digestive and non-digestive (bacterial killig) lysozymes.

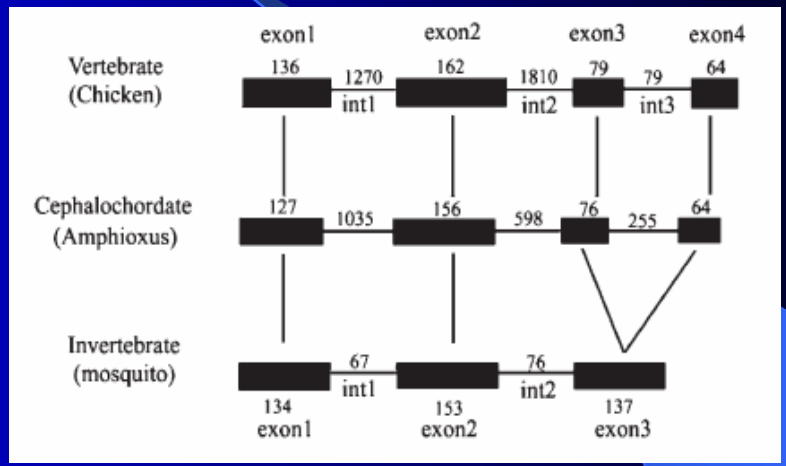




BbLysC **is an enzyme with a dual function of digestion (predominant expression in gut and presence of all features typical of digestive c-type lysozyme genes such as low pI values and pH-optimum in acidic range) and bacteriolysis (ubiquitous expression).**

Genomic organization

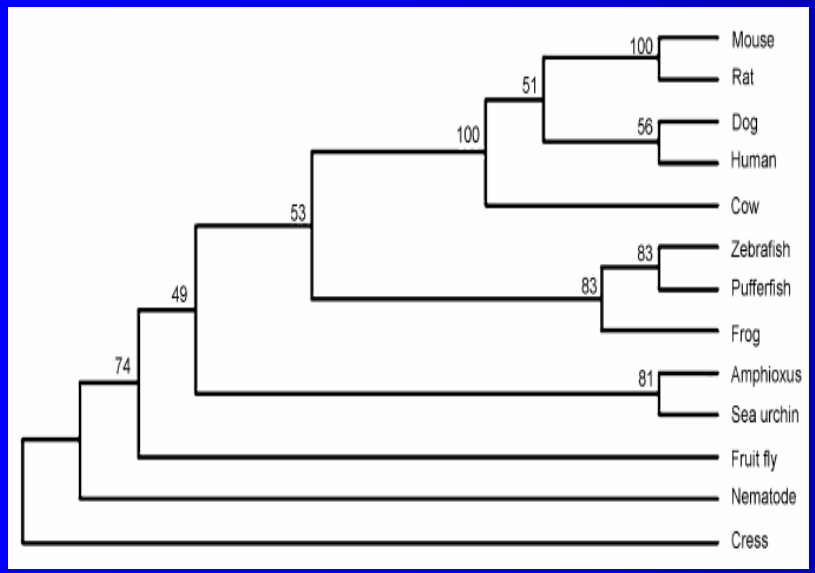


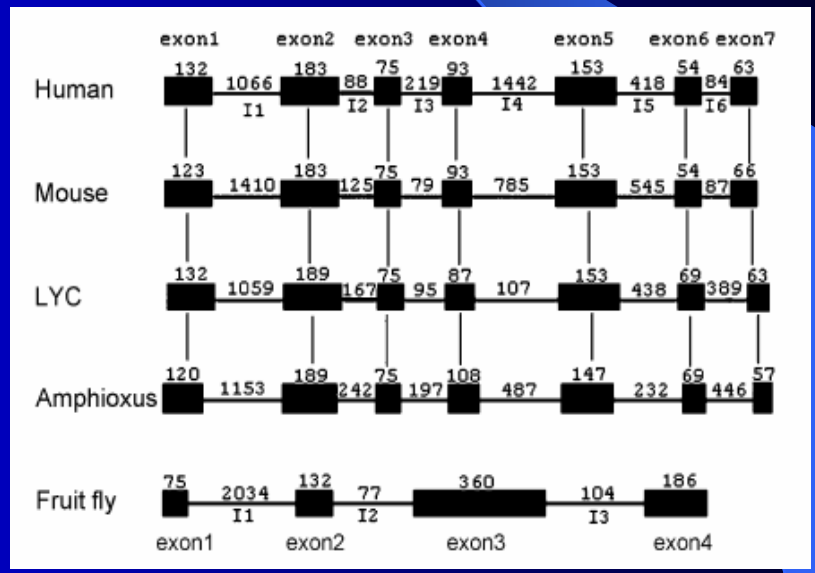


- Two copies of *BbLysC* is found in genome.
- Genomic organization of *BbLysC* is similar to vertebrate c-type lysozyme genes with respect to number and size of both exons and introns.

