解剖學與細胞生物學研究主題之選擇、發展、與願景

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解剖學與細胞生物學研究主題之選擇

探討生物體自細胞起各層次結構與功能之關係。
範圍除涵蓋細胞生物、解剖、胚胎、 組織等領域外，並包括細胞組織分化、變異、退化與再生，以及內、外在環境改變後從細胞到個體各層級形態功能變異與重整之基礎與臨床等相關研究領域。

細胞生物學: 包山包海，大小通吃，老少咸宜

選對上天堂！選錯住套房！

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INTERNATIONAL SOCIETY FOR STEM CELL RESEARCH

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Concurrent Topics

- Cardiac Development and Disease
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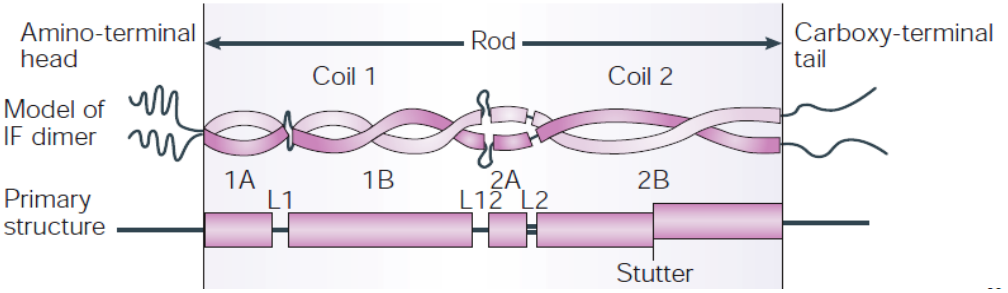
- Epigenetics and Genetic Regulatory Networks
- Epithelial Stem Cells
- Gene Editing
- Hematopoiesis
- Metabolism and Aging
- Mechanisms of Reprogramming I: To Pluripotency
- Mechanisms of Reprogramming II: Transdifferentiation Between Lineages
- Muscle Stem Cells
- Nervous System Disease
- Neural Development
- Organoids in Modeling Disease and Development
- Road to the Clinic I
- Road to the Clinic II
- Stem Cell Niches
- Stem Cells and Cancer
- Stem Cells in Organ Development and Maintenance
- Technology Frontiers
- Tissue Engineering

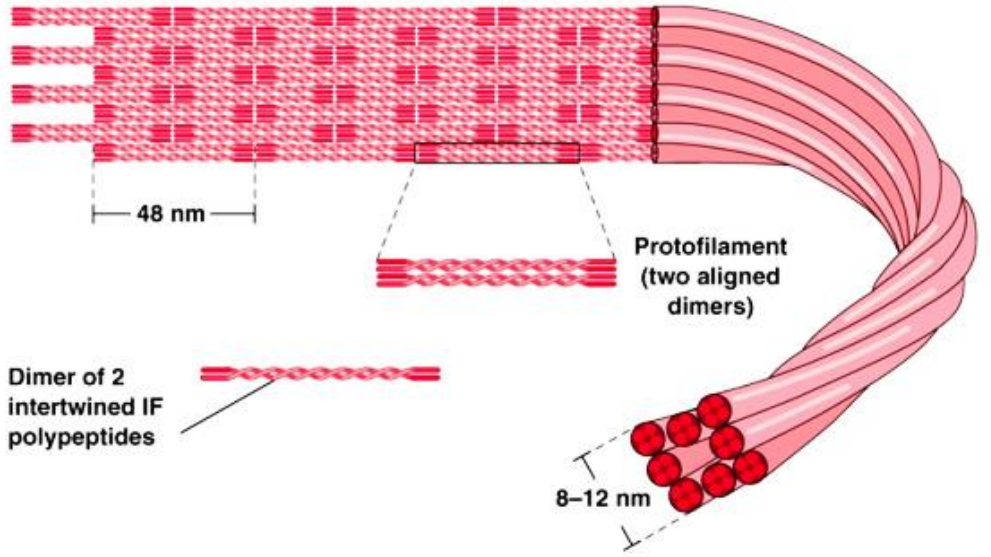
Board Numbers	Session Titles
B200-B228	Science Education 1
B230-B250	Light and Electron Microscopy in New Imaging Technologies
B251-B271	New Technologies for Cell Biology 1
B273-B293	Actin and Actin-Associated Proteins 1
B294-B299; B300-B311	Regulation of Actin Dynamics 1
B313-B330	Kinesins 1
B332-B347	Microtubule Dynamics and Its Regulation 1
B350-B371	Ciliary Signaling and Ciliopathies
B373-B389; B400-B406	Cytokinesis 1
B407-B419	Kinetochore Assembly and Functions 1
B420-B432	Centrosome Assembly and Functions 1
B433-B454	Chromosome Organization
B455-B471	Spindle Assembly 1
B473-B489; B500-B511	Cancer Therapy 1
B512-B530	Oncogenes and Tumor Suppressors 1
B531-B548	Invasion and Metastasis
B549-B567	Tumor Microenvironment 1
B900-B913	Regulatory and Non-Coding RNAs
B914-B920	RNA Localization, Transport, Stability, and Modification
B922-B941	The Nuclear Envelope and Nuclear Pore Complexes 1
B943-B971	Endocytic Trafficking 1
B972-B989; B1000-B1001	ER and Golgi Transport
B1002-B1014	Rab GTPases
B1016-B1043	Cell Signaling and Polarity
B1045-B1067	Neuronal Morphogenesis and the Cytoskeleton
B1069-B1088	Establishing and Maintaining Organelle Structure 1
B1100-B1118	Mitochondria, Chloroplasts, and Peroxisomes 1
B1119-B1139	Lipids and Membrane Microdomains 1
B1140-B1165	Signaling from the PM/Cytoplasm to the Nucleus
B1200-B1216	Signaling Scaffolds and Microdomains
B1218-B1241	Mechanotransduction 1
B1242-B1247	Intermediate Filaments
B1249-B1265	Cell-Cell Junctions 1
B1266-B1289	Integrins and Cell-ECM Interactions
B1301-B1323	Cell Death
B1324-B1343	Chaperones, Protein Folding, and Quality Control 1
B1345-B1361	Physical Approaches to Cell Biology
B1362-B1368; B1400-B1404	Systems and Synthetic Biology, and Tissue Engineering
B1406-B1423	Germ Cells, Gametogenesis, and Fertilization
B1424-B1445	Cell-Cell Interactions in Tissue Development and Morphogenesis
B1446-B1457	Cell Fate Determination 1
B1459-B1473	Prokaryotic Cell Biology
B1475-B1493	Defining Therapeutic Targets and New Therapeutics 1

Intermediate Filaments (IFs)

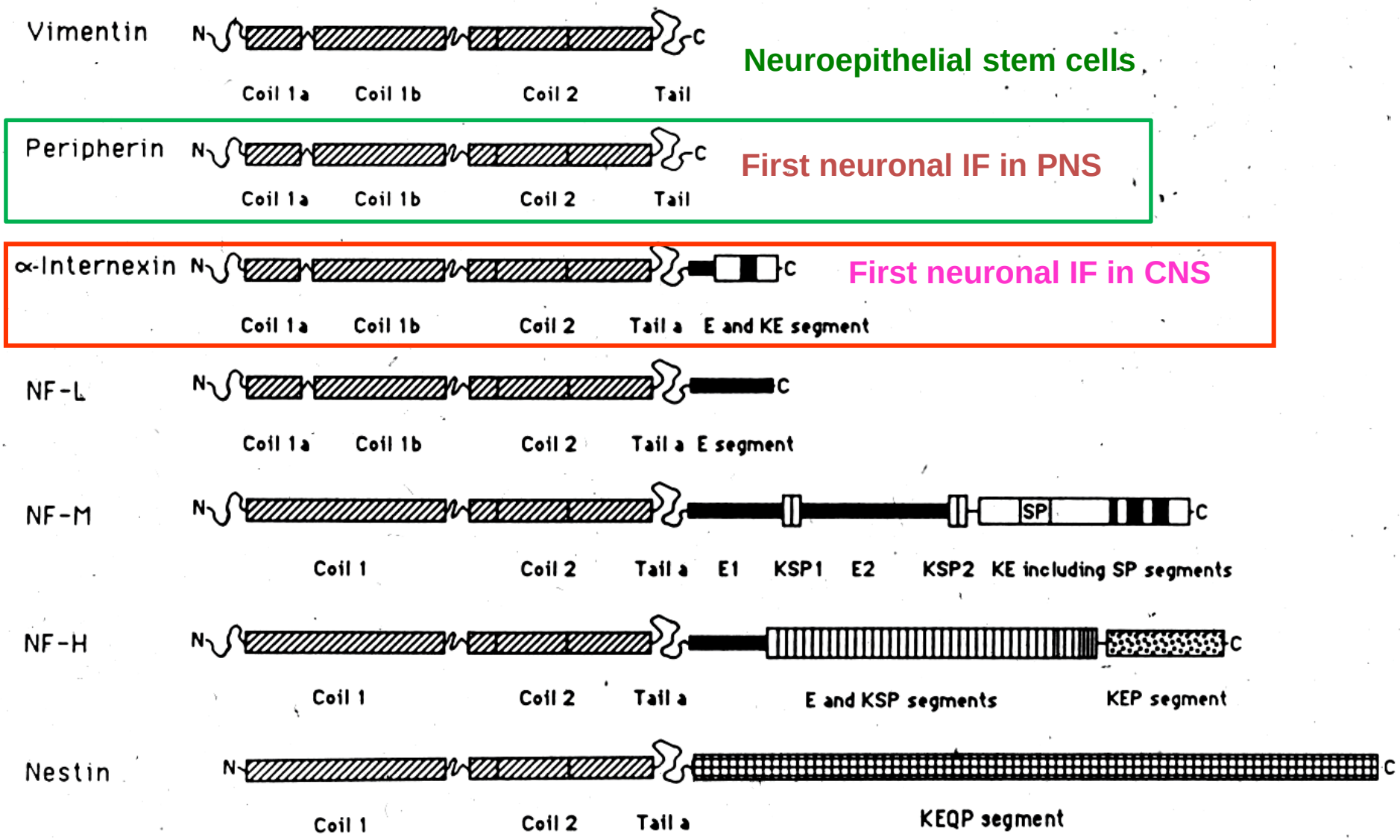
– 10 nm filaments
– Cytoskeletal component
– Tissue-specific proteins
– 6 types:
  - Type I (acid keratins)
  - Type II (basic keratins)
  - Type III (**vimentin**, desmin, **GFAP**, and **peripherin**)
  - **Type IV [neuronfilaments (NFs), α-internexin**, synemin, and syncoilin]
  - Type V (lamins)
  - Type VI (**nestin**)



(Chang and Goldman, 2004)



Seven Intermediate Filament Proteins in Neural Differentiation



Neuroepithelial stem cells

Gene, 149 (1994) 289–292

GENE 08245

Characterization of the mouse gene encoding the neuronal intermediate filament protein α-internexin

(Neurofilament; sequence homology; genomic sequence; recombinant DNA)

Chung-Liang Chien and Ronald K.H. Liem

Departments of Pathology and Anatomy and Cell Biology, Columbia University College of Physicians and Surgeons, New York, NY10032, USA

Received by R.W. Davies: 18 February 1994; Revised/Accepted: 16 April/10 May 1994; Received at publishers: 11 July 1994

