

非損傷微測技術在斑馬魚離子細胞的應用： 從生理到環境毒理



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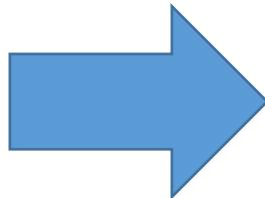


SUNSHINE DL01646

研究主題與實驗動物

基礎研究

1. 環境生理學
2. 環境毒理學
3. 斑馬魚醫學模式
4. 斑馬魚微測技術



應用

1. 魚類養殖技術
2. 環境毒物檢測
3. 藥物篩選開發
4. 生醫微測系統

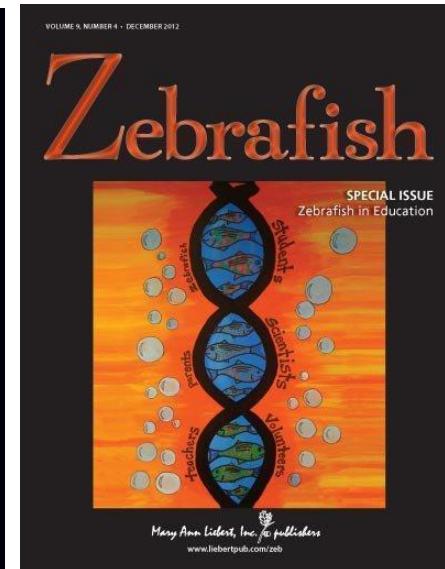
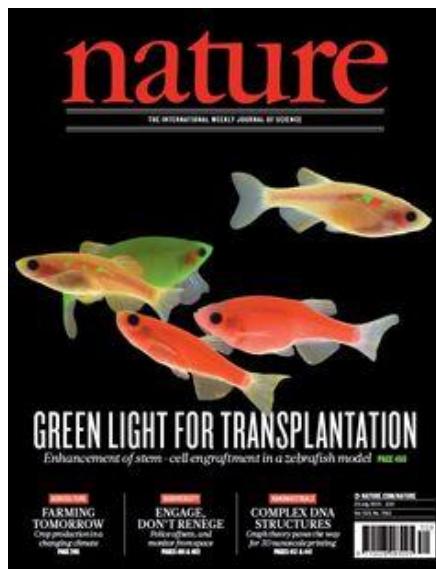
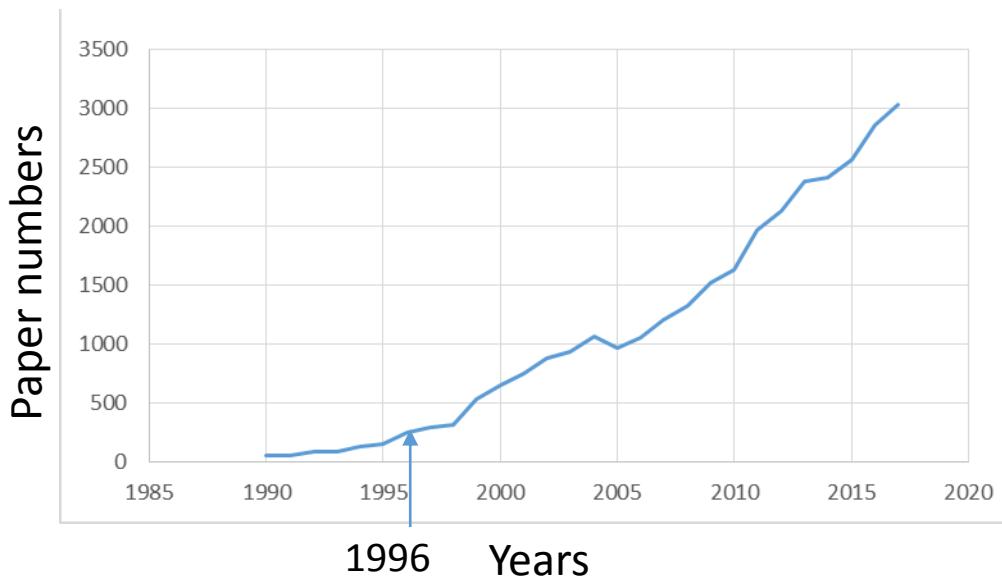
Zebrafish (*Denio rerio*) 斑馬魚



Medaka (*Oryzias latipes*) 青鱂魚



以斑馬魚為模式動物的論文數量 (Pubmed)



美國斑馬魚專利(~2017) 12699件

Google Patents (Google專利搜索) 是Google公司旗下的一個專業進行專利和專利申請搜索的搜尋引擎，資料來自美國專利及商標局 (USPTO)。

Google Patents

zebrafish

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G01N33/5005?

Chemical analysis of biological material, e.g. blood, urine; Testing involving biospecific ligand binding methods; Immunological testing involving human or animal cells

Methods of screening agents for activity using teleosts

[Grant US6656449B1](#) • George Serbedzija • Phylonix Pharmaceuticals, Inc.

Priority 1998-02-23 • Filing 2000-08-23 • Grant 2003-12-02 • Publication 2003-12-02

, wherein step (c) is performed on a subset of wells containing teleosts that survive incubation with the compound. 17. The method of claim 1, wherein the teleosts are **zebrafish**. 18. The method of claim 1, wherein the teleosts are embryos, ...

Methods of screening agents for activity using teleosts

[Grant US6299858B1](#) • George N. Serbedzija • Phylonix Pharmaceuticals, Inc.

Priority 1998-02-23 • Filing 1999-02-22 • Grant 2001-10-09 • Publication 2001-10-09

wherein the fluorescent dye is benzothiazonium-4-quinolium dye. 21. The method of claim 1, wherein the living teleost is a **zebrafish**. 22. The method of claim 1, wherein the candidate agent is a compound and a

Top 1000 results by filing date

Relative count of top 5 values

Assignees Inventors CPCs

Assignees	Inventors	CPCs
President And Fellows Of Harvard College	A01K67/033 A01K2227/50 A01K67/0339 A01K67/0337	2.5%
The General Hospital Corporation	A61K47/00 A01K67/0276 C07D C07K14/461	1.7%
The Johns Hopkins University School Of Medicine	C07K2319/50 C07K2319/23 C07K2319/21 C07K2319/20	1.7%
Curis, Inc.	A61K38/1709 A61K38/1703 A61K31/496 A61K38/17	1.5%
Children's Medical Center Corporation	A61K31/19 A61K31/185 A61K35/28 A61K35/12	1.3%
山东省科学院生物研究所	G01N G01N30/02 A61K A61K49/00	1.3%