Characterization and expression of GILT (gamma-interferon-inducible lysosomal thiol reductase) gene in amphioxus with implications for GILT in innate immune response

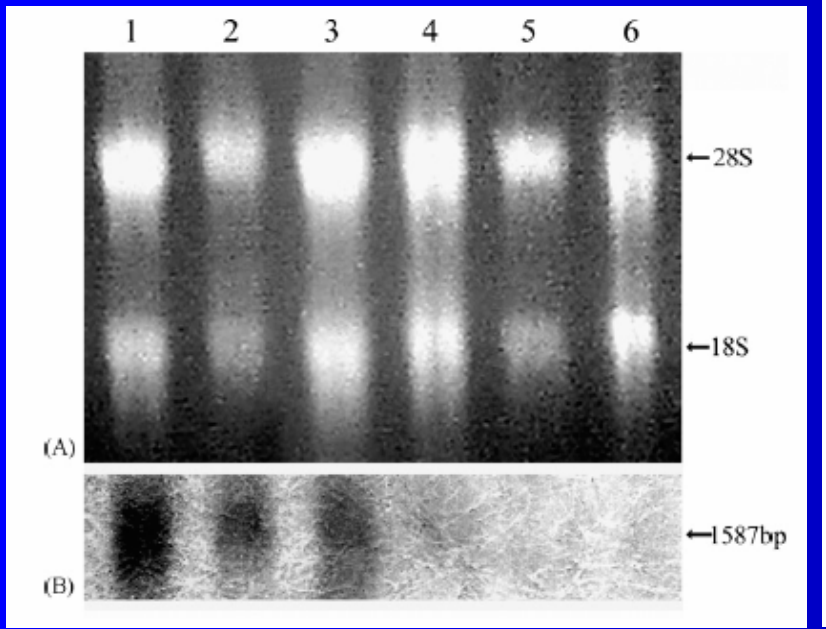
-GILT is involved in MHC-mediated antigen processing.