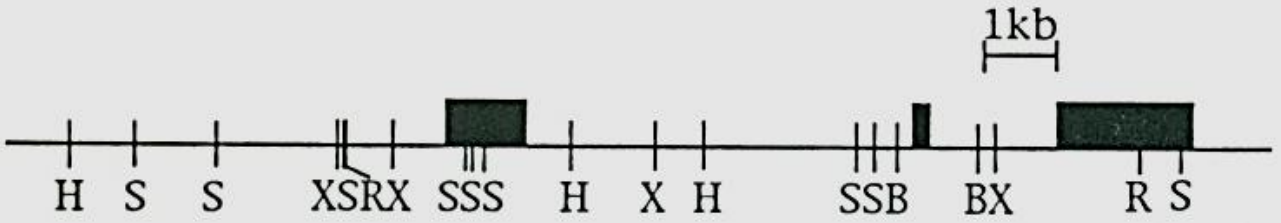
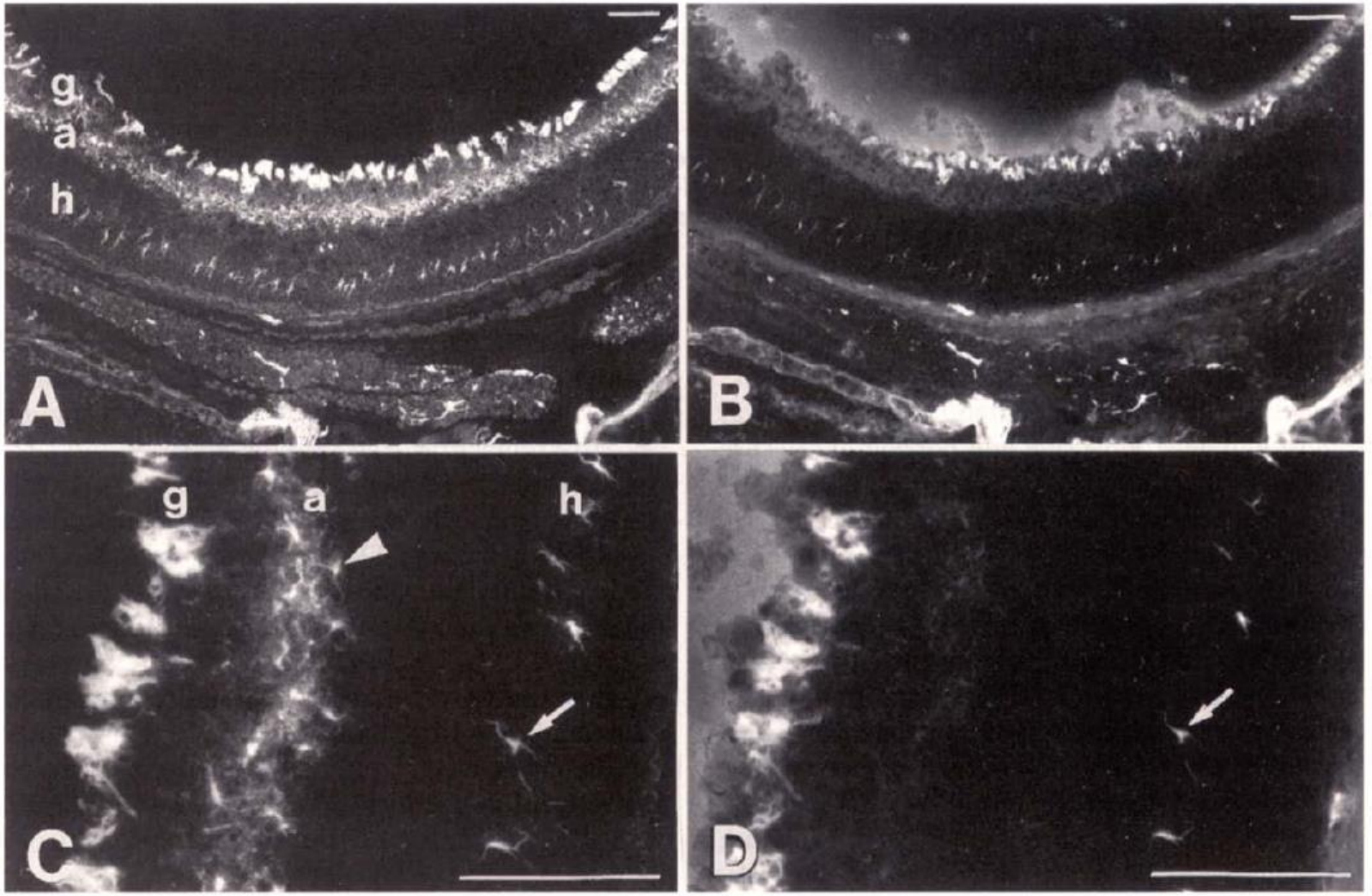


Fig. 1. Restriction map and genomic structure of the m*αINX*. Exons I, II and III are shown as filled boxes in the gene structure. The lengths of intron I and II are about 5.6 kb and 1.6 kb, respectively. B, *Bam*HI; H, *Hind*III; R, *Eco*RI; S, *Sac*I; X, *Xba*I.

α-internexin and NFM in new-born mouse retina



Chien and Liem, 1995. The neuronal intermediate filament, alpha-internexin is transiently expressed in amacrine cells in the developing mouse retina. ***Exp. Eye Res.*** 61:749-756.

Mouse α-internexin is transiently expressed in amacrine cell

Immunoreactivity of neuronal intermediate filament proteins in the developing mouse retina

Age	Horizontal cell processes	Amacrine cell processes	Ganglion nerve fibers	Glial (Müller) processes	Glial processes in ganglion nerve fibers
newborn	α-internexin, NFL, NFM, Vimentin	α-internexin,	α-internexin, NFL, NFM	Vimentin	Vimentin
P 3	↓	↓	↓	↓	↓
P 5	α-internexin, NFL, NFM, Vimentin, (NFH)	α-internexin, (NFM)	α-internexin, NFL, NFM, (NFH)	↓	↓
P 7	α-internexin, NFL, NFM, NFH, Vimentin	(α-internexin)	α-internexin, NFL, NFM, NFH	↓	↓
P 9	↓	n.a.	↓	↓	↓
P 11	↓	n.a.	↓	↓	↓
P 14	↓	n.a.	↓	↓	↓
P 21	↓	n.a.	↓	↓	Vimentin, (GFAP)
4 weeks	↓	n.a.	↓	↓	Vimentin, GFAP
6 months	α-internexin, NFL, NFM, NFH, Vimentin	n.a.	α-internexin, NFL, NFM, NFH	Vimentin	Vimentin, GFAP

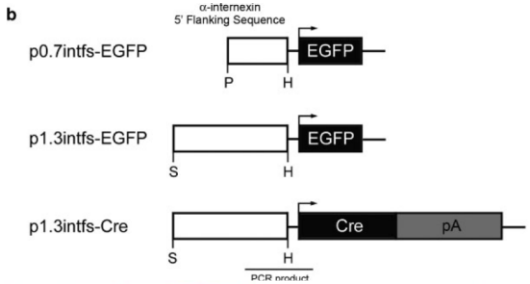
n.a.: no detectable immunoreactivity; arrow indicates the same level of immunoreactivity as detected at earlier stage(s); parenthesis indicates the weak immunoreactivity.

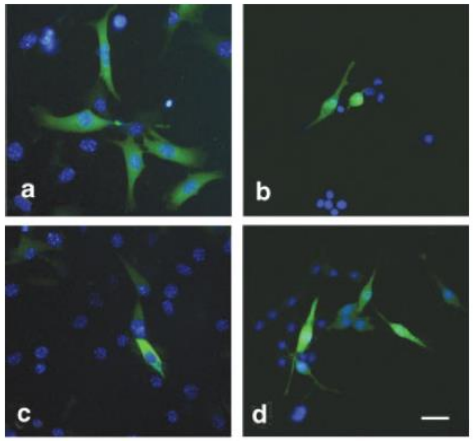
Neural Expression of α-*Internexin* Promoter In Vitro and In Vivo

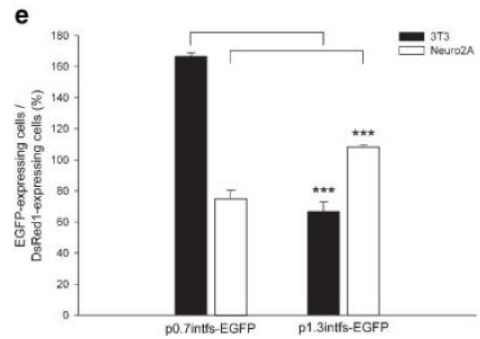
Journal of Cellular Biochemistry 9999:1–13 (2005)

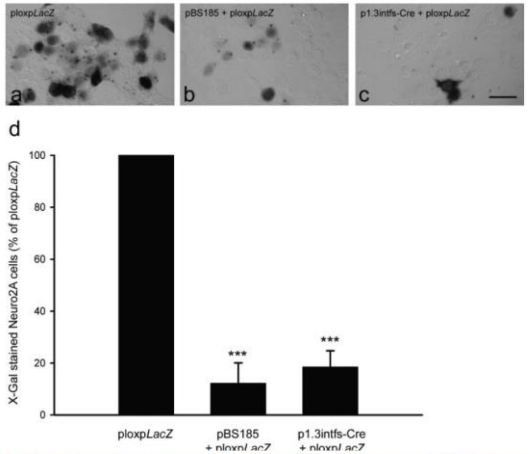
Pei Wang, Seu-Mai Wang, Chia-Ju Hsieh, and Chung-Liang Chien*

Department of Anatomy and Cell Biology, College of Medicine, National Taiwan University, Taipei, Taiwan









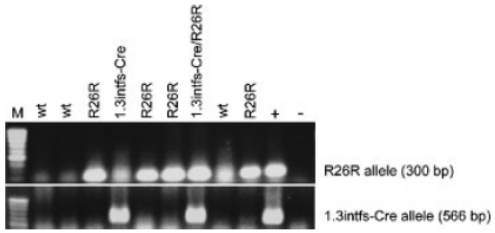
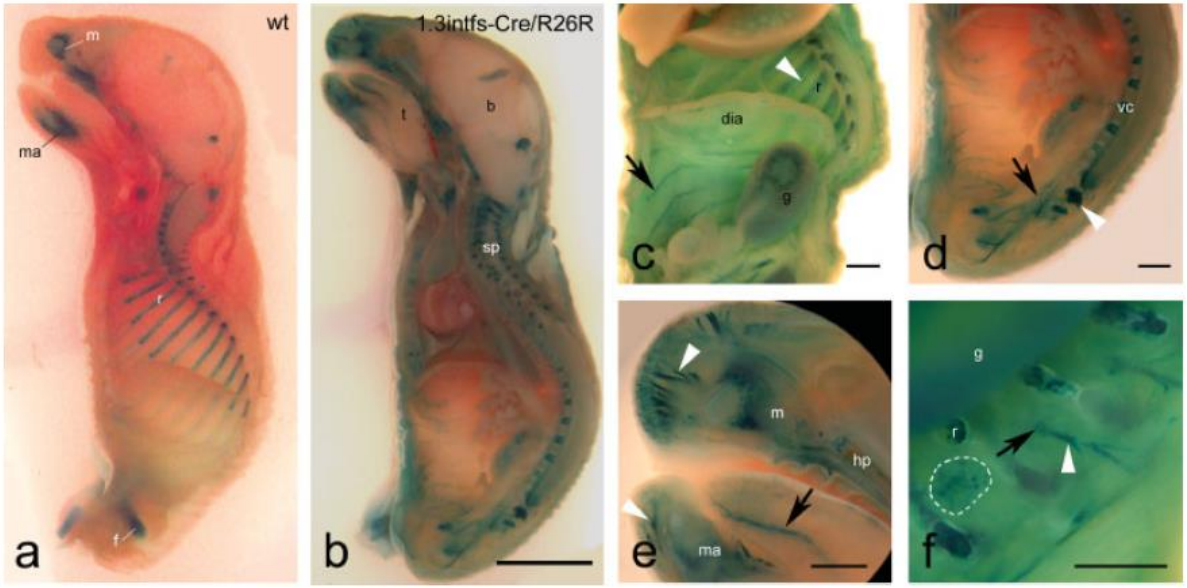


Fig. 4. PCR screening of 1.3intfs-Cre/R26R transgenics. The data represent a typical example (this litter contains nine newborns) from three independent litters. R26R allele-specific PCR and 1.3intfs-Cre allele-specific PCR were performed on mouse tail genomic DNA from the1.3intfs-Cre transgenic line crossed with the R26R strain. One double-transgenic 1.3intfs-Cre/R26R mouse can be identified from the wild type (wt), 1.3intfs, and R26R mice. M: 100 bp DNA ladder marker, +: positive control, −: negative control.