生物醫學上使用的模式動物

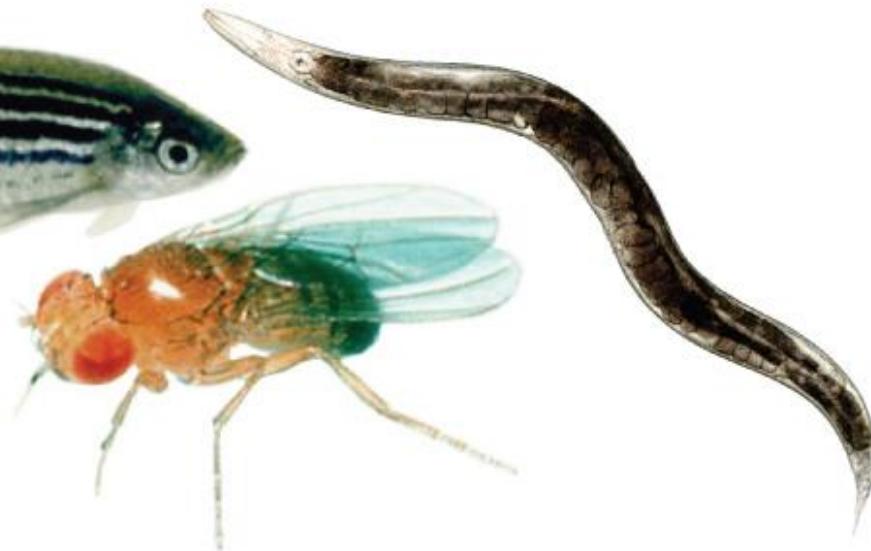
哺乳類



魚類

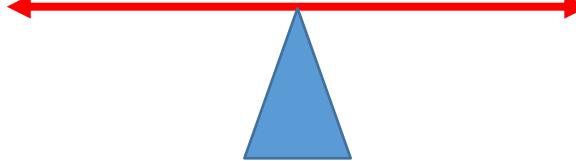


無脊椎動物



動物福祉的問題

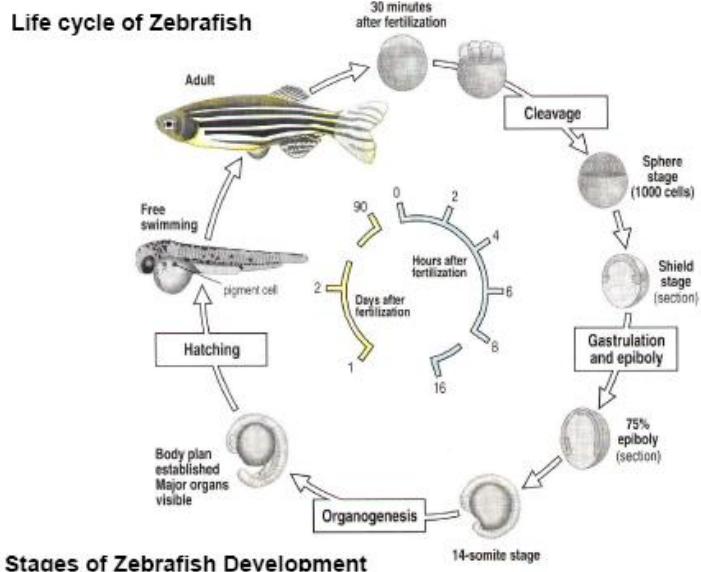
與人類的相似度低



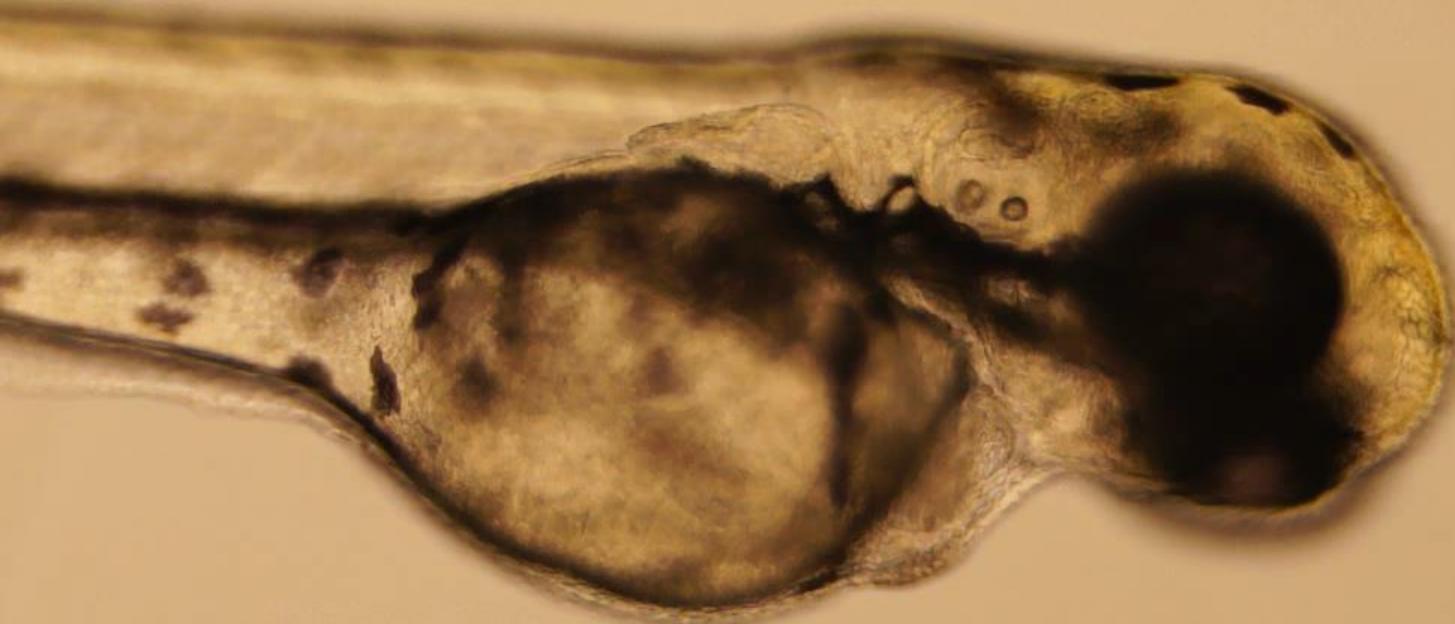
動保法規定，每一間進行動物實驗的機構皆須設置「實驗動物照護及使用委員會」（英文簡稱IACUC），其任務為審查、監督機構所進行之動物實驗及動物飼養情況。

斑馬魚模式動物的優點

1. 受精卵體外發育取得容易，可全年產卵，每隔一周可產下300-500顆卵。
2. 胚胎全身透明，容易觀察胚胎發育情況，並進行篩選突變個體。
3. 胚胎發育快速，48小時(hpf)內可以發育出大多數的器官。
4. 世代週期短，受精卵發育到成魚約3個月，可進行許多隔代遺傳實驗。
5. 可以在實驗室內大量養殖，照顧成本低。
6. 基因體與人類相似度高達70%。
7. 基因操作容易(轉殖、剔除、弱化)。
8. 豐富研究資源(mutants, gene expressions, database (zfin), bioinformatics, services...)
9. 胚胎(0-96hpf)不須動物實驗審查。



顯微鏡下的斑馬魚胚胎 (72hpf)



[点击观看《斑马鱼胚胎镜下观察》视频](#)

斑馬魚作為疾病模式與藥物篩選動物



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NATURE REVIEWS DRUG DISCOVERY | REVIEW

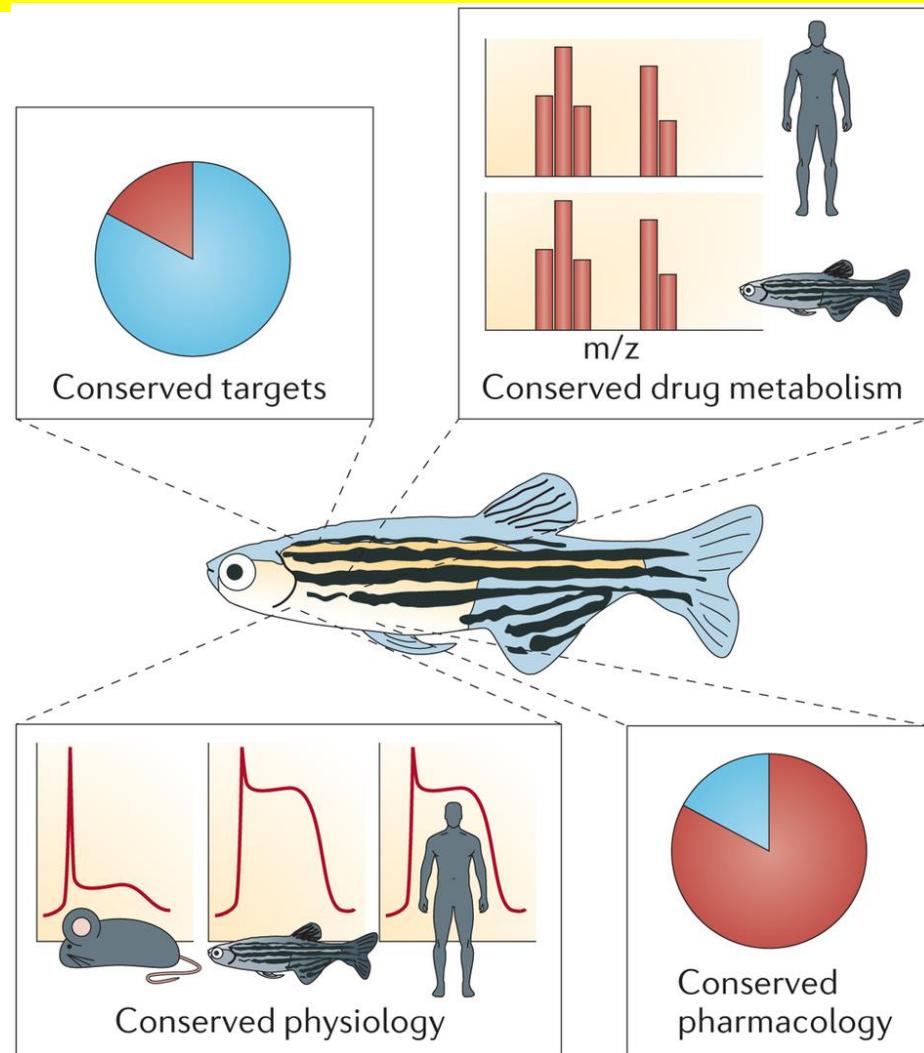
Zebrafish as tools for drug discovery

Calum A. MacRae & Randall T. Peterson

Affiliations | Corresponding authors

Nature Reviews Drug Discovery 14, 721–731 (2015) | doi:10.1038/n

Published online 11 September 2015



斑馬魚作為毒性試驗模式

經濟合作發展組織『魚類急毒性試驗』指引（*OECD Guidelines for Testing of Chemicals. Guideline 203: Fish, Acute Toxicity Test. Adopted*）與美國環保署『淡水和海水魚之急毒性試驗』指引（*USEPA Ecological Effects Test Guidelines . OPPTS 850.1075: Fish Acute Toxicity Test, Freshwater and Marine*）都推薦的斑馬魚作為供試魚種，來檢測試驗物質對斑馬魚之 96 小時急性致死毒性，求取半數致死濃度（LC₅₀），可供評估試驗物質對淡水脊椎動物毒性的影響。

法規資訊

臺灣環保署公告的環境毒性檢測方法

法規名稱：生物急毒性檢測方法—斑馬魚胚胎半靜水式法（NIEA B909.10C）

訂定時間：中華民國106年3月28日

立法沿革：中華民國106年3月28日行政院環境保護署環署檢字第1060021982號公告訂定發布，並自106年7月15日生效

法規體系：[/ 行政](#) [/ 環境保護](#) [/ 檢驗](#)

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歷史沿革

1. 中華民國106年3月28日行政院環境保護署環署檢字第1060021982號公告訂定發布，並自106年7月15日生效

附件下載

[附件-生物急毒性檢測方法—斑馬魚胚胎半靜水式法（NIEA B909.10C）](#)