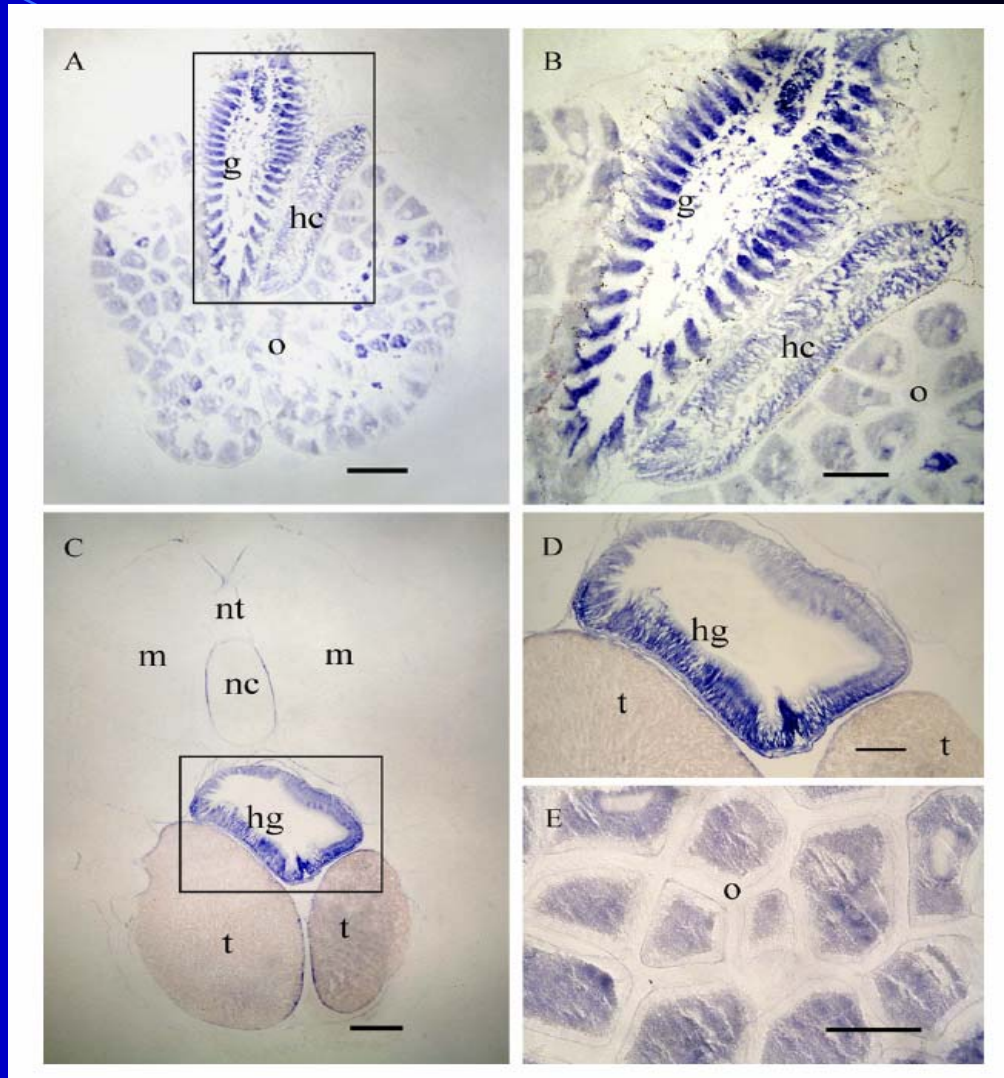




***BbGILT* is archytype of vertebrate GILT genes.**

***BbGILT* is expressed in a tissue-specific manner with most abundant levels in hepatic caecum and hind-gut.**

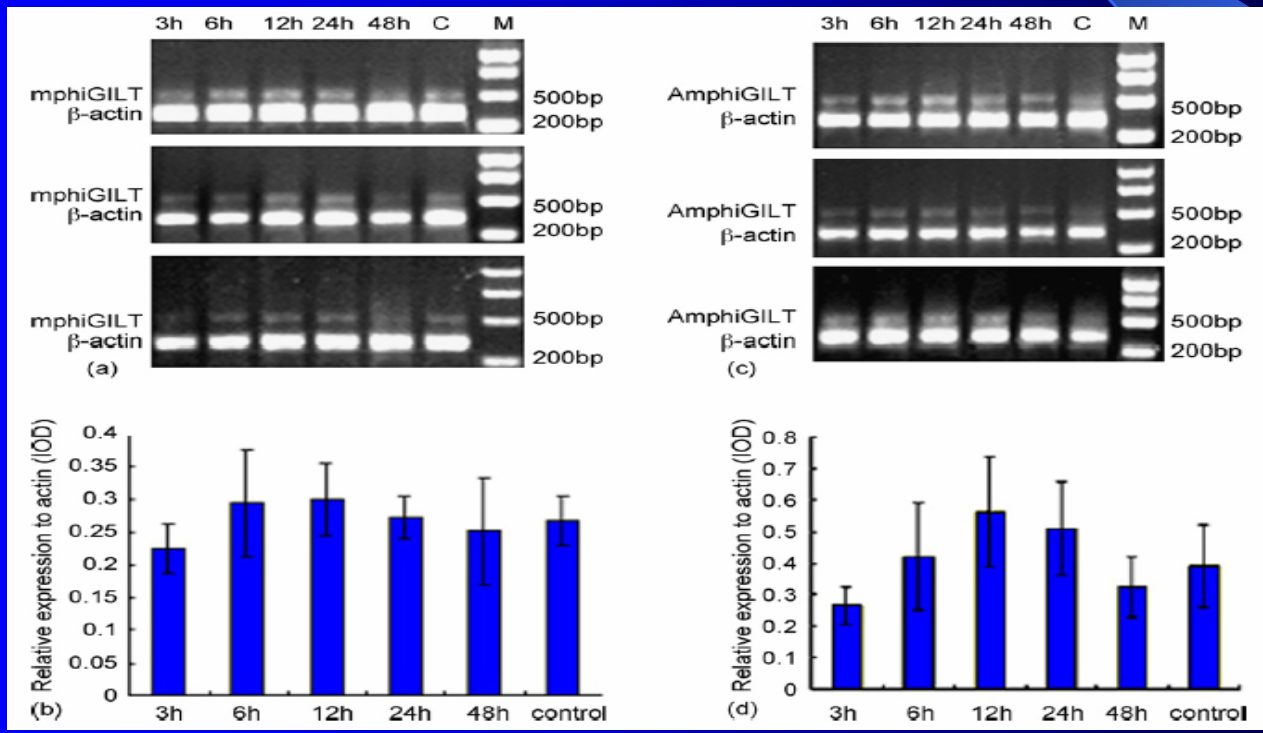




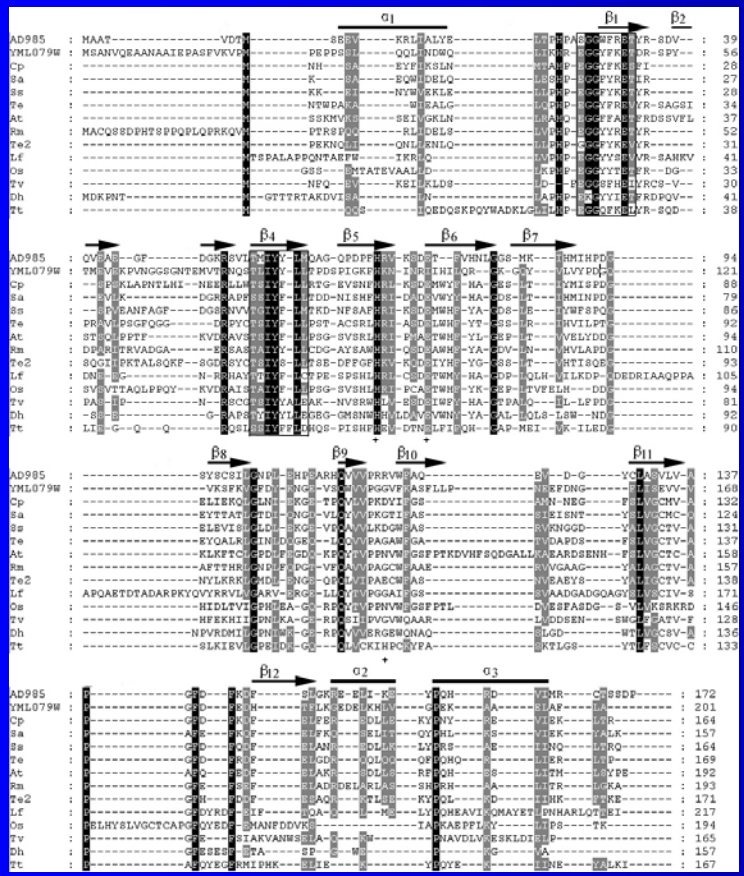
Mammalian IFN-gamma induced significant expression of human and mouse GILT genes.