Example for a PhD student training

The Journal of Comparative Neurology | Research in Systems Neuroscience 521:2147–2164 (2013)

Molecular Cloning and Characterization of Chicken Neuronal Intermediate Filament Protein α-Internexin

Chi-Hsiu Liu and Chung-Liang Chien*
Department of Anatomy and Cell Biology, College of Medicine, National Taiwan University, Taipei 10051, Taiwan R.O.C

Experimental Eye Research
Experimental Eye Research 110 (2013) 18–25

Neuronal intermediate filament α-internexin is expressed by neuronal lineages in the developing chicken retina

Chi-Hsiu Liu [a], I-Jong Wang [b], Fong-Di Wei [a], Chung-Liang Chien [a,*]

[a] Department of Anatomy and Cell Biology, College of Medicine, National Taiwan University, No. 1, Section 1, Jen-Ai Road, Chung-Cheng Dist., Taipei 10051, Taiwan, ROC
[b] Department of Ophthalmology, National Taiwan University Hospital, No. 7, Chung Shan S. Rd., Chung-Cheng Dist., Taipei City 10002, Taiwan, ROC


43rd Annual Meeting for
the Japanese Society of Developmental Biologists
Jointly Sponsored by the Asia-Pacific Developmental Biology Network
June 20 – 23, 2010
Kyoto International Conference Center, Kyoto City, Japan

Best Presentation Award

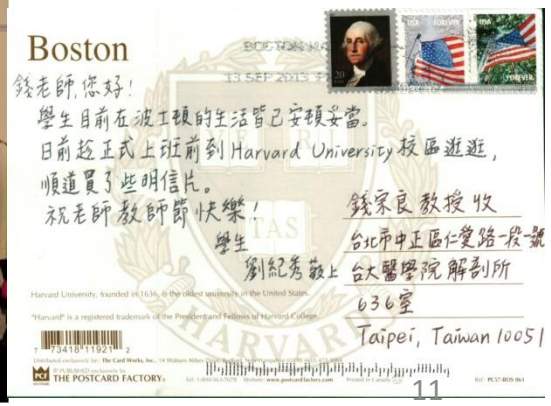
for an excellent presentation
to
Chi-Hsiu Liu

Dr. Tadashi Uemura
Chairperson of the 2010 JSDB-APDBN Meeting Organizing Committee