斑馬魚胚胎藥物測試

	1	2	3	4	5	6	7	8	9	10	11	12
A	✓											
B	●	●	●	●	●	●	●	●	●	●	●	●
C	●	●	●	●	●	●	●	●	●	●	●	●
D	●	●	●	●	●	●	●	●	●	●	●	●
E	●	●	●	●	●	●	●	●	●	●	●	●
F	●	●	●	●	●	●	●	●	●	●	●	●
G	●	●	●	●	●	●	●	●	●	●	●	●
H	●	●	●	●	●	●	●	●	●	●	●	●



成魚交配



收集大量的受精卵

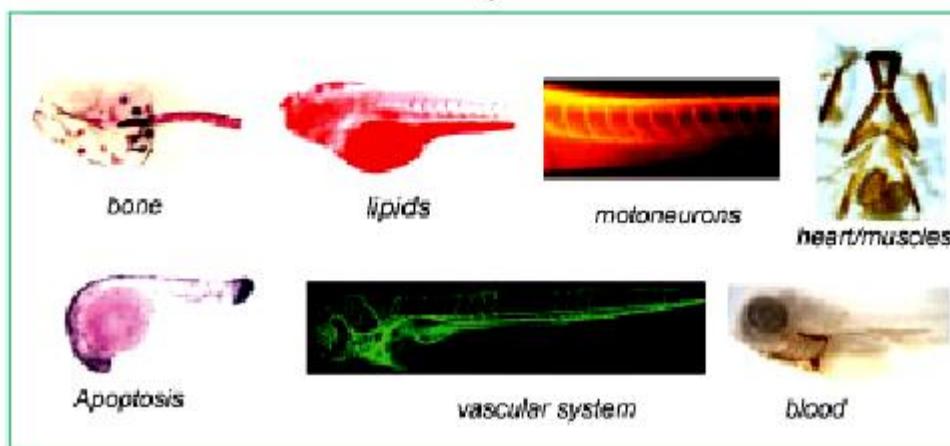


篩選受精卵



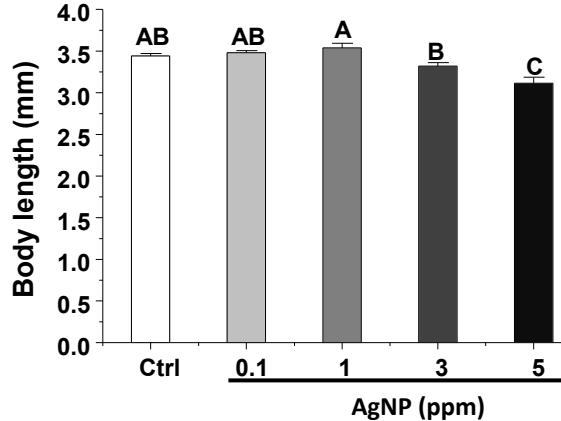
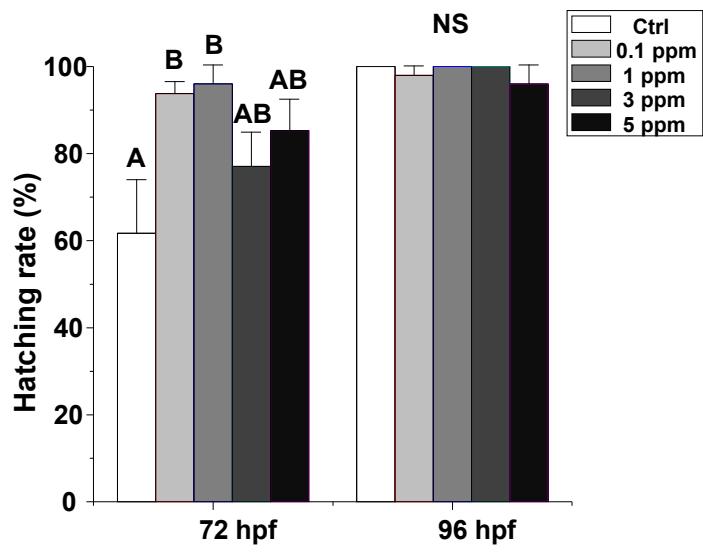
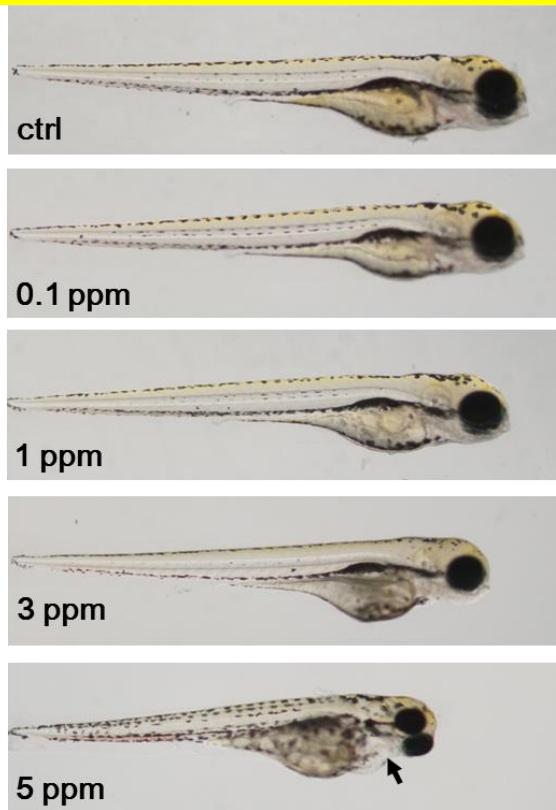
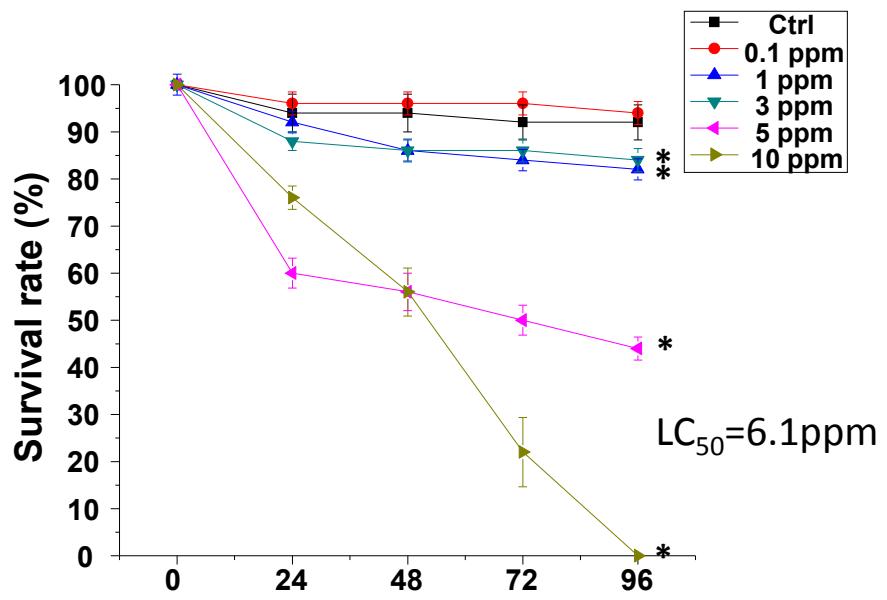
96-well microplates
(100~200 ul)

藥物處理

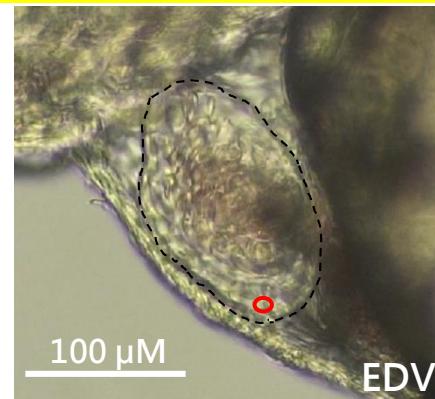
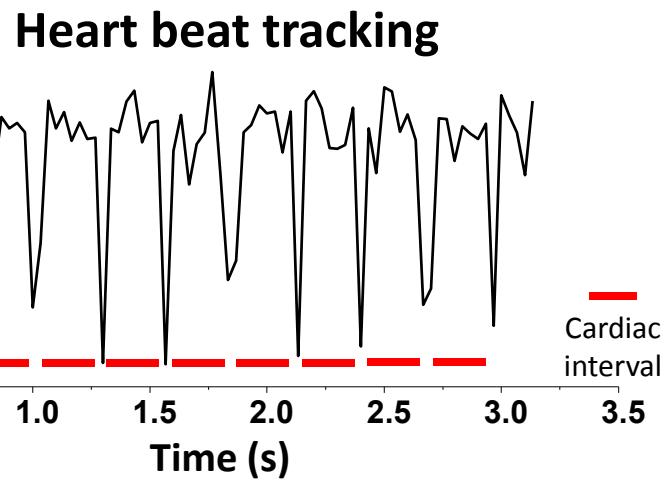
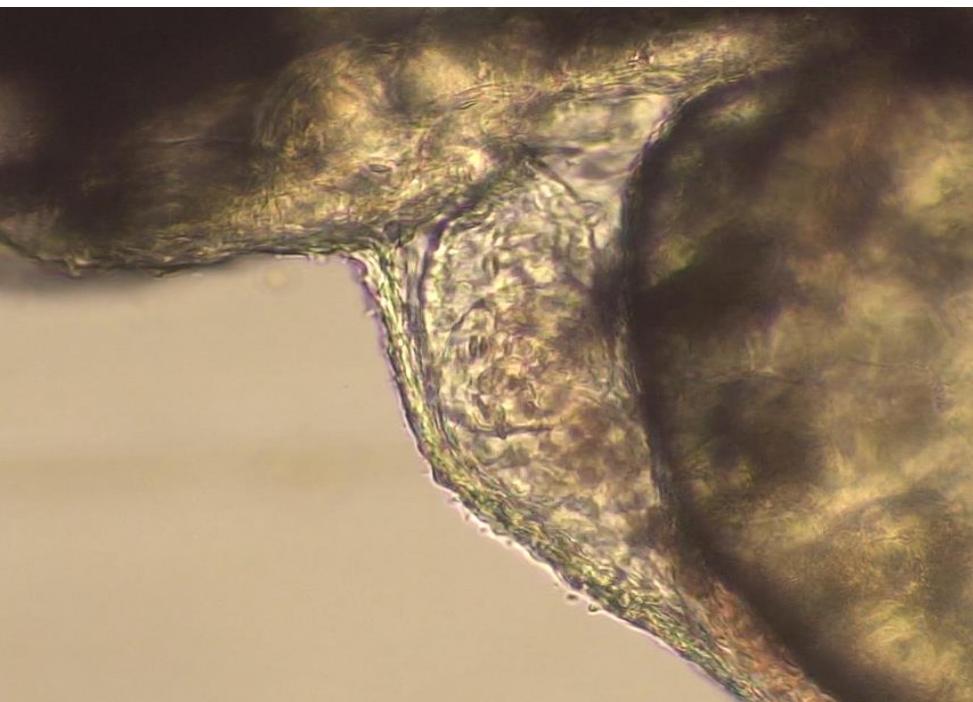