Mammalian IFN-gamma only exerted a slight effect on expression of *BbGILT*, forming a contrast to the marked expression of human and mouse GILT genes by IFN-gamma.

***BbGILT* is highly likely to play a role in the innate immune responses.**

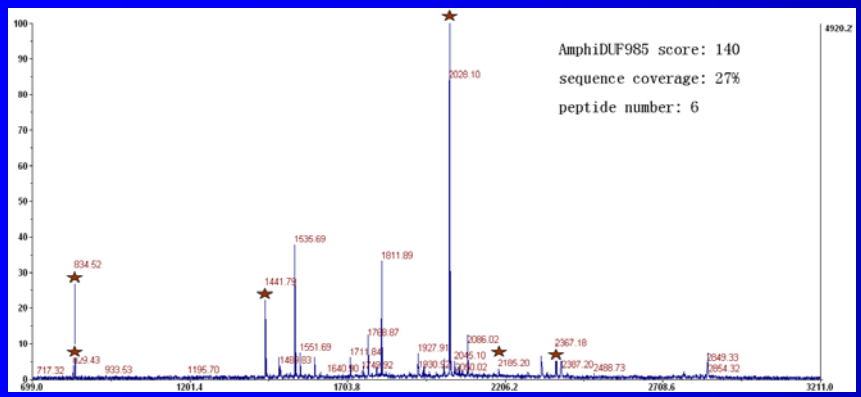


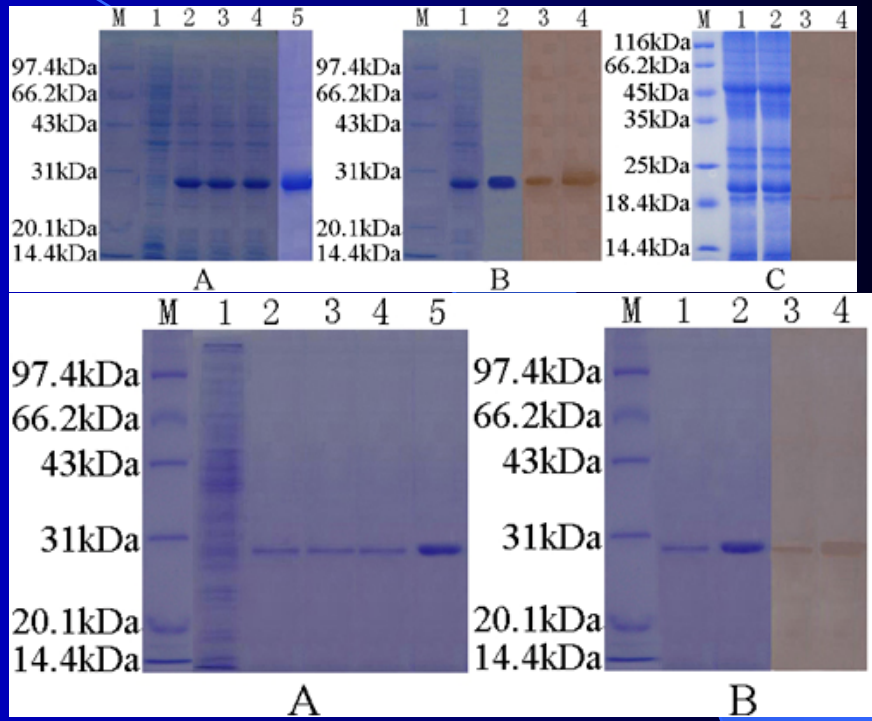
Identification of a DUF985 domain-containing hypothetical gene from amphioxus (*Branchiostoma belcheri*) as a novel member of phosphoglucose isomerase: expression, function and localization



The progress in genome sequencing has led to an increasing submission of uncharacterized hypothetical genes with the domain DUF985 in GenBank, and none of these genes is experimentally related to a known protein.