Dr. Atsuko Sehara
Head of Program Committee of the 2010 JSDB-APDBN meeting

Dr. Shinichi Aizawa
President of Japanese Society of Developmental Biologists

劉紀秀博士接受 楊院長撥穗後合影



cDNA and Protein Sequences of Chicken α-Internexin (chkINA)

```
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CGCCGCCTGCTCGCCCAGTCCCCGCGGCGCACGGAGGGCGCTGAGCCGCGCCGTGCTAGC
 R  R  L  L  A  Q  S  P  R  R  T  E  G  A  E  P  R  R  A  S
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 E  K  E  Q  L  R  G  L  N  E  R  F  A  G  Y  I  E  R  V  R
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 P  E  P  R  R  L  G  Q  L  L  G  G  E  L  R  A  L  R  A  R
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 L  E  E  A  H  G  E  R  A  Q  A  A  L  E  R  A  R  L  A  E
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 E  T  Q  R  L  R  A  R  C  E  E  E  A  R  G  R  A  E  A  E
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 Q  A  L  R  A  R  Q  Q  A  A  D  G  A  A  R  A  R  A  D  L
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 T  A  R  P  D  L  A  A  A  L  R  E  L  R  A  Q  Y  E  A  L
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 P  A  R  N  L  Q  A  A  E  D  W  Y  R  A  R  C  A  S  L  H
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 D  T  I  G  Q  L  E  A  D  L  R  S  T  K  T  E  M  A  R  H
```

```
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 Y  R  K  L  L  E  G  E  E  N  L  F  S  M  G  S  V  G  L  P
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 A  M  N  P  L  P  N  P  T  Y  S  F  R  P  R  S  S  T  P  S