分析藥物對
器官發育、功能
影響

斑馬魚胚胎發育毒性分析 (以AgNP為例)



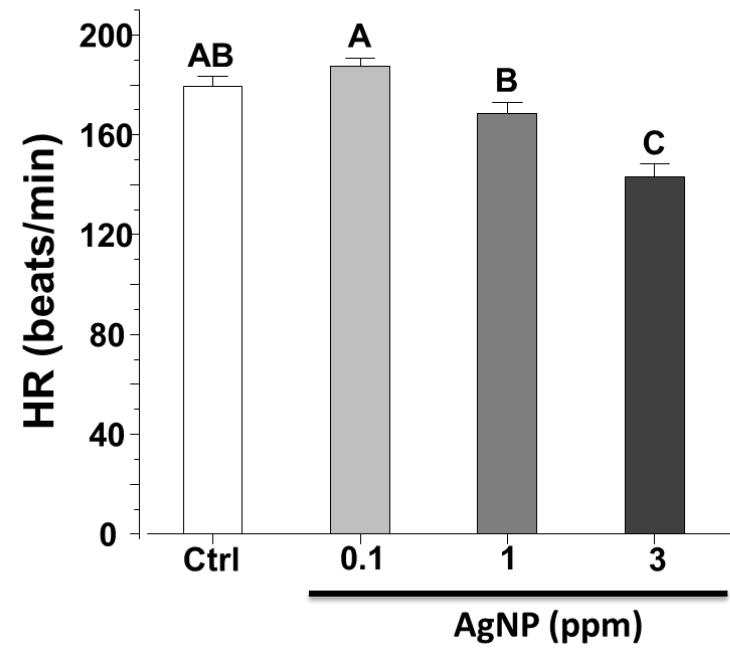
斑馬魚胚胎心血管功能分析: 心律



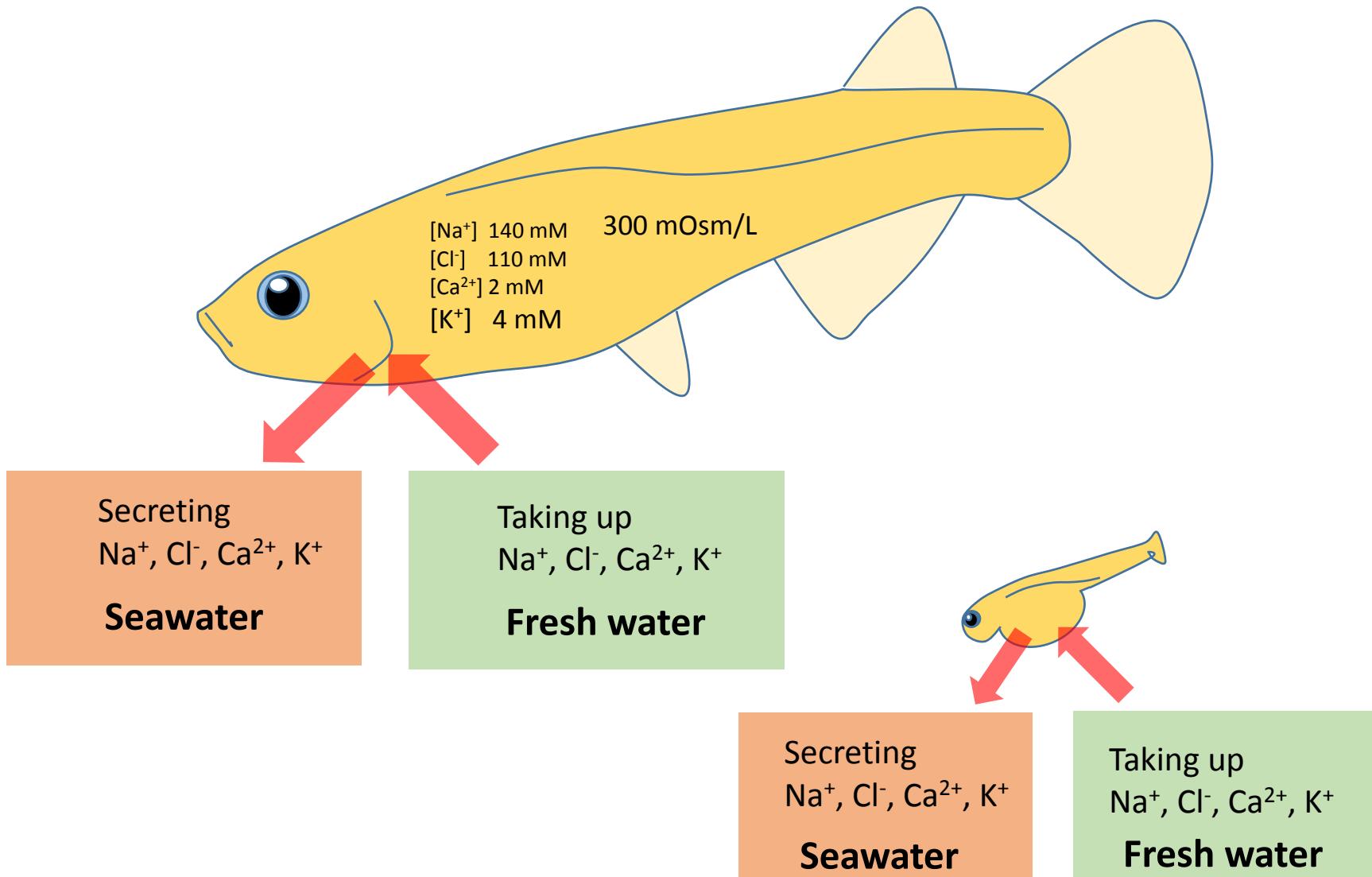
心室舒張末期



心室收縮末期

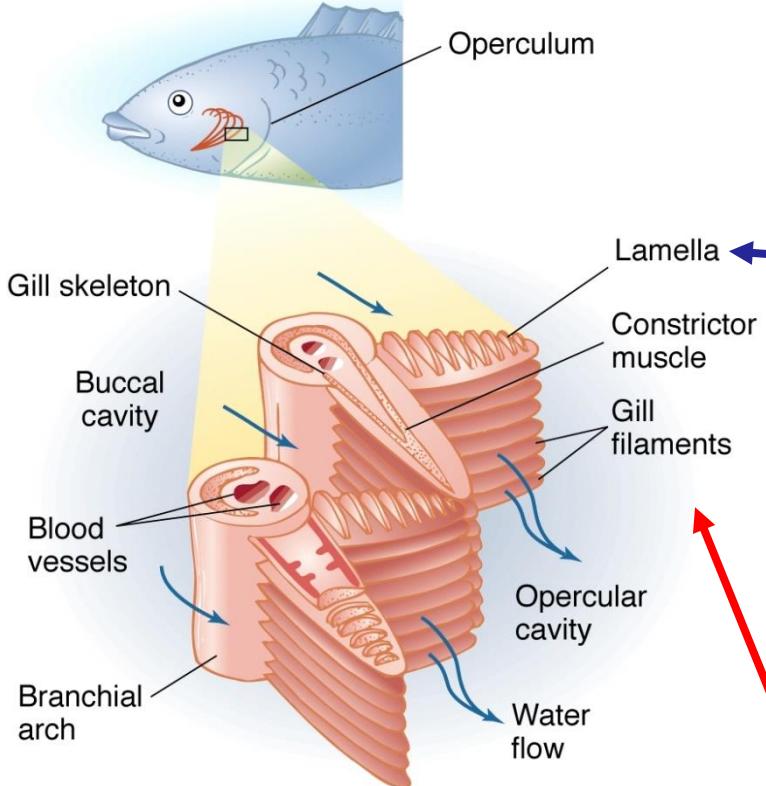


魚類的離子調節的重要性

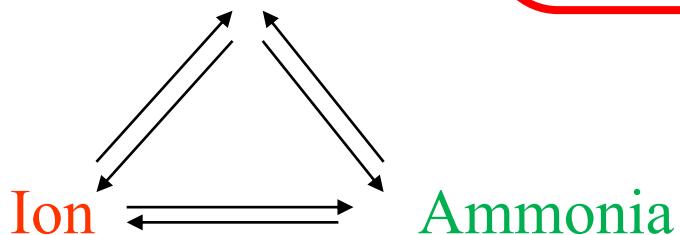


離子細胞在魚類的功能

(a) Teleost gill



Acid-base



Pavement cells

1. Gas exchange (O_2, CO_2)

Ionocytes (離子細胞)