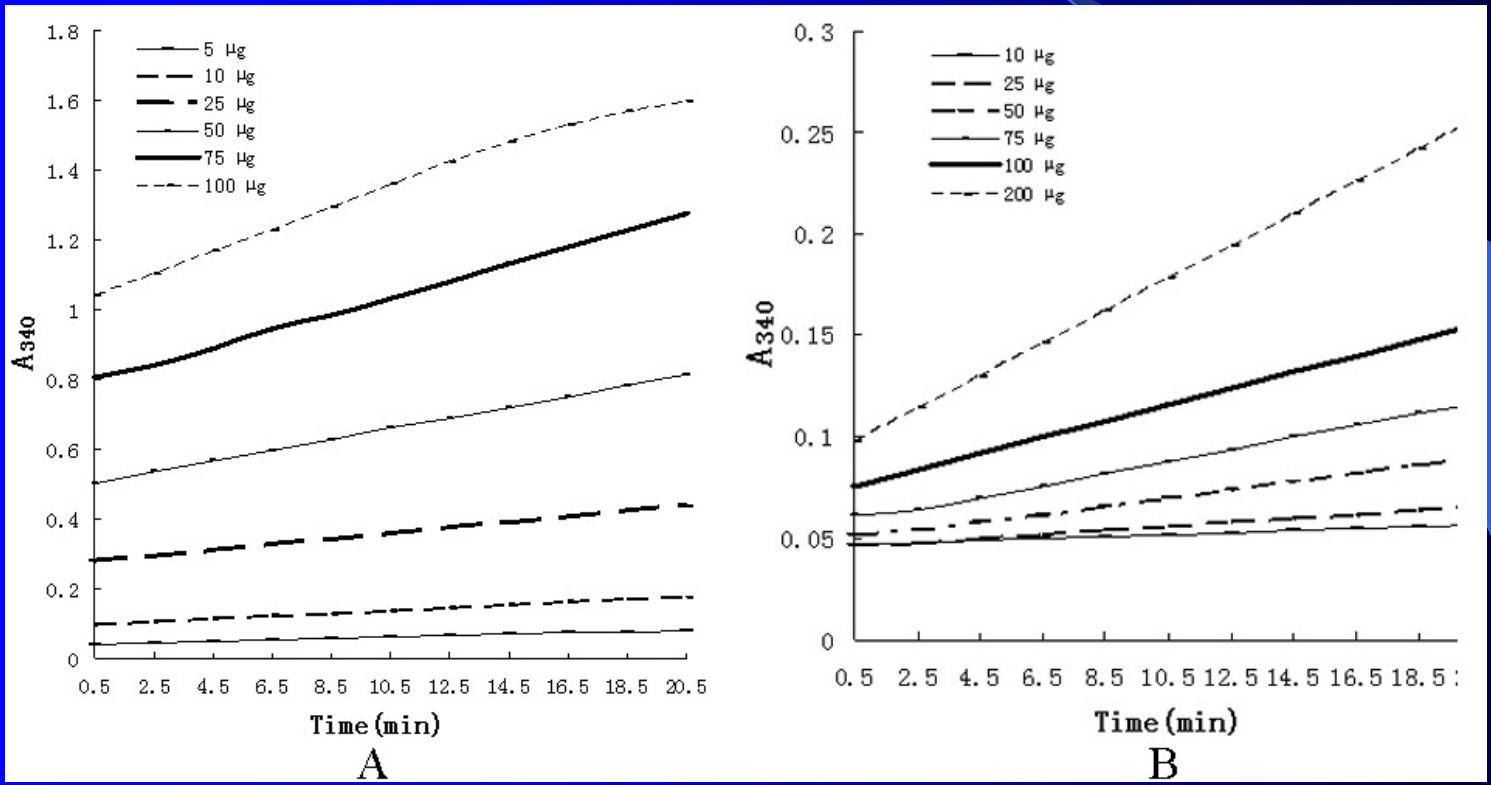
***BbDUF985* is expressed in both prokaryotic (*E. coli*) & eukaryotic (*Pichia pastoris*) systems.**





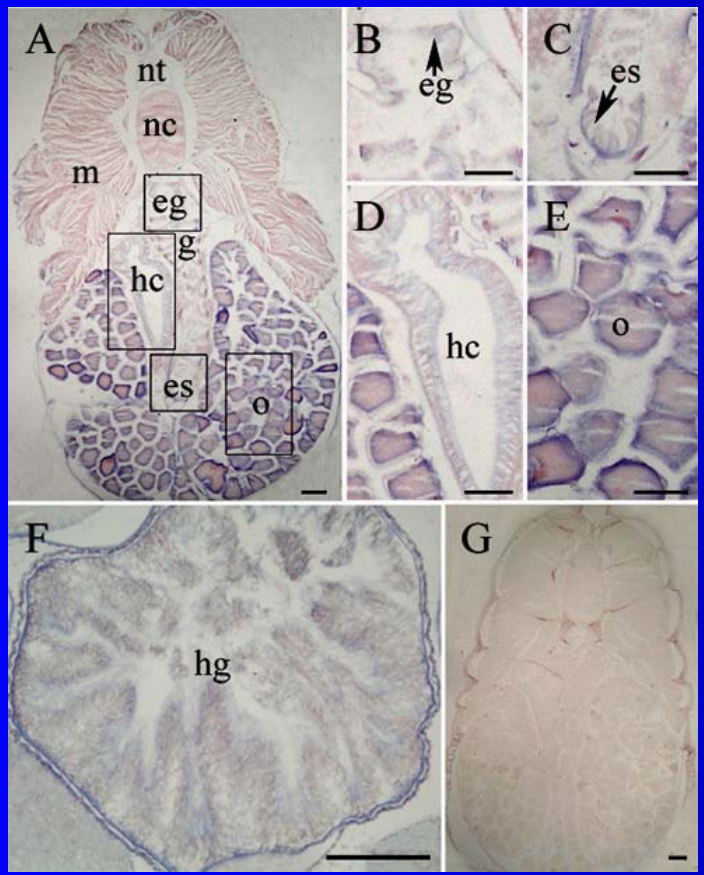
***BbDUF985* did code for an actual protein.**