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 F  K  K  E  E  Q  R  E  A  V  R  A  T  S  K  I  P  S  G  Q
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 A  G  V  L  D  G  T  I  T  T  A  K  R  T  E  R  F  N  V  H
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 G  G  I  I  A  N  A  K  V  Q  W  W  E  P  H  P  F  C  I  *
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AAGTTAATAATTAAAAAGAAAGAAATAGTTATGTGAACCAAAAAAAAAAAAAAA
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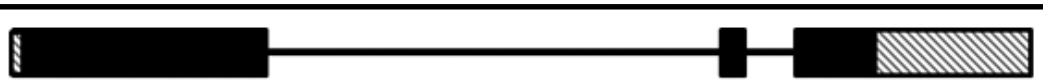
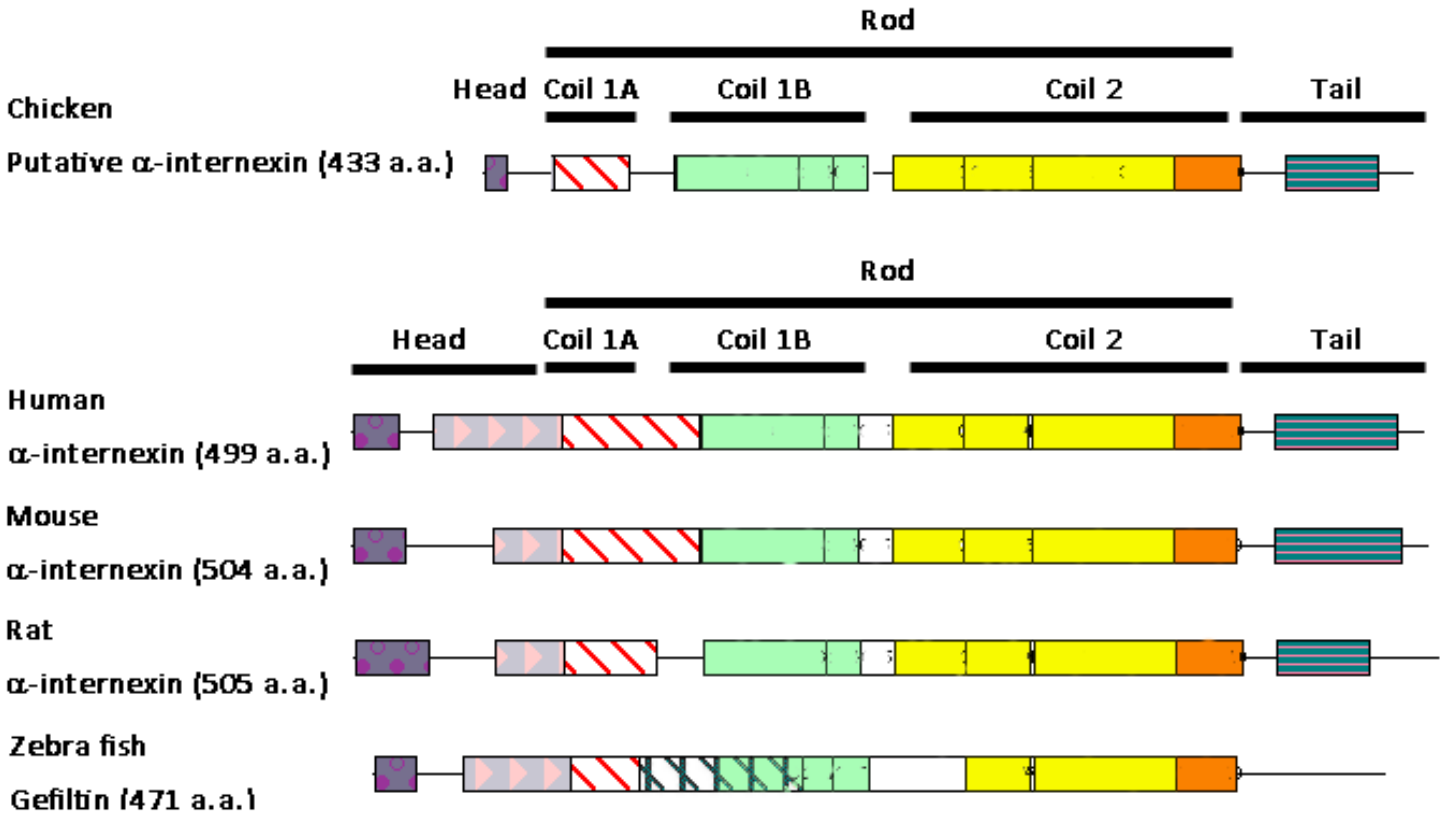
CDS: 1302 bp
Putative protein: 433 a.a.

Gene structure



Percent Identity and Similarity between Predicted Protein Sequence of chkINA and Other Species



	Identity	Similarity
Human α-internexin (499 a.a.)	55%	72%
Mouse α-internexin (504 a.a.)	55%	71%
Rat α-internexin (505 a.a.)	56%	72%
Zebra fish Gefiltin (471 a.a.)	52%	71%

Sequence alignments were done by online tool, **ProDom**, and modified by Photoshop software.
http://prodom.prabi.fr/prodom/current/html/form.php

- ✓ High Similarity
- ✓ Conserve structure

Custom Antibody – CHK 366-384

Predicted chicken α-internexin (chkINA)

- 433 a.a.
- Theoretical pI/Mw: 9.37 / 48435.59 (**48.4 kDa**)

MSYSVEPPALAASSRRLLAQSPRRTEGAEPRRASEKEQLRGLNERFAGYIERVRALEERN
RALAGELAELRRLPPEPRRLGQLLGGELRALRARLEEAHGERAQAALERARLAEETQRLR
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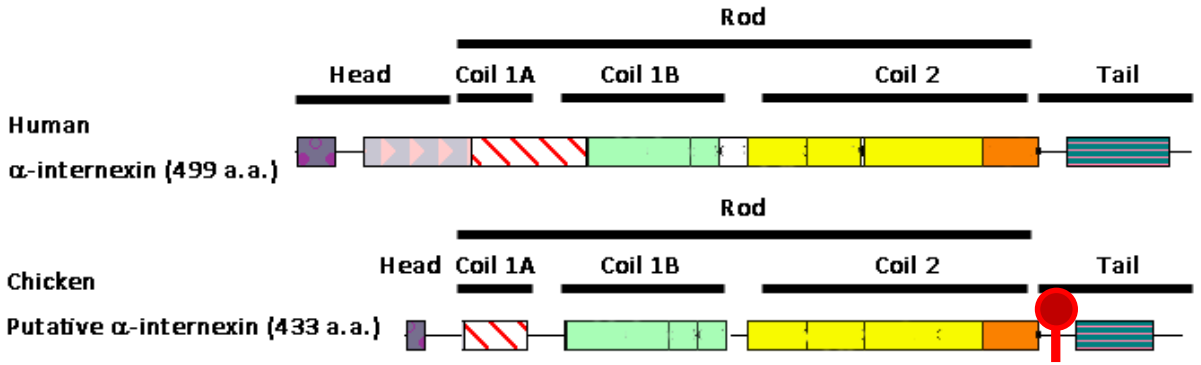
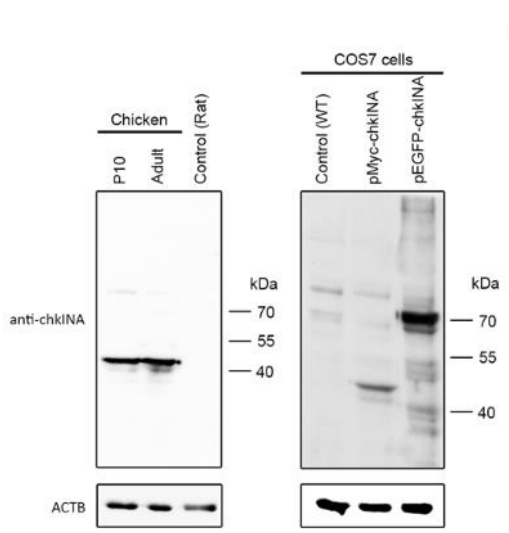
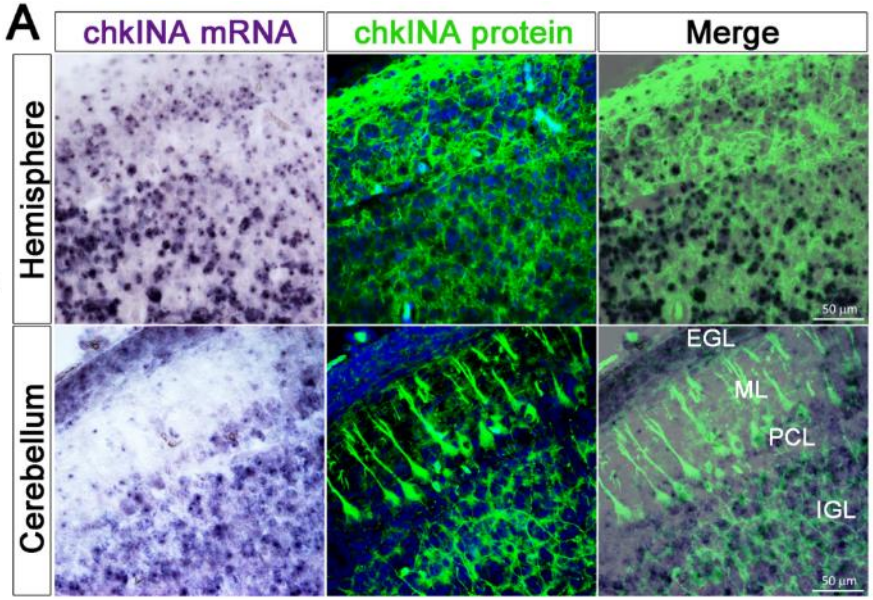
Epitope

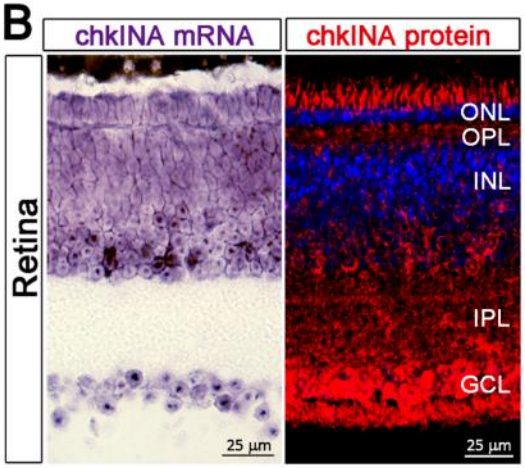