2. Ion uptake (Na^+, Cl^-, Ca^{2+})
3. Ion secretion (Na^+, Cl^-, K^+)
4. Ammonia excretion (NH_3, NH_4^+)
5. Acid-base regulation (H^+, HCO_3^-)

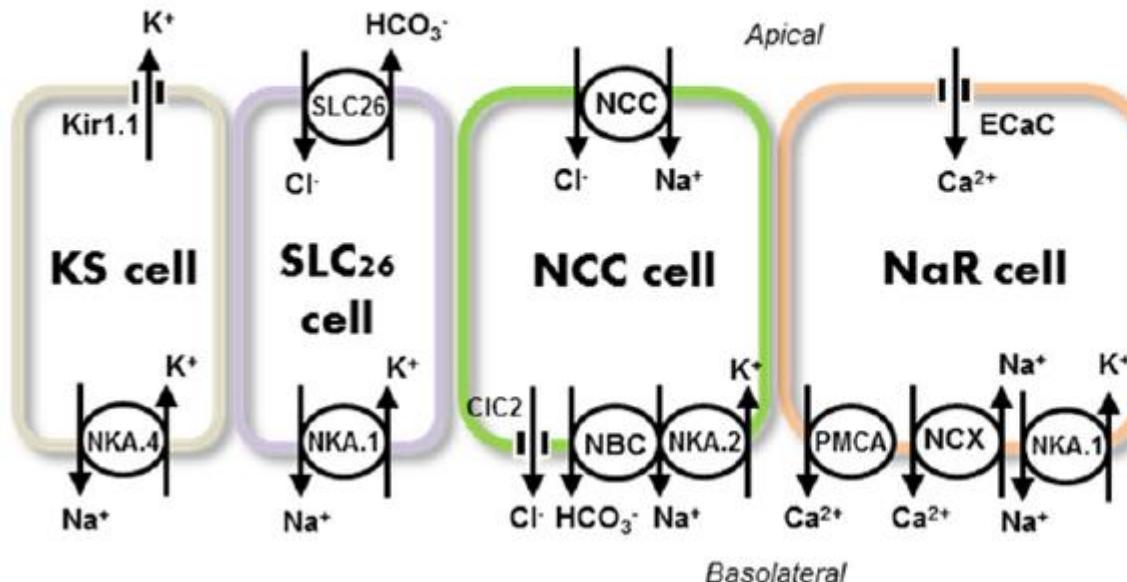
斑馬魚五型離子細胞



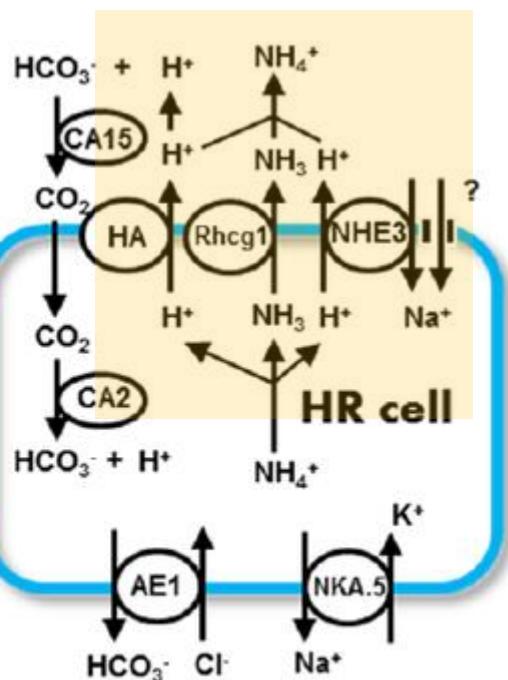
K^+
secretion

Cl^-
uptake

Na^+, Cl^-
uptake

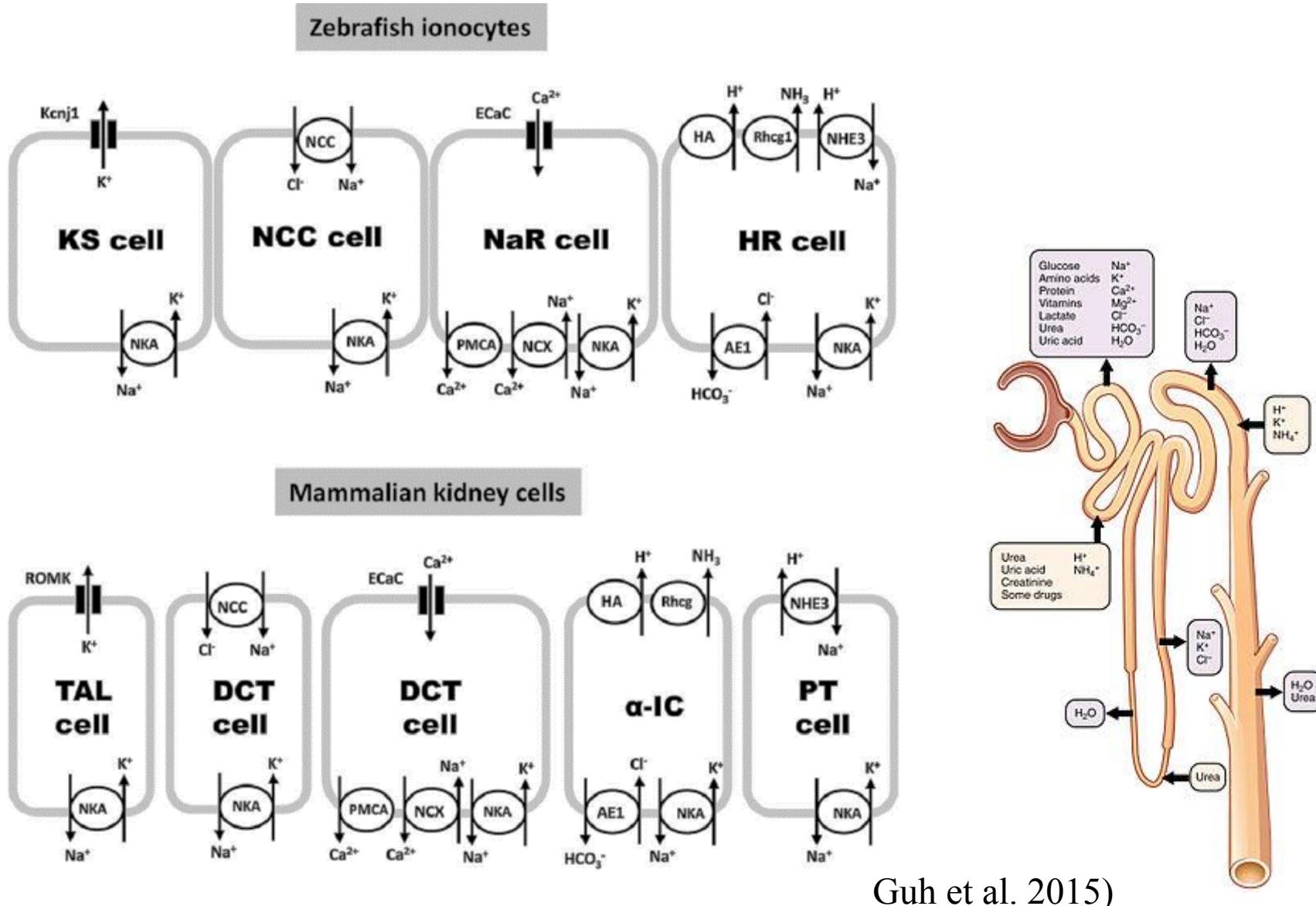


Ammonia excretion
Acid secretion
 Na^+ uptake

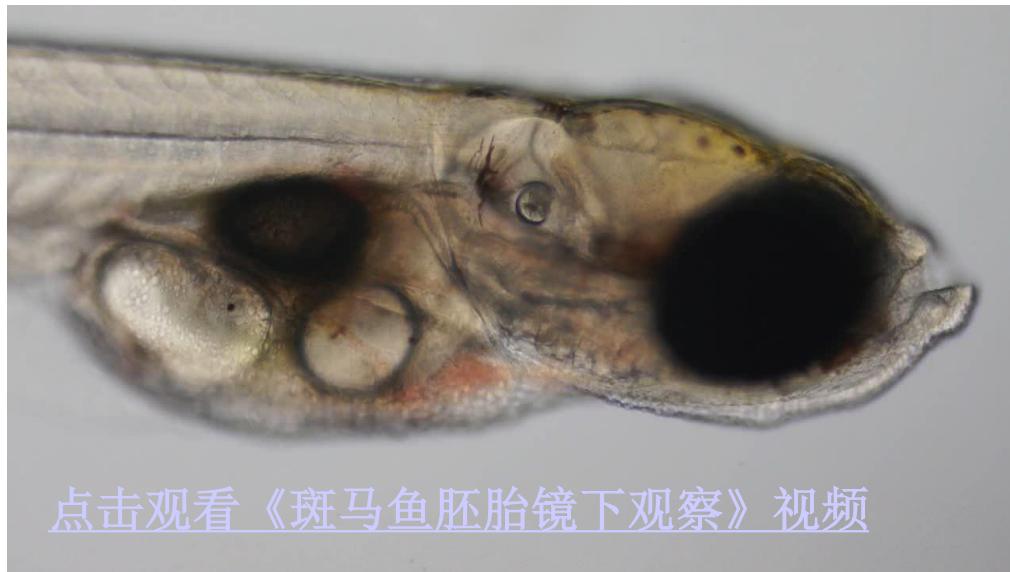


(Hwang and Lin 2013)