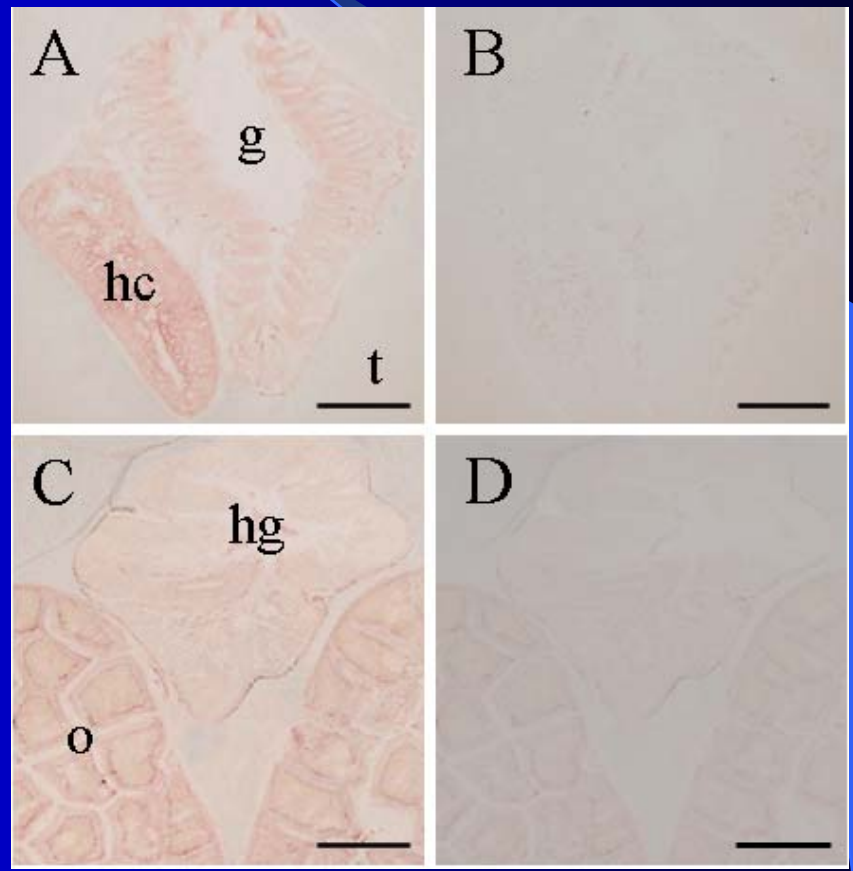
The recombinant proteins expressed in prokaryotic and eukaryotic systems both exhibited an activity of phosphoglucose isomerase (PGI), suggesting that the DUF985-containing hypothetical gene from amphioxus *Branchiostoma belcheri*, *BbDUF985*, encoded a novel functional member of PGI.



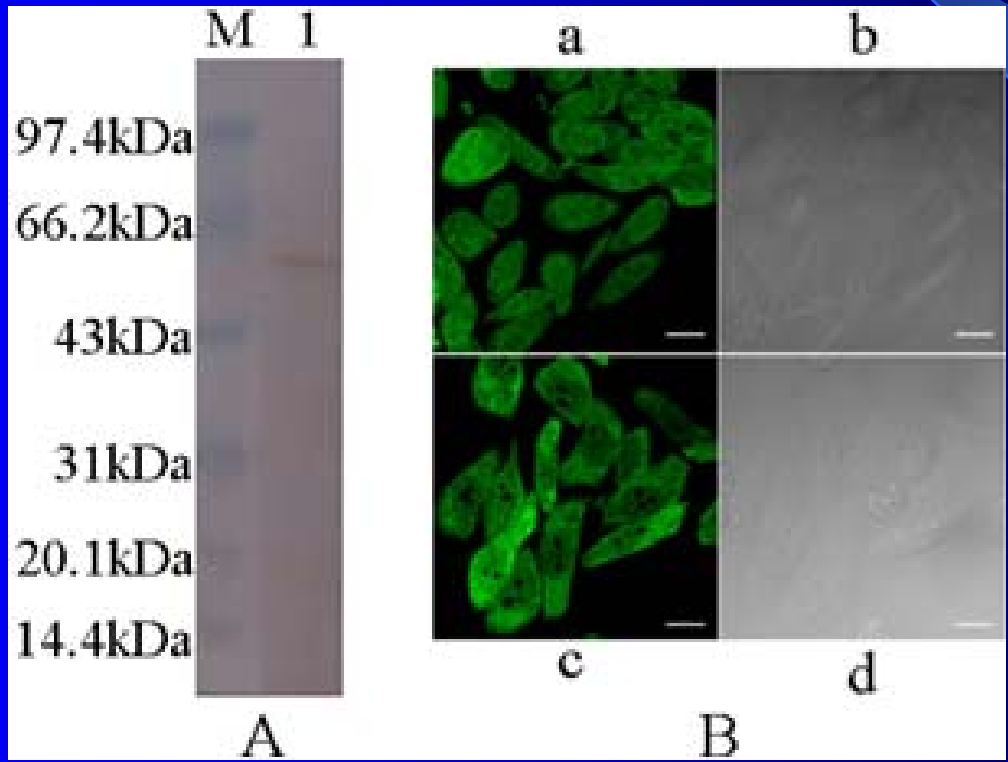
Oxalate decarboxylase and epimerase acyivities: No!