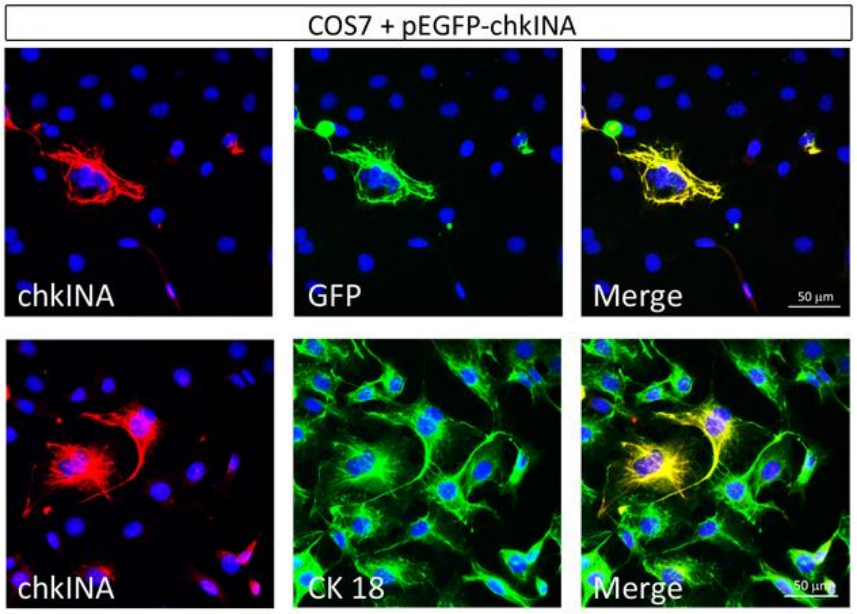
Antibody Characterization

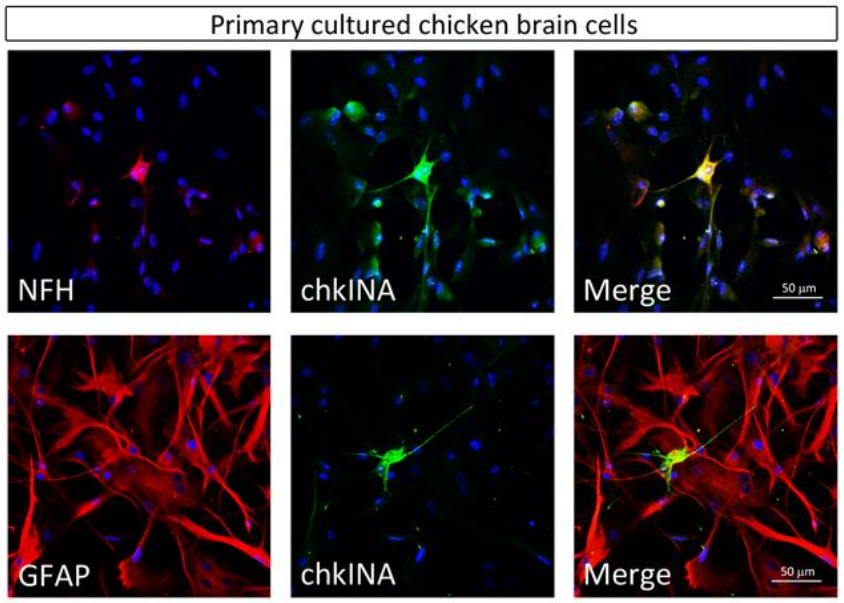
J. Comp. Neurol. 2013; 521:2147-64





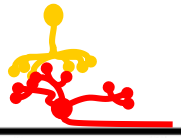




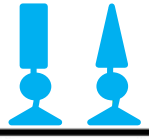


The antibody is specific for recognizing chkINA protein.

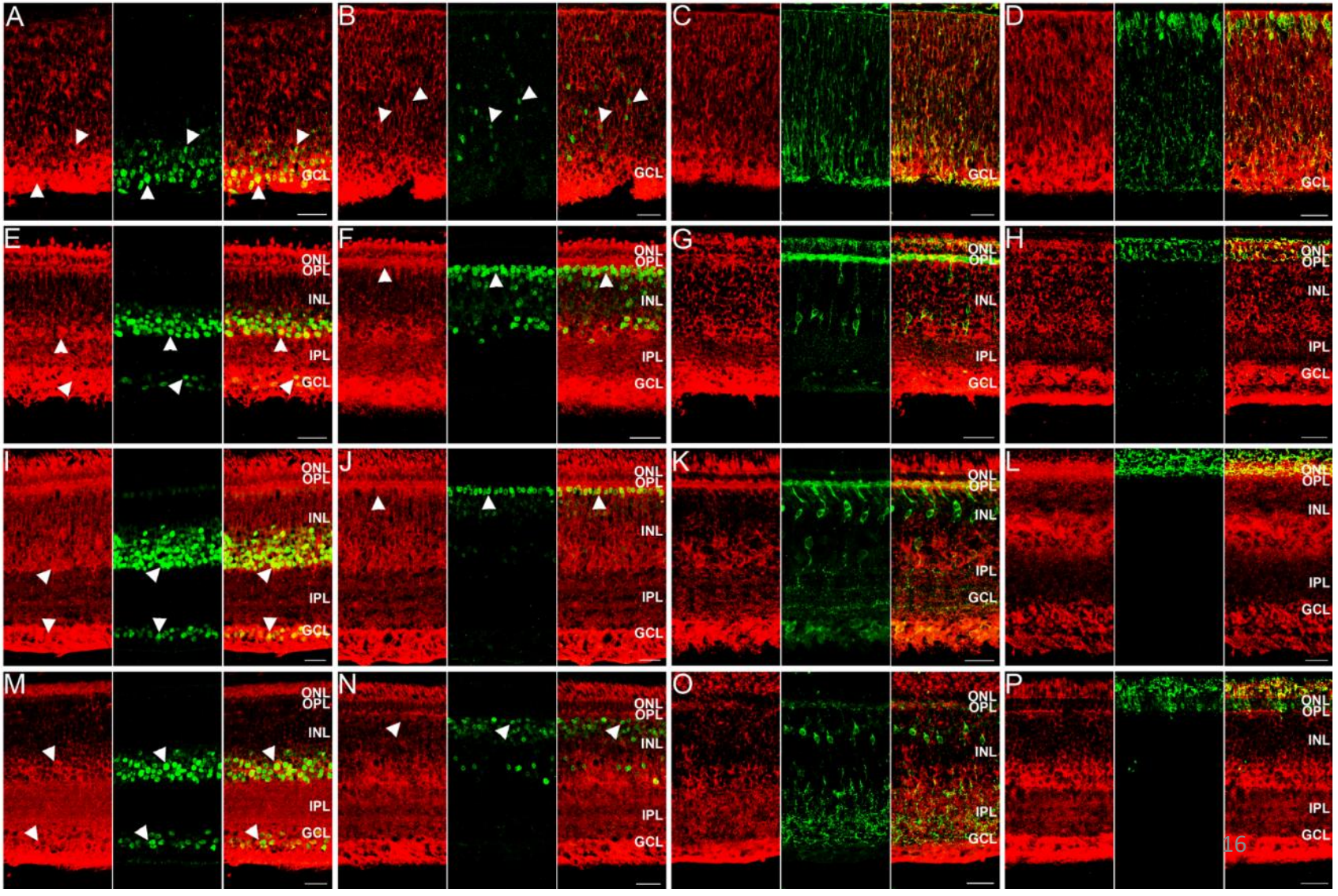
Expression of chkINA and Cell-type-specific Markers in the Developing Retina



Experimental Eye Research. 2013; 110:18-25.

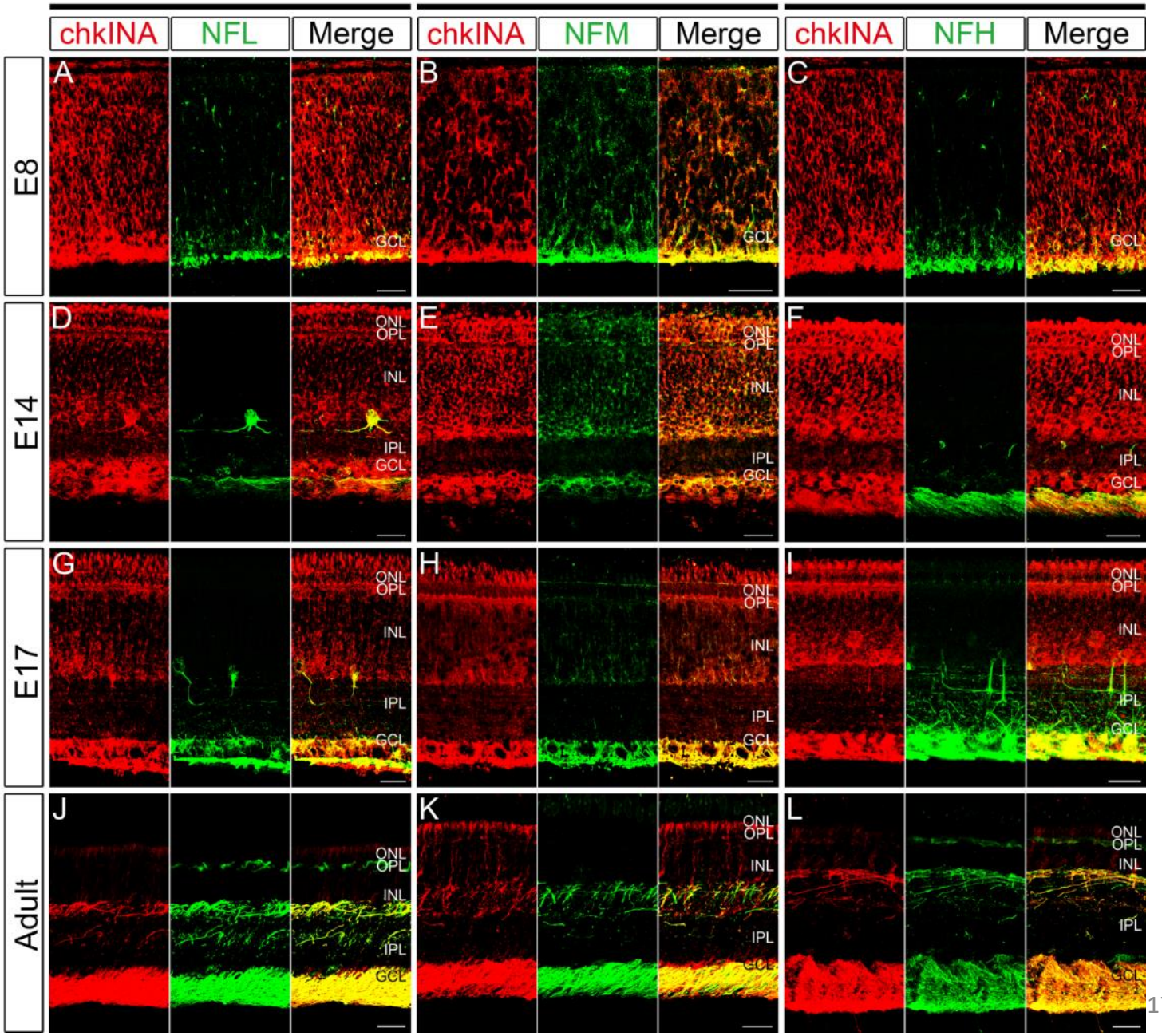


Scale bars = 25 µm



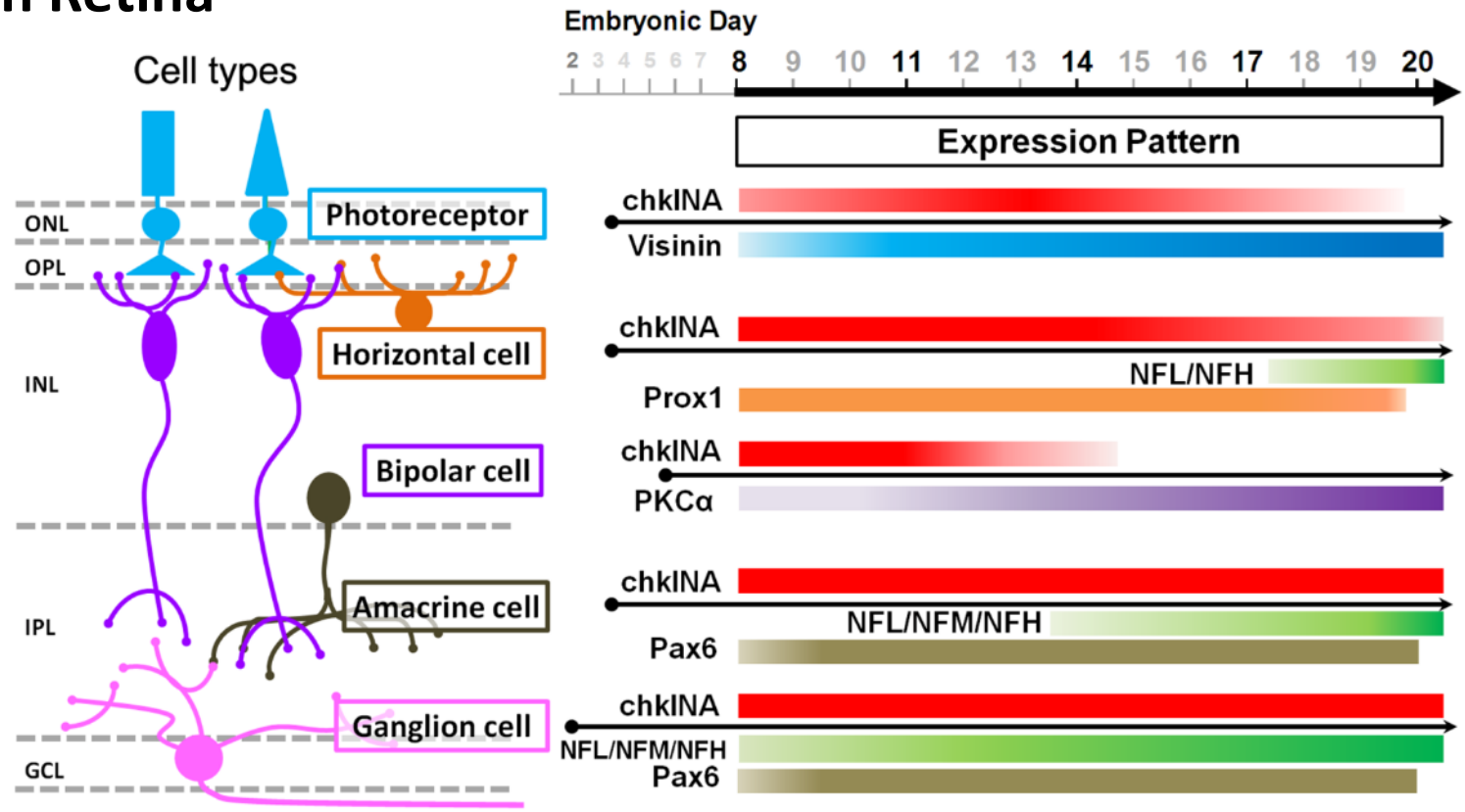
Distribution of chkINA and NFs in the Developing Retina

Experimental Eye Research. 2013; 110:18-25.



Scale bars = 25 µm

Chicken Retina



Dynamic pattern of **chkINA** during development

- Early stage → **all neuronal lineages**
- Late stage → **ganglion cells**, **amacrine cells**, and **horizontal cells**.

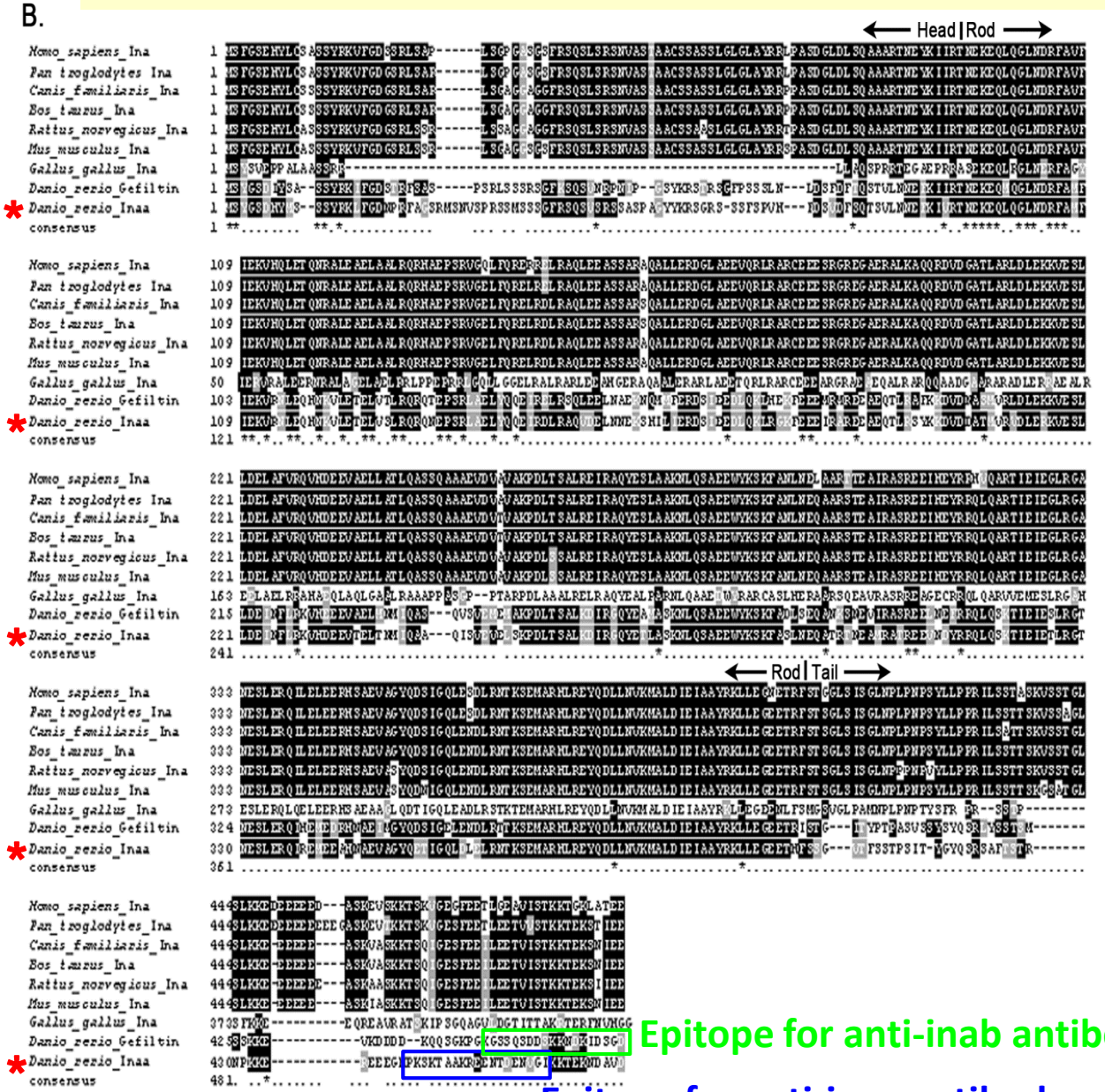
Dynamic pattern of **NF triplet proteins** during development

- Early stage → **ganglion cells**
- Late stage → **ganglion cells**, **amacrine cells**, and **horizontal cells**.

The developmental expression of neuronal intermediate filament inaa in the zebrafish retina

Dr. Meng-Lin Liao
2013 Travel Fellowship from the Japanese Society of Developmental Biologists (JSDB)

zebrafish α-internexin Homologs



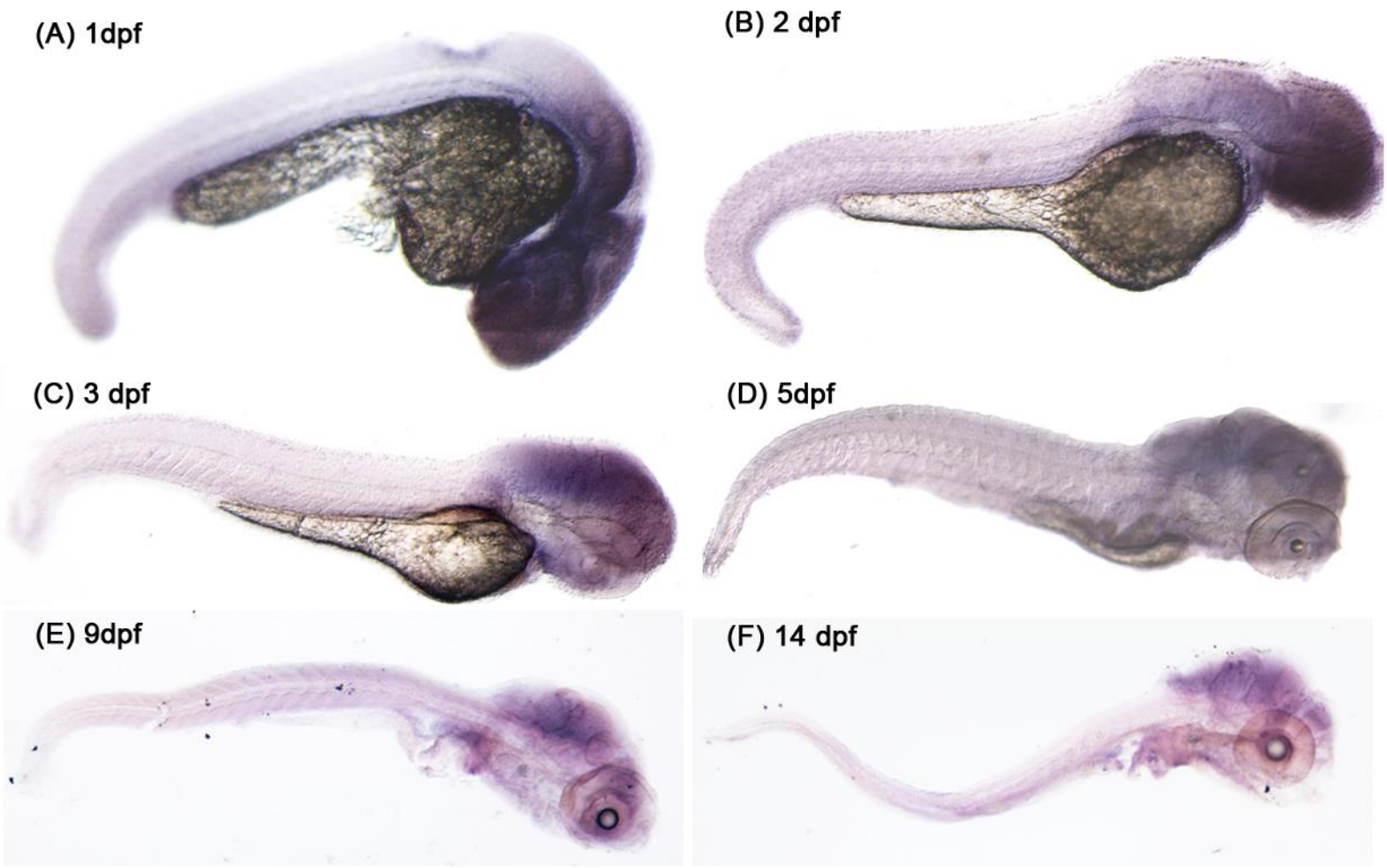
* Inaa
- NP_001138256
- 474 a.a. in length
- ~ 58 kDa

* Inab
- NP_571107.2
- 469 a.a. in length
- ~ 58 kDa

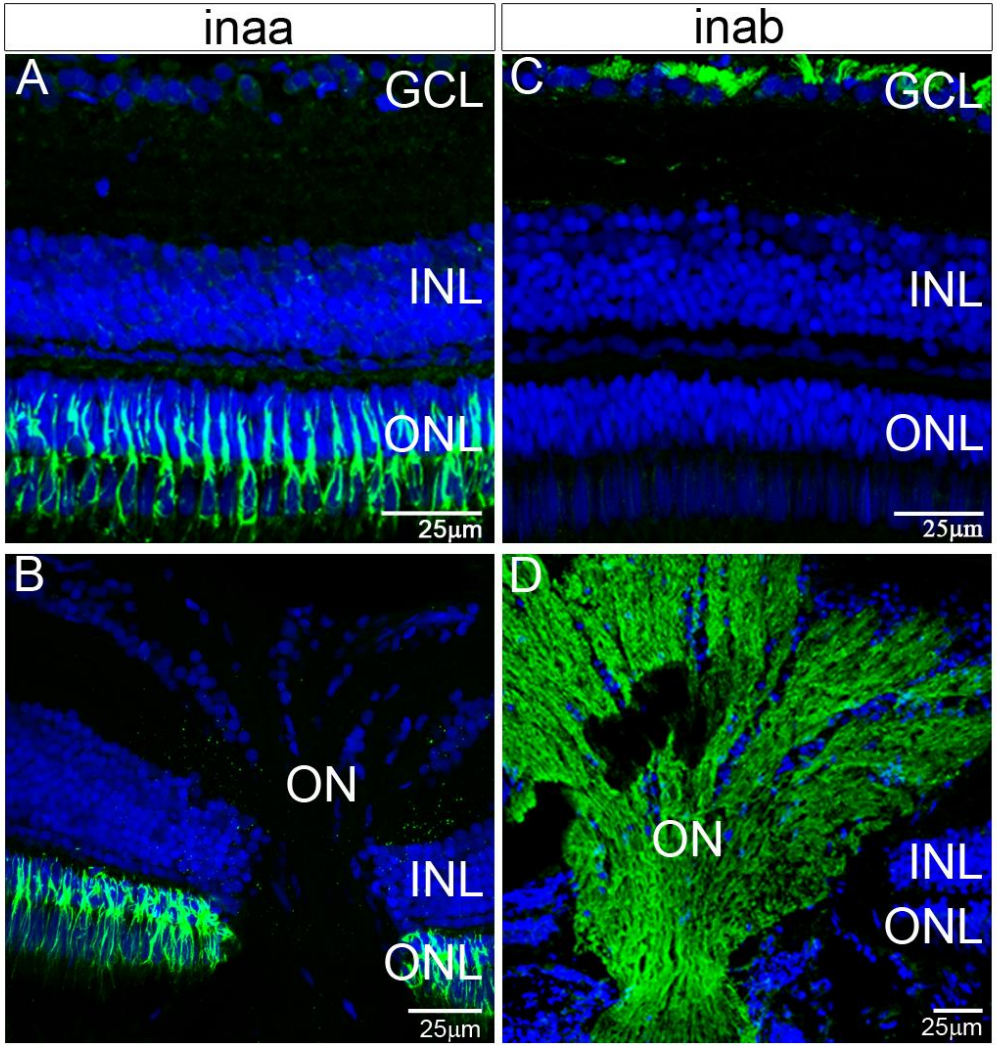
* Both genes have highly conserved central rod domain.