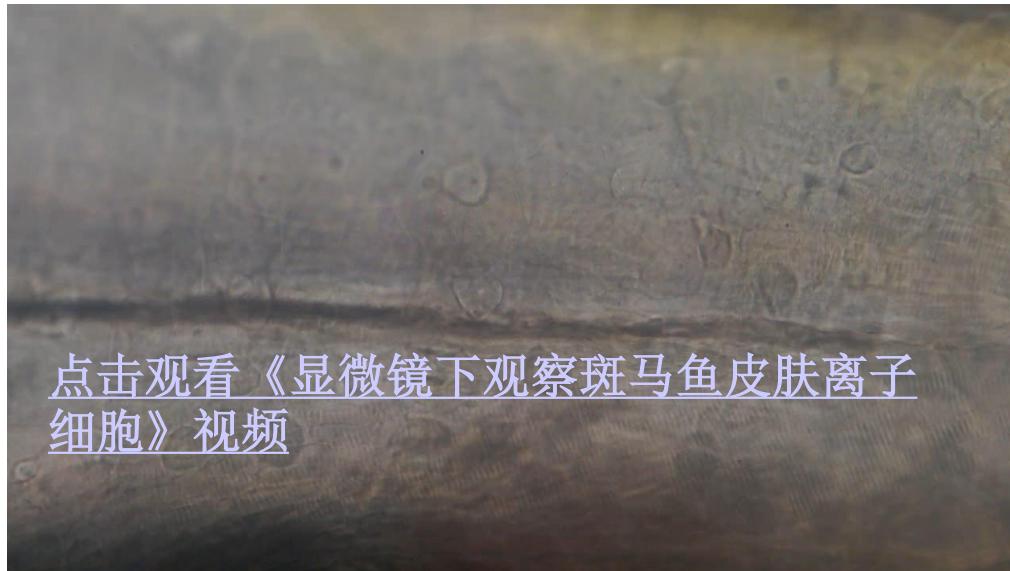
斑馬魚離子細胞與人類腎臟細胞相似



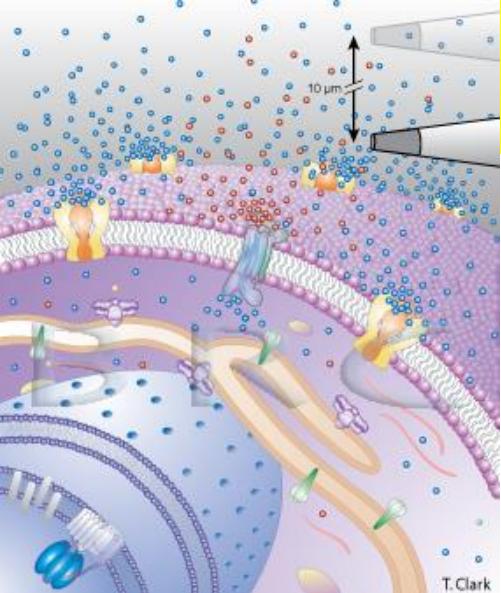
魚類胚胎皮膚上離子細胞可以在顯微鏡下被觀察



[点击观看《斑马鱼胚胎镜下观察》视频](#)



[点击观看《显微镜下观察斑马鱼皮肤离子细胞》视频](#)



非損傷微測系統原理(NMT)

Fick's equation:

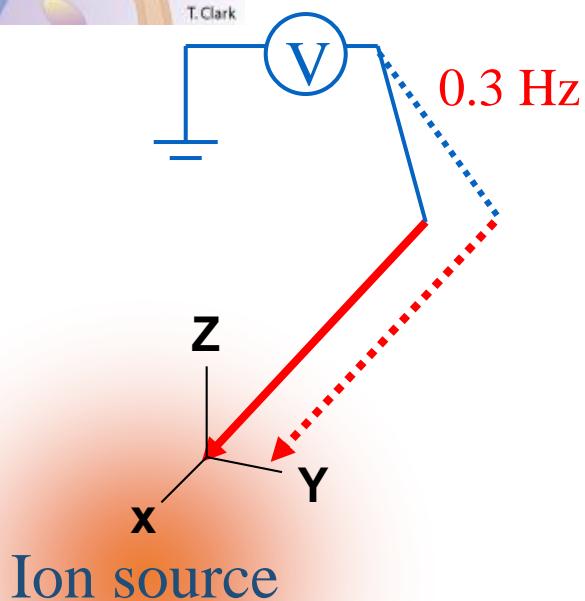
$$\mathbf{J} = -\mathbf{D} (\mathbf{dC}/\mathbf{dr})$$

\mathbf{J} = ion flux (mole $\text{cm}^{-2} \text{ sec}^{-1}$)

\mathbf{D} = diffusion coefficient ($\text{cm}^2 \text{ sec}^{-1}$)

\mathbf{dC} = concentration difference (mole ml^{-1})

\mathbf{dr} = distance between measurements (cm)



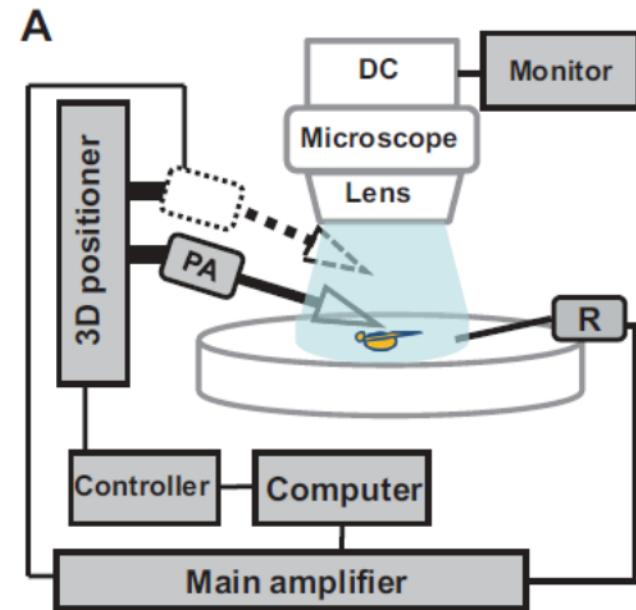
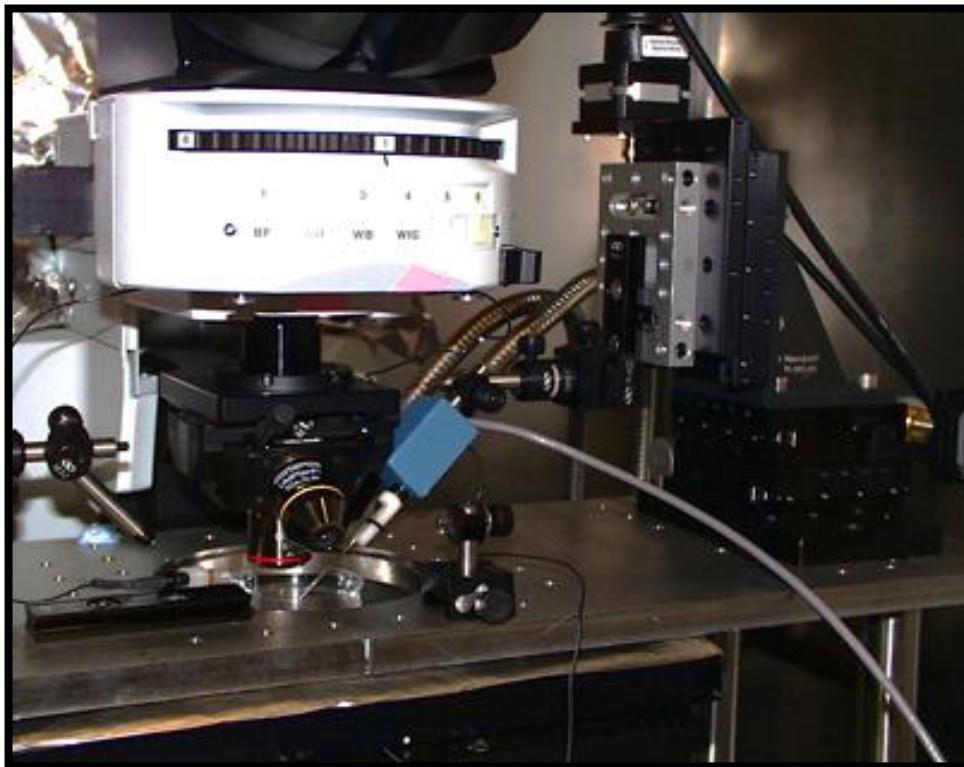
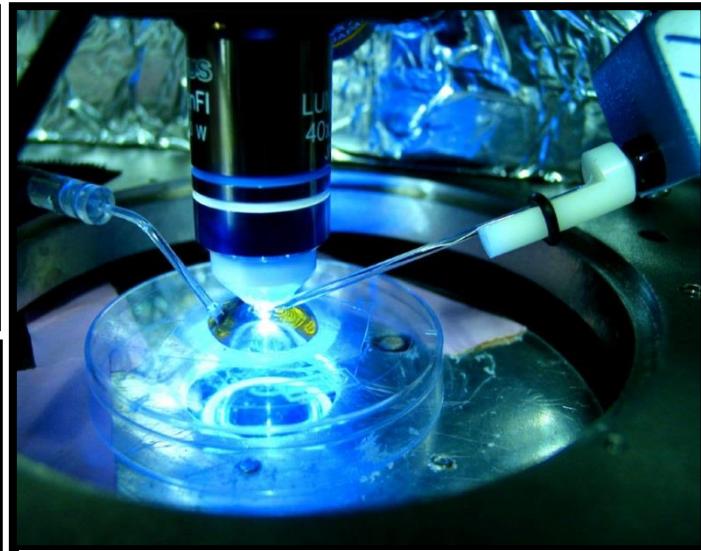
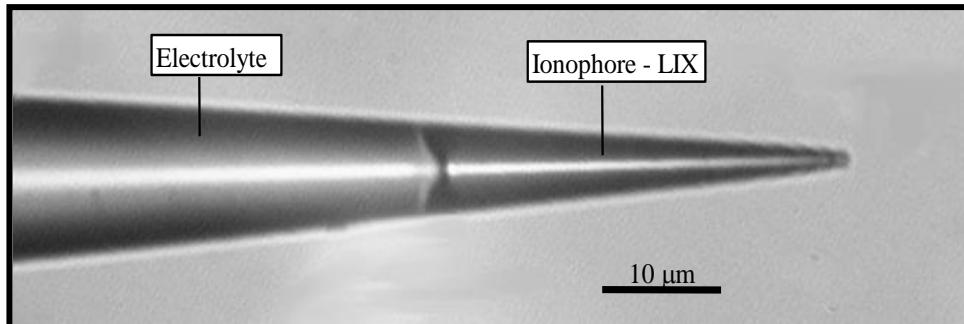
$$dV \xrightarrow{\text{calibration}} dC$$