Both tissue-section *in situ* hybridization and immunohistochemistry demonstrated that BbDUF985 was expressed in a tissue-specific manner, with most abundant levels in the hepatic caecum and ovary.





In the CHO cells transfected with the expression plasmid pEGFP-N1/BbDUF985, the fusion protein was targeted in the cytoplasm of CHO cells, suggesting that BbDUF985 is a cytosolic protein.

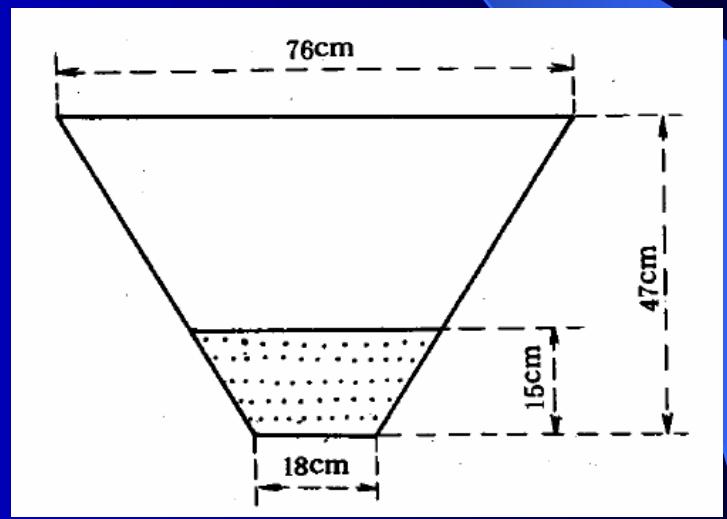


Amphioxus-Rare & endangered animals

- World-wide but patchy distribution
- 29 Species
- Qingdao & Xiamen
- Pollution
- Change in living environment
- Reproduction-necessary

Maintenance in Lab

- Container with 15 cm layer of sand
- Room temperature
- Ambient photoperiod
- Unicellular algae
- Water change



Gonads developed normally in the animals maintained in lab.

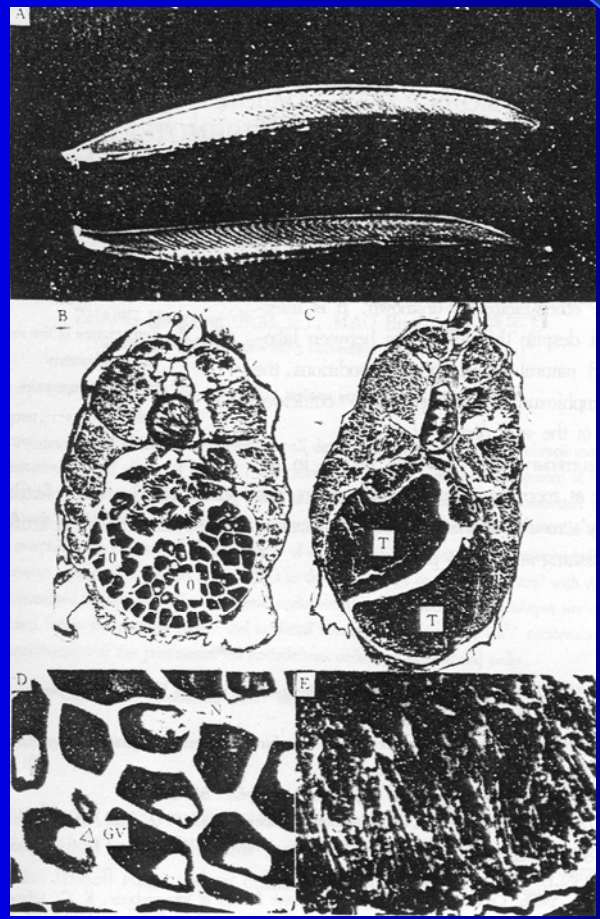


Table 1. The statistics of gonadal development and gamete release of the animals maintained in the laboratory.

Group	1996~1997			1998~1999	
	♀	♂	♀+♂	♀	♂
Total	136	136	136	60	60
No. of animals dead	4	0	1	5	5
No. of animals with fully grown gonads	122*	128	116	49*	55
Percentage of animals with fully grown gonads	92% (122/132)	94% (128/136)	85% (116/135)	92% (49/53)	100% (55/55)
No. of animals spawned	0	0	116	0	0
Percentage of animals spawned	0 (0/122)	0 (0/128)	100% (116/116)	0 (0/49)	0 (0/55)

*Five and 2 sexually reversed males had been excluded, respectively.