Transcription of *inaa* gene in zebrafish developing CNS

Whole-mount *in situ* hybridization

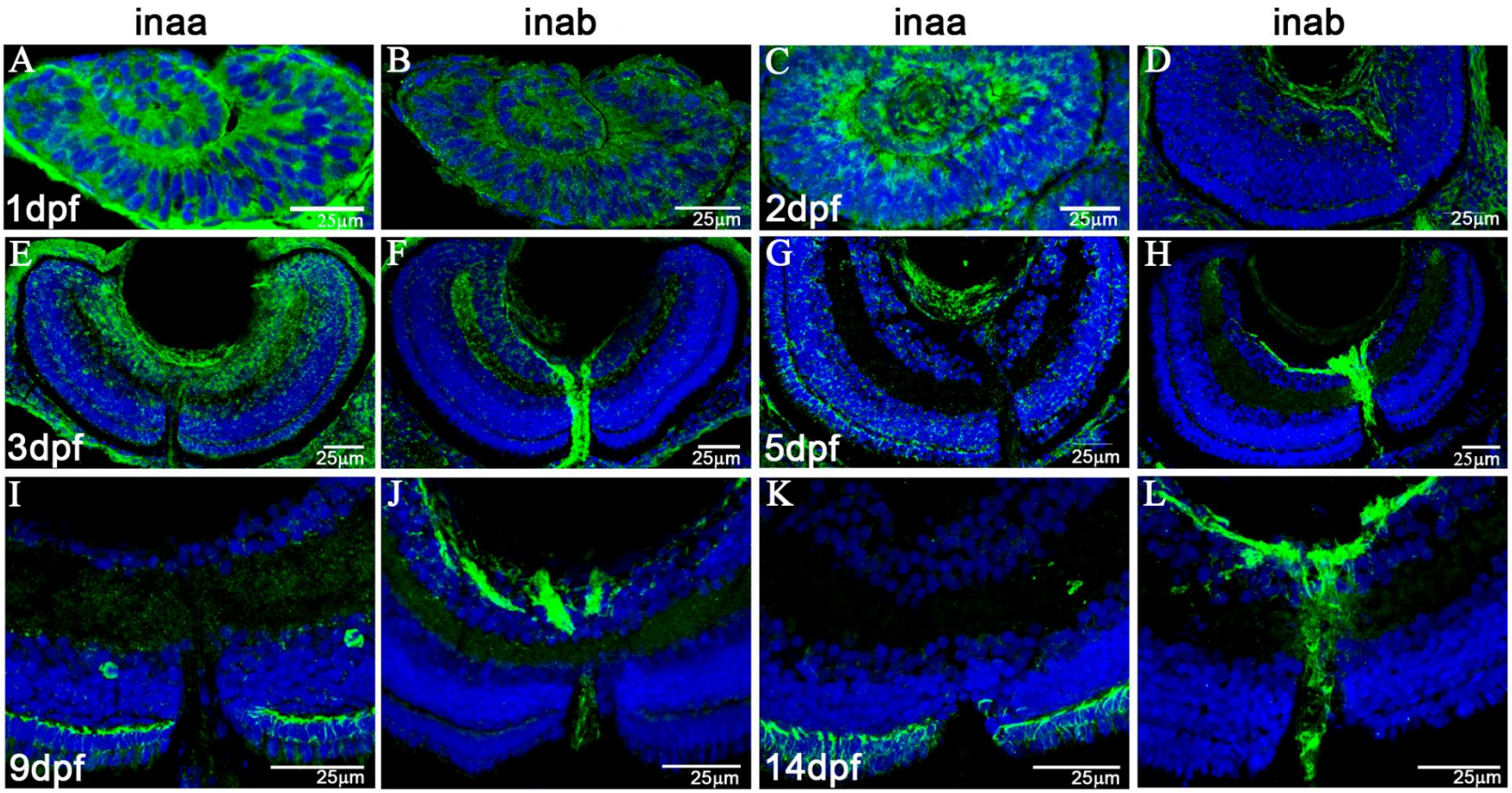


Distribution of inaa and inab protein in adult zebrafish retina

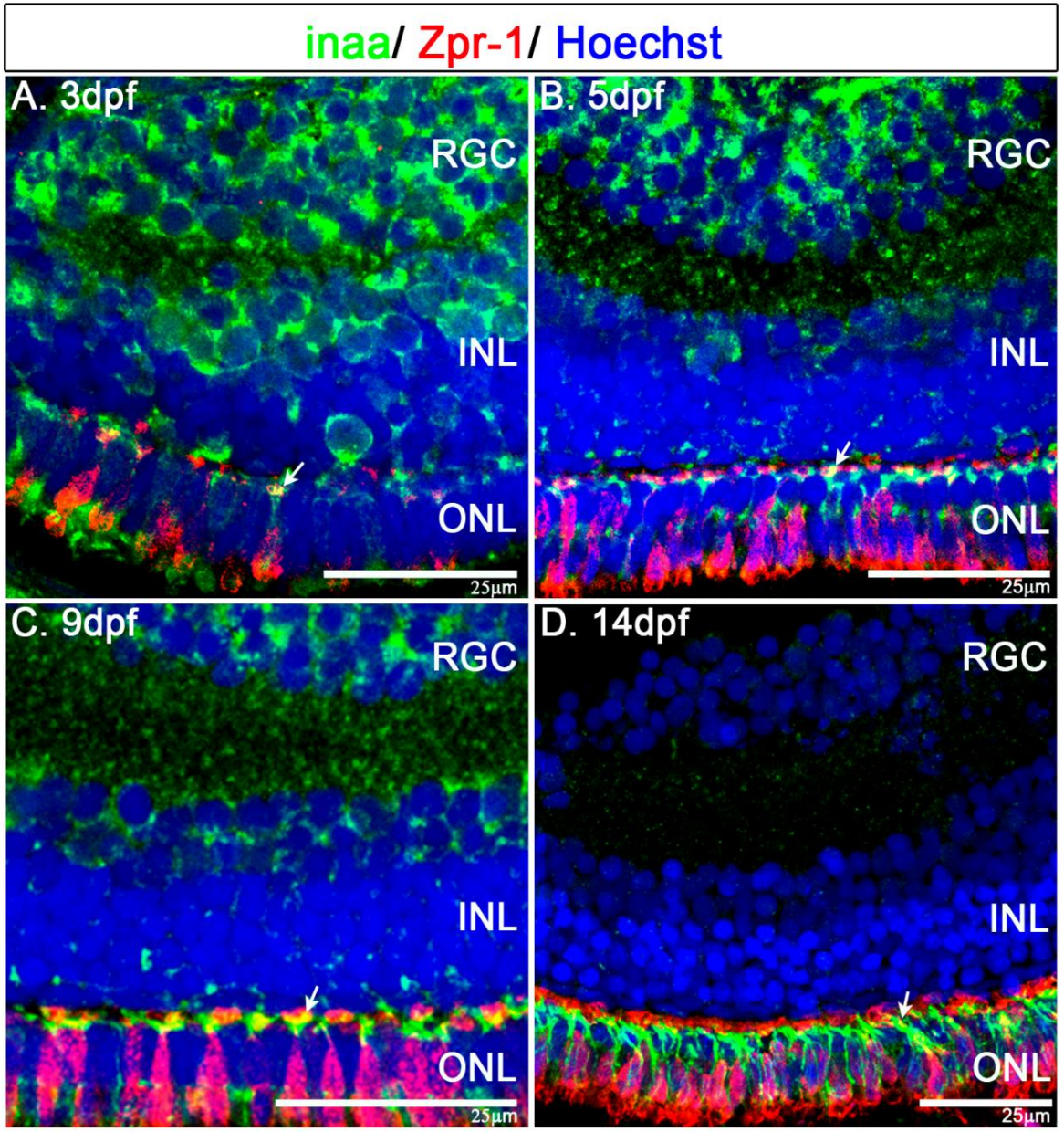


Inaa: mainly in outer nuclear layer (ONL)
Inab: mainly in the ganglion cell layer (GCL), optic fiber layer and optic nerve (ON)

Expression patterns of inaa and inab in developing zebrafish retina

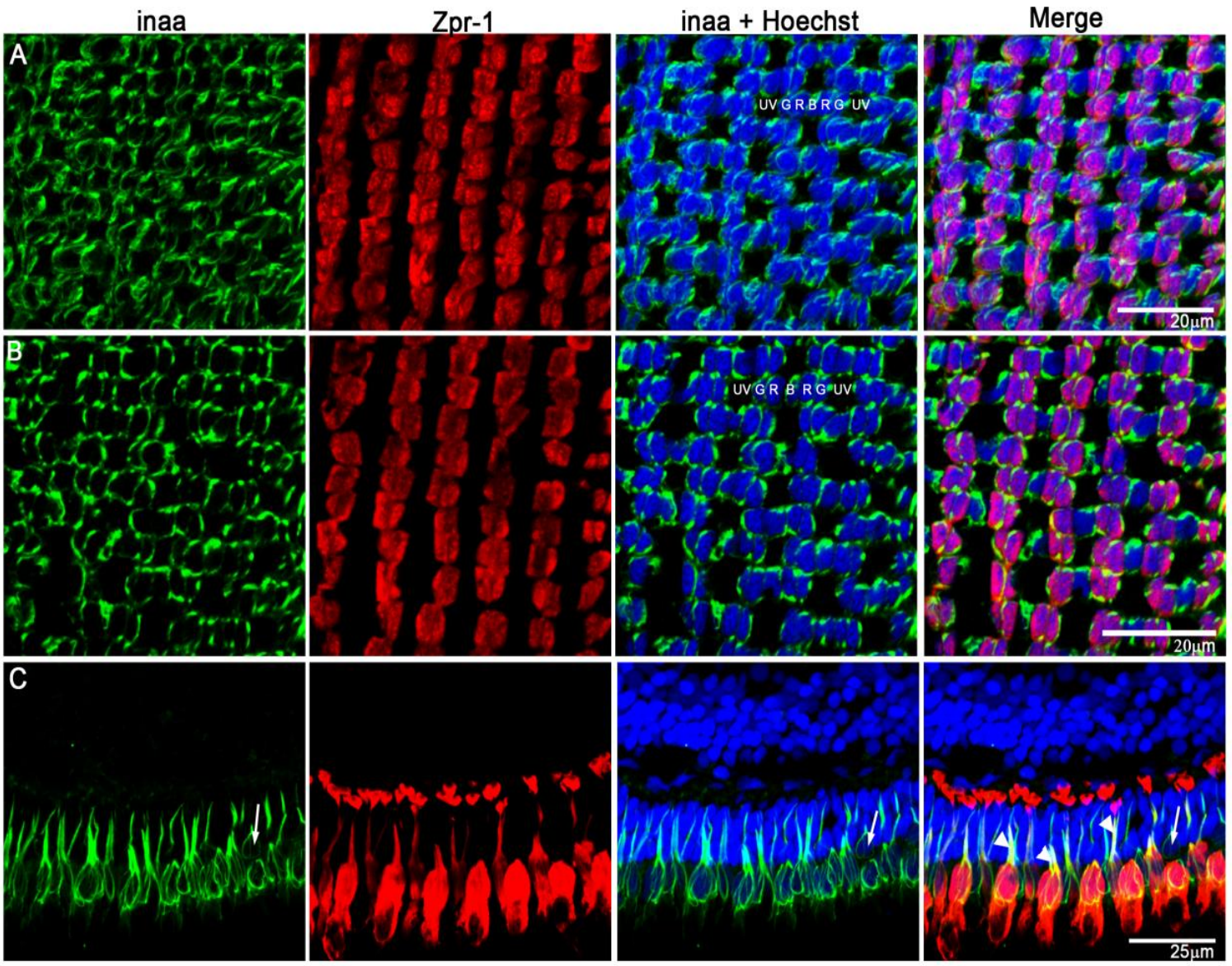


Inaa localized to the double-cone photoreceptors

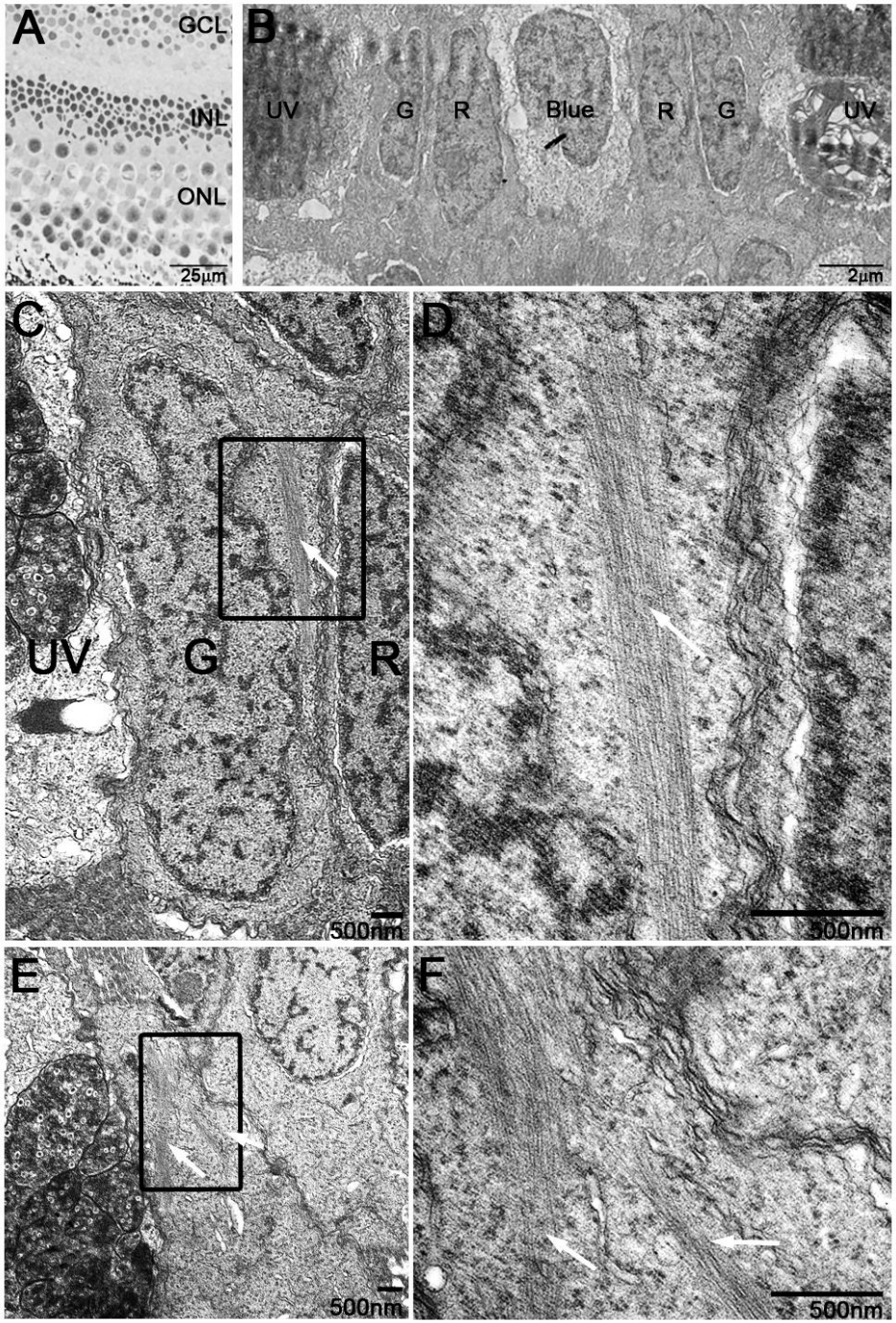


Zpr-1:
marker for double-cone

Inaa is predominantly expressed in cone photoreceptors of adult retina.



Zpr-1: marker for double-cone

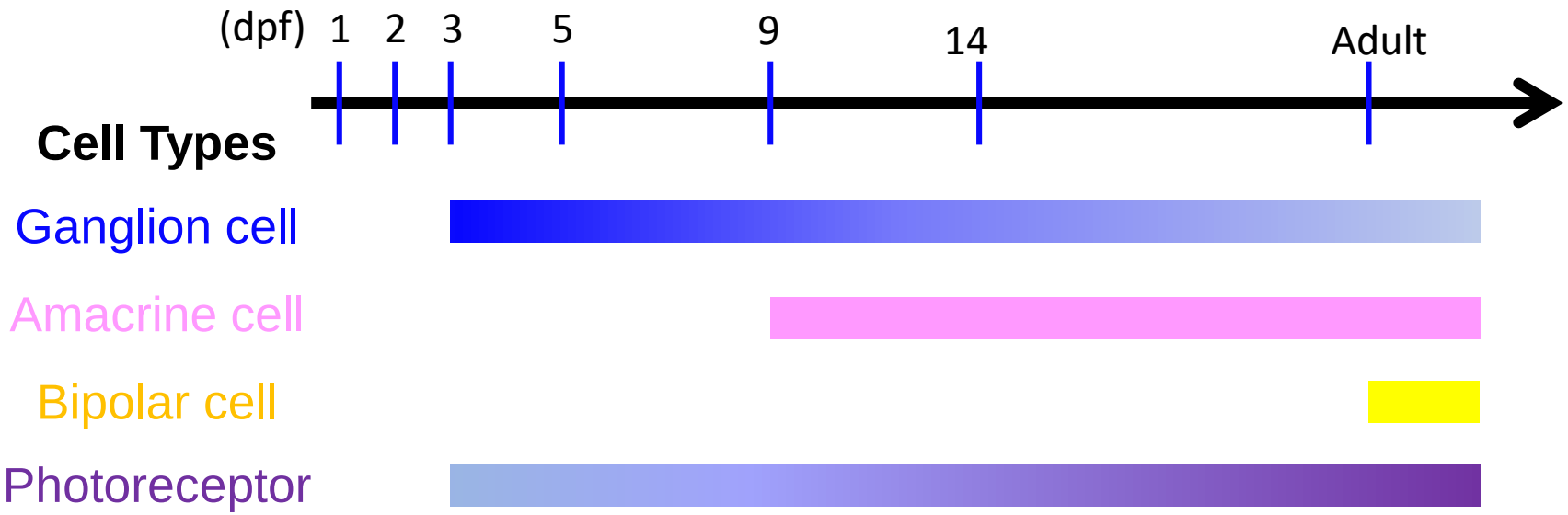


IFs could be found in the cell body and the inner fiber of the cone photoreceptors.

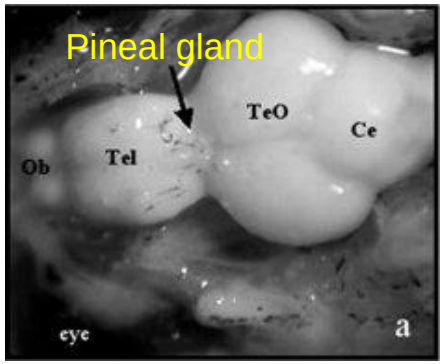
Inaa could be a good marker for identifying the cone photoreceptors in the zebrafish.

Inaa dynamically distributed in the developing retina

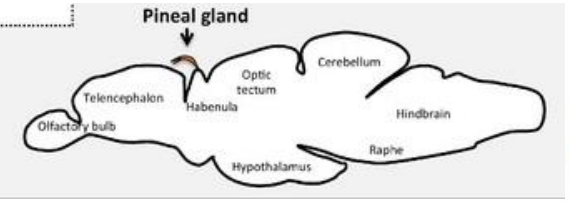
Cell types \ dpf	3	5	9	14	Adult
Ganglion cell	++	++	++	+	+
Amacrine cell	-	-	+	+	+
Bipolar cell	-	-	-	-	+
photoreceptor	+	+	+	++	++



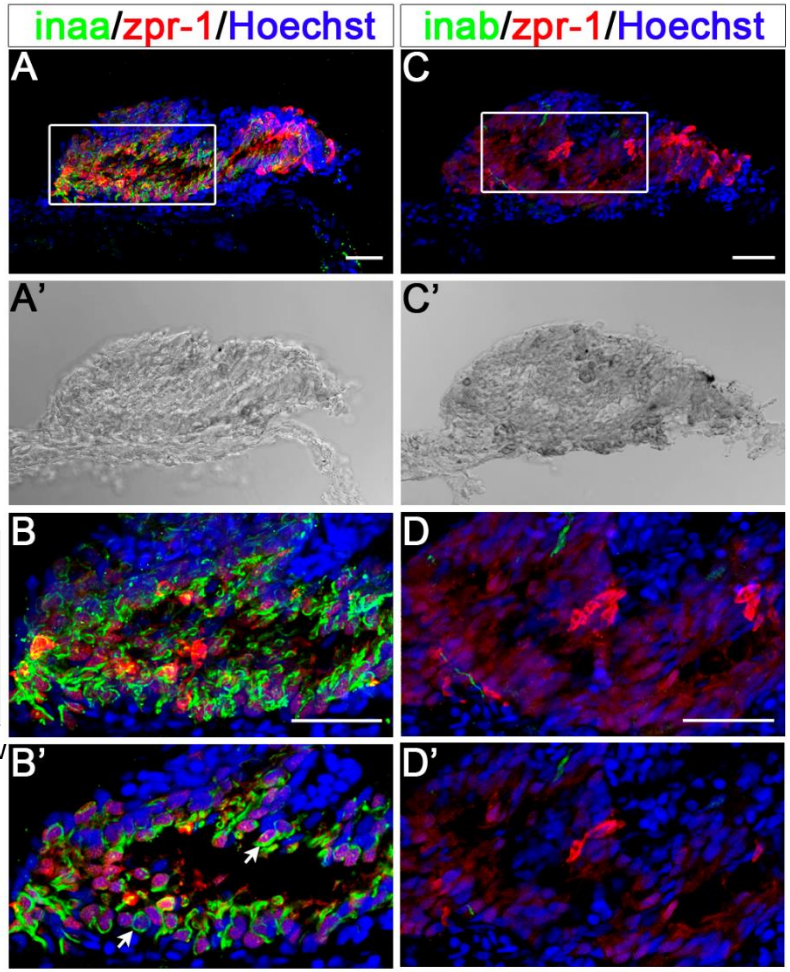
Inaa is expressed in photoreceptor-like cells in the adult pineal gland



(Laura et al., 2012) Sup. view



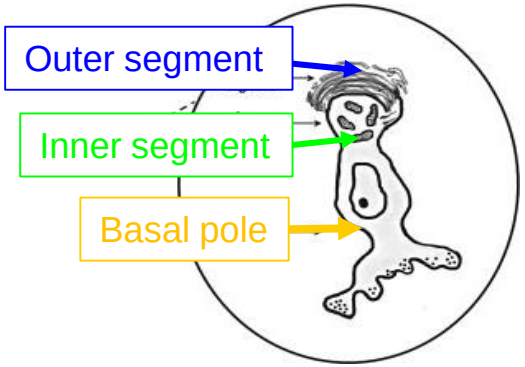
(Balasubramanian et al., 2014) sagittal view



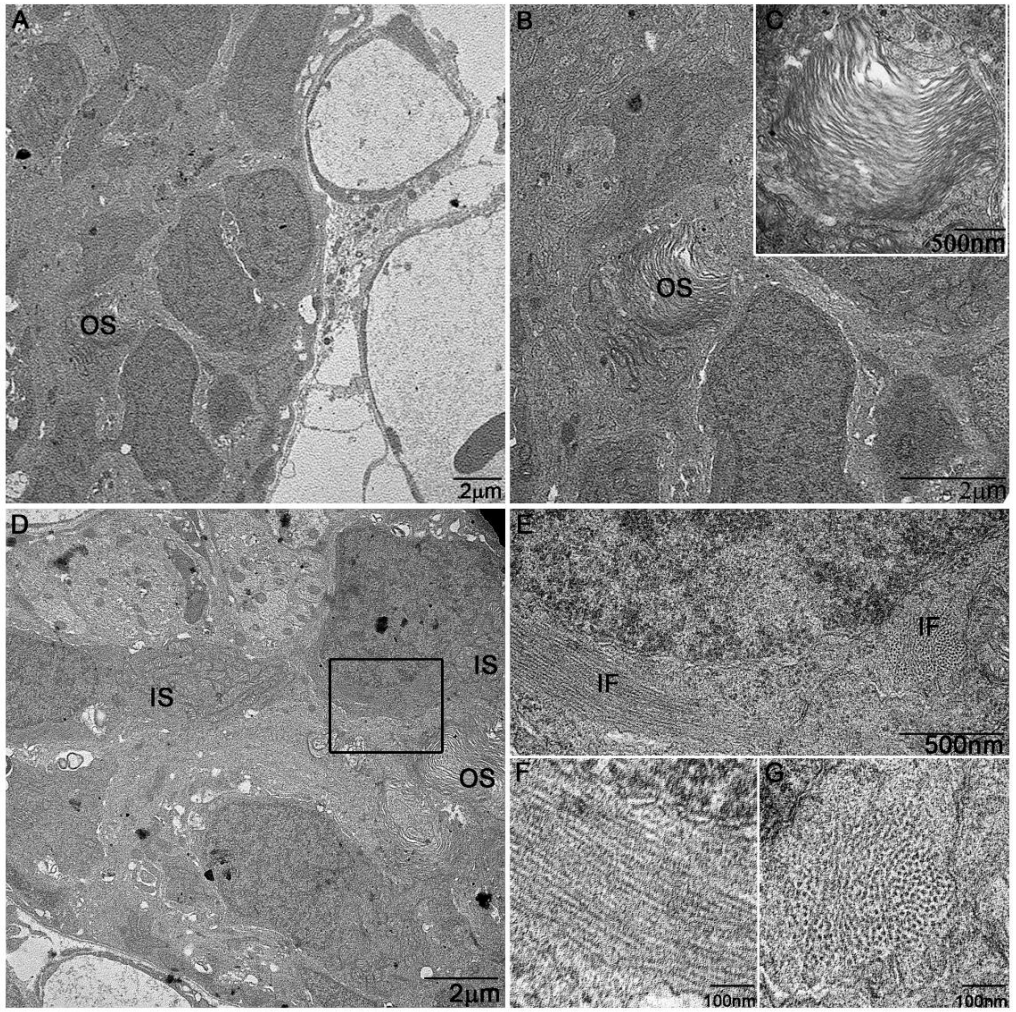
Zpr-1:
marker for double-cone photoreceptor
In retina

Pineal photoreceptor-like cells could be identified by the anti-inaa antibody.

Ultrastructure of IFs in the cell body of zebrafish pineal photoreceptor-like cells



(Laura et al., 2012)



(OS: outer segment;
IS: inner segment)

Neuronal specific IF, inaa, could be one of cytoskeletal components in zebrafish pineal photoreceptor-like cells.

Cell Tiss. Res. 158, 409—424 (1975)

Comparative Ultrastructure of Cerebrospinal Fluid-Contacting Neurons and Pinealocytes

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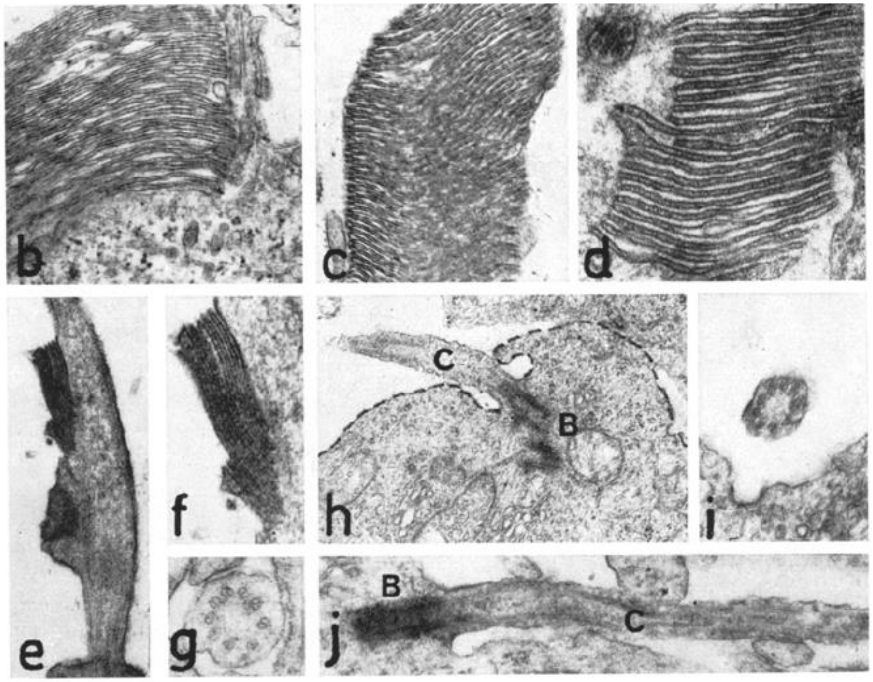

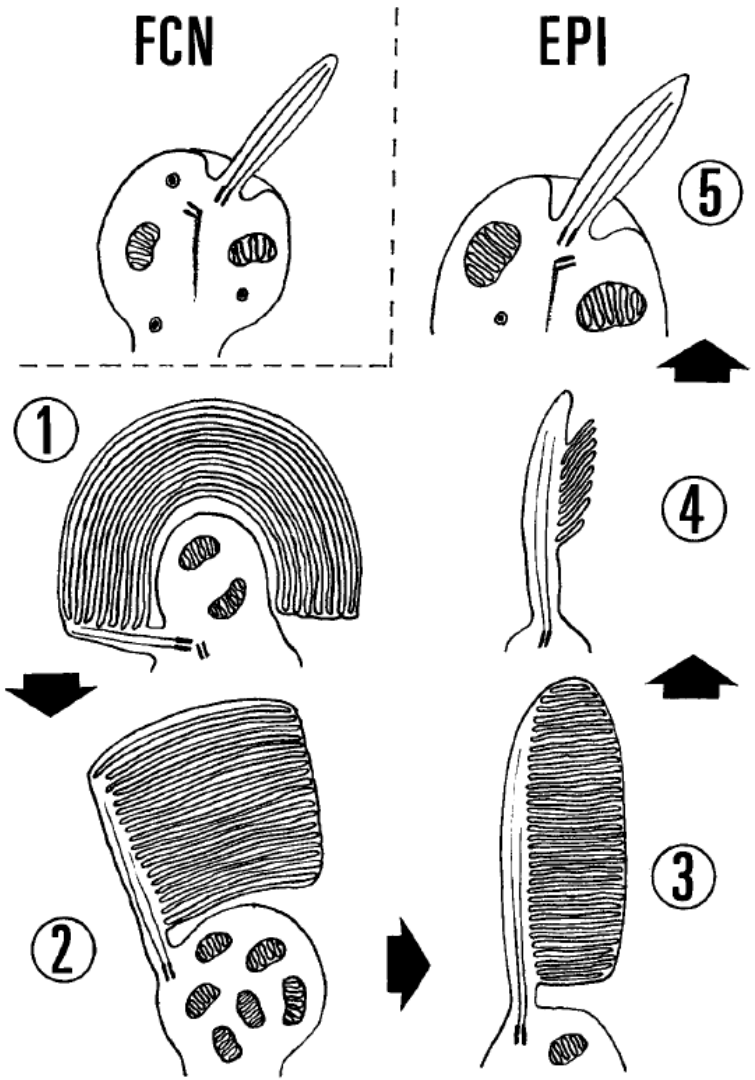

Fig. 6. Schematic drawing comparing the intraventricular dendritic terminal (*FCN*) of the CSF contacting neuron with pinealocytic outer and inner segments (*EPI* 1–5) in *1* fishes, *2* amphibians, *3* reptiles, *4* birds, *5* mammals. *Arrows* indicate the direction of the regression of photoreceptor lamellae. In mammals (*EPI* 5) the structure of the outer segment is identical with that of the cilium of the CSF contacting terminal (*FCN*)

What a PhD student learned from my Lab.

DNA level	Molecular cloning
	Transfection / expression (*Ex ovo* electroporation for chick embryo)
RNA level	*in situ* hybridization & RT-PCR
	morpholino knockdown (for Zebrafish)
Protein level	QC for the antibodies, Western blot、immunohistochemistry、immunocytochemistry
Cell level	Transmission electron microscopy Confocal microscopy Live Image of YFG-product (Tagged with GFP)
Organism level	Developmental/ Species differences

解剖學與細胞生物學發展與願景

教學熱忱，研究初衷

擴大視野，跨域合作

兄弟登山，各自努力

經驗傳承，提攜後進

Acknowledgement