$$dr = 10 \mu\text{m}$$

The NMT/SIET can measure:

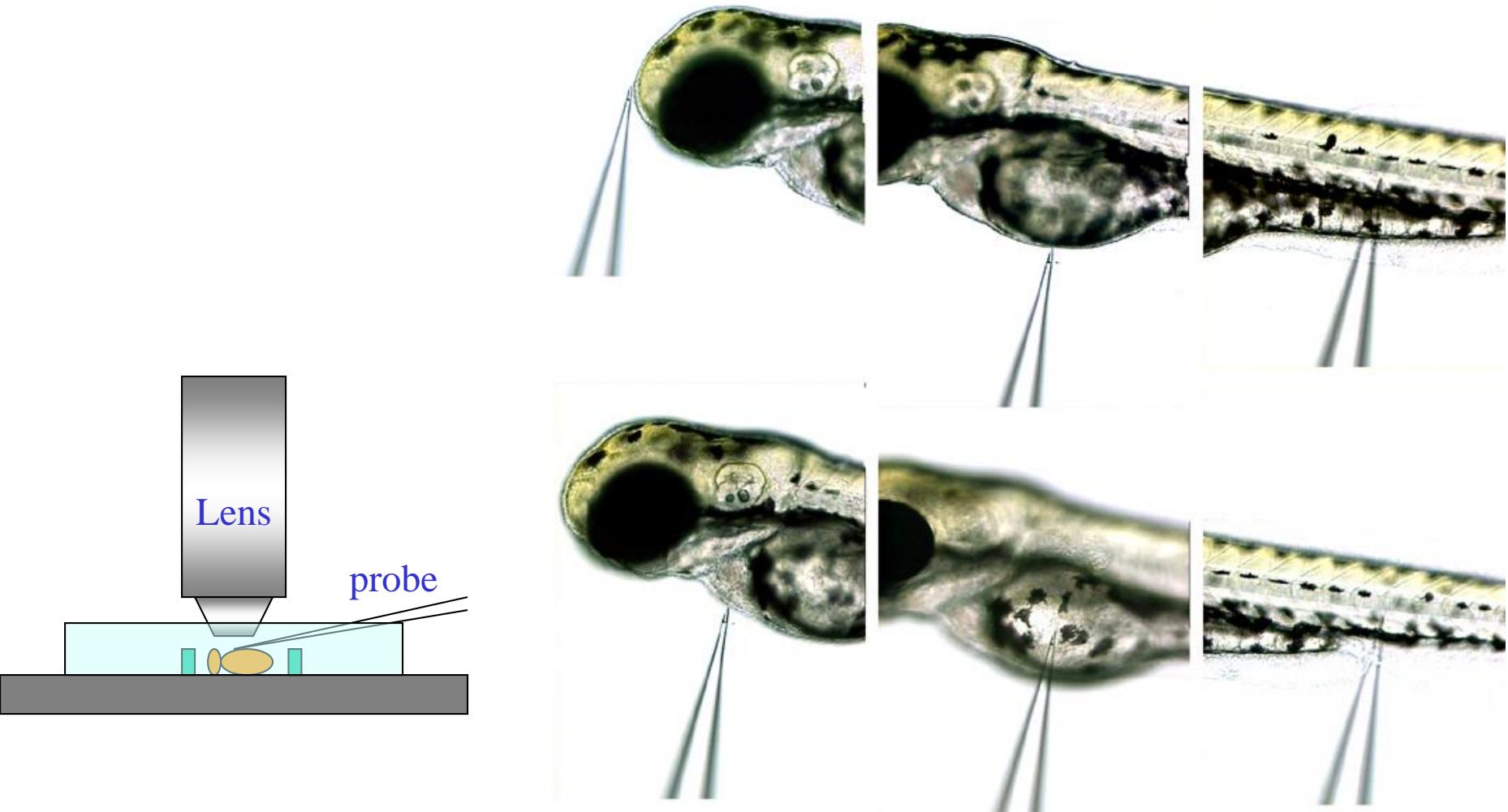


非損傷微測系統測量活體斑馬魚胚胎

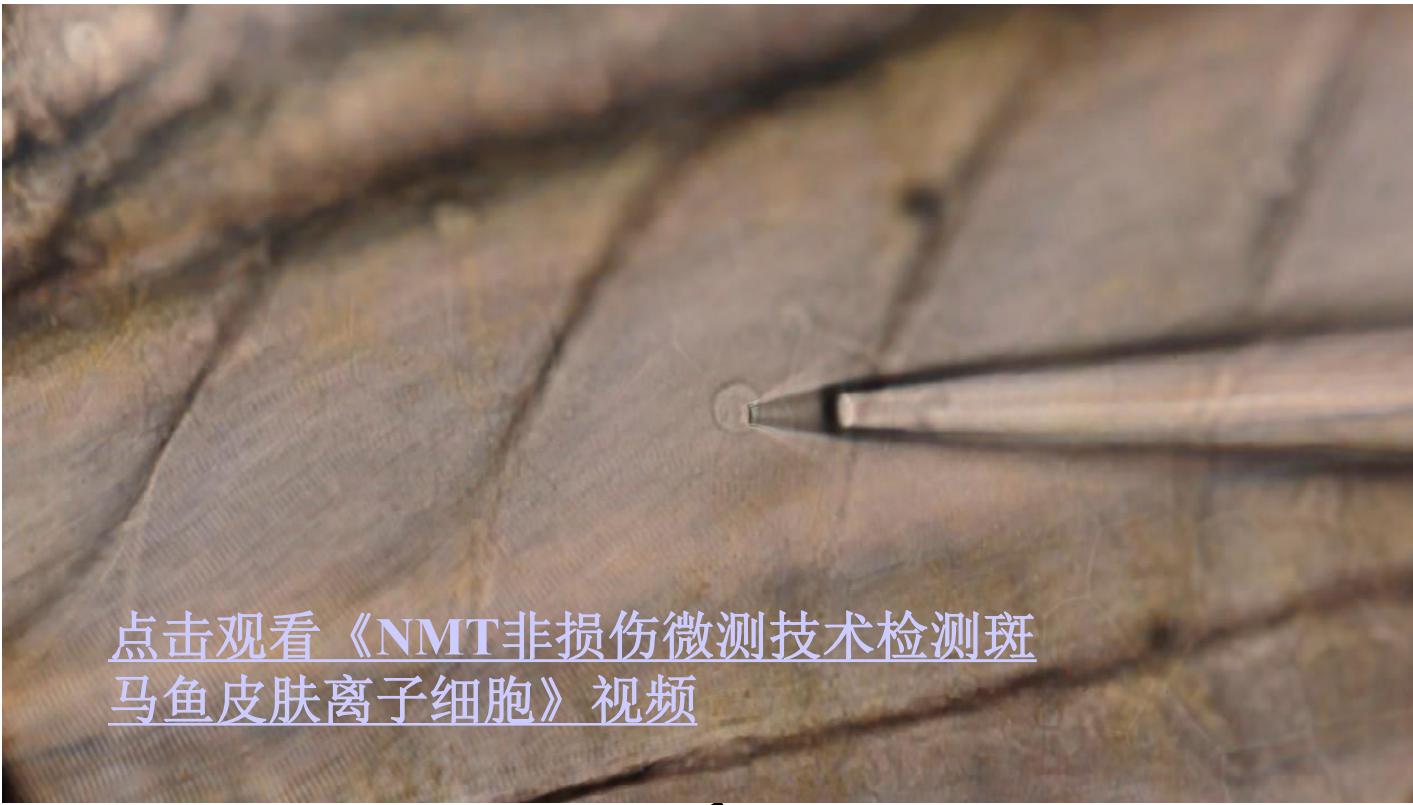


斑馬魚胚胎皮膚表面的氫離子測量

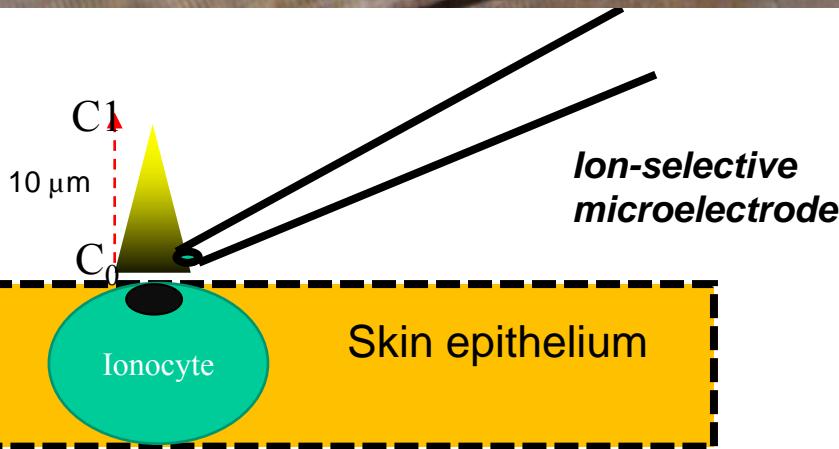
H⁺-selective electrode technique



離子細胞表面進行離子流測量



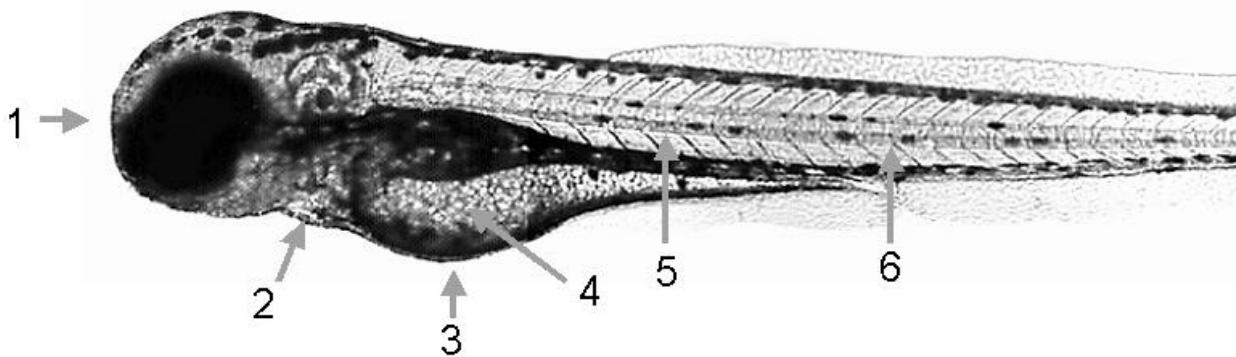
点击观看《NMT非损伤微测技术检测斑
马鱼皮肤离子细胞》视频



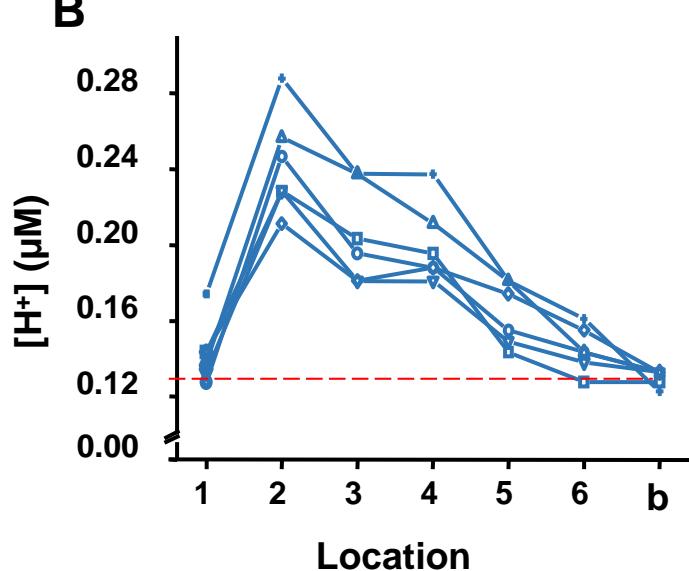
- H^+ flux (Lin et al. 2006 etc.)
- NH_4^+ flux (Shih et al. 2008 etc.)
- Na^+ flux (Wu et al. 2010 etc.)
- Cl^- flux (Horng et al. 2009 etc.)
- Ca^{2+} flux (Lin et al. 2013 etc.)
- K^+ flux (Horng et al. 2017)

斑馬魚皮膚表現氫離子濃度

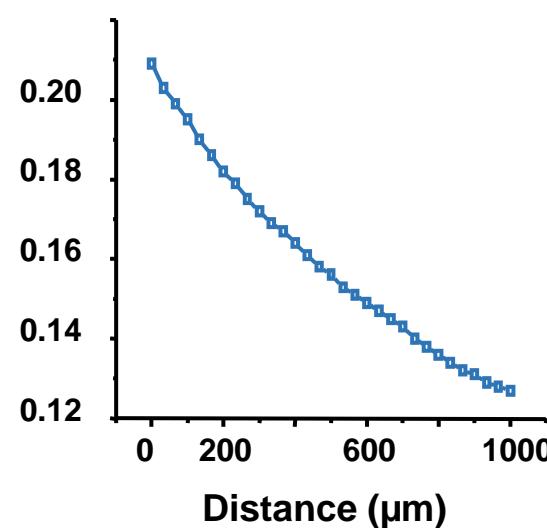
A



B



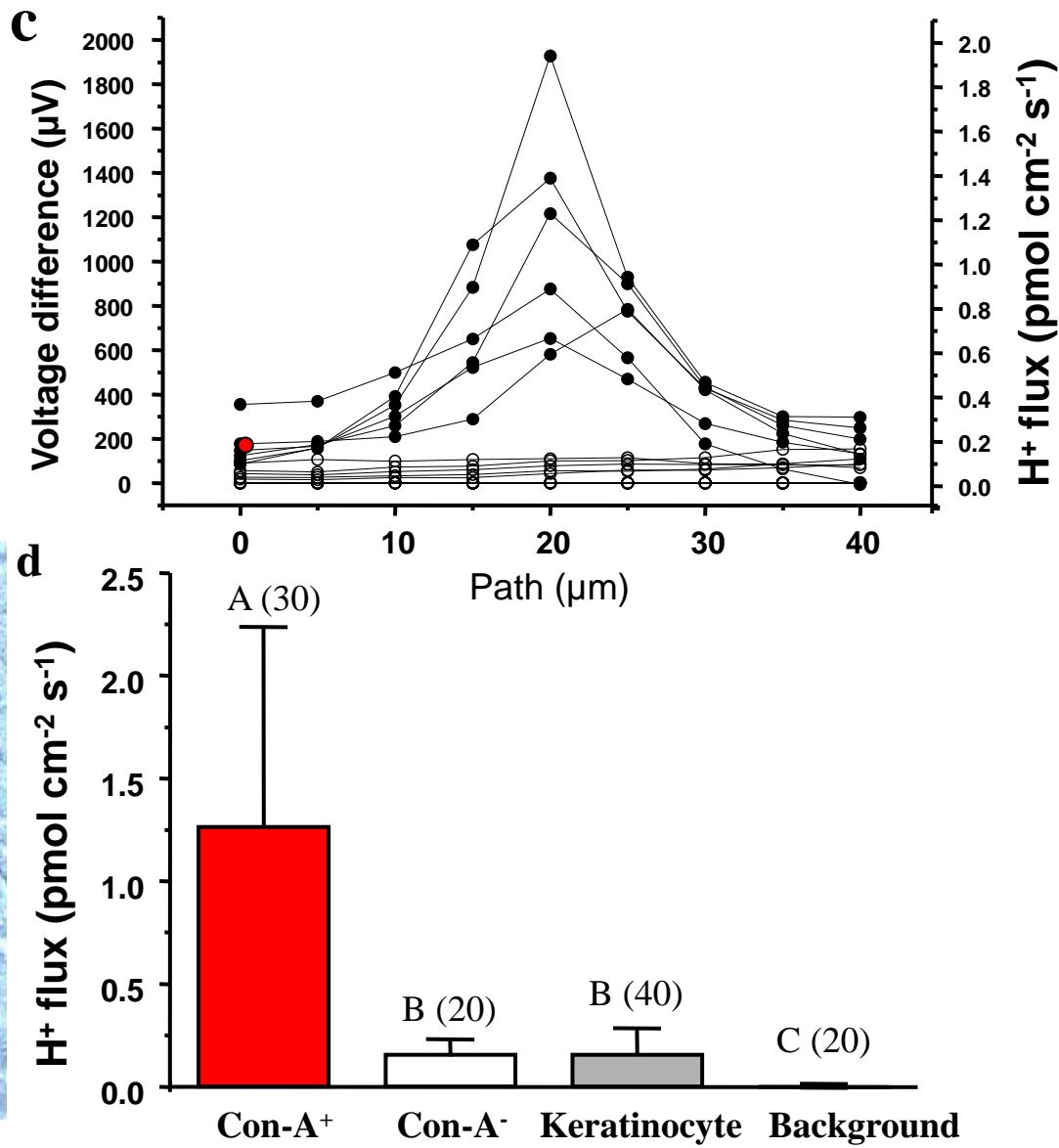