Presence of pheromone?

Table 2. Comparison of the number of ovaries, the fecundity and the diameter of oocytes between the laboratory-maintained and wild lancelets.

No.		1	2	3	4	5	6	Average
Body length (mm)		35	36	37	38	39	40	
Laboartory maintained animals	No of ovaries (L:R)	24:25	25:27	25:27	25:27	25:29	N	25:27
	Fecundity	2695	2900	3250	3640	4680	N	3433
	Diameter of oocytes (μm)	140	140	135	142	144	N	140
Wild animals	No of ovaries (L:R)	N	25:27	24:26	25:27	25:28	24:26	25:27
	Fecundity	N	2990	3200	3770	4740	5200	3980
	Diameter of oocytes (μm)	N	144	138	134	140	144	140

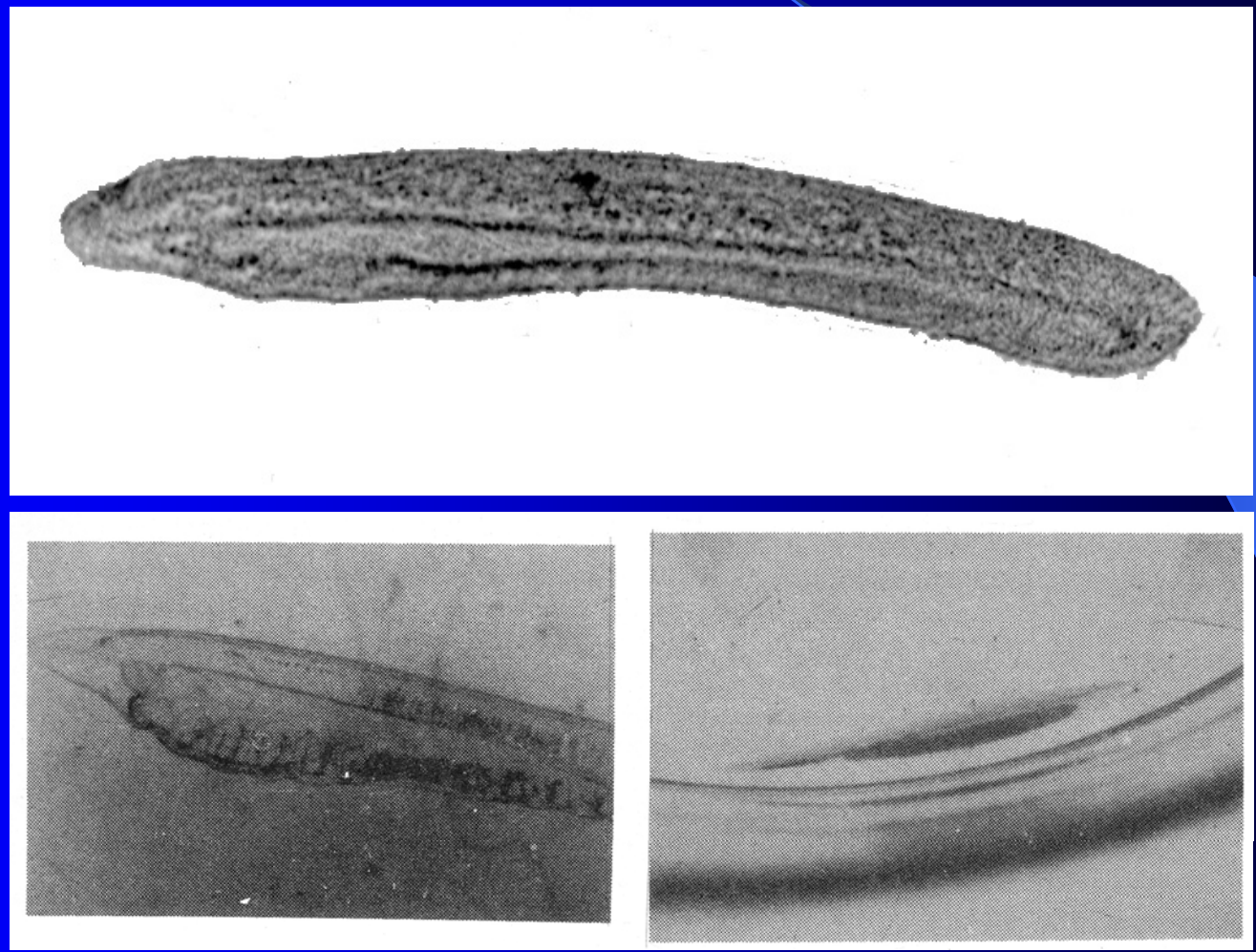
N=not determined. L:R=Left:Right.

Table 3. Comparison of the number of testes between the laboratory-maintained and wild lancelets.

No.	1	2	3	4	5	6	Average
Body length (mm)	36	37	38	39	39	40	
Laboratory maintained animals	25:27	26:28	26:28	25:27	25:27	N	25:27
Wild animals	26:27	25:27	25:27	26:28	N	25:28	25:27

N=not determined.

Nornal development of eggs released from amphioxus maintained in lab




Thank you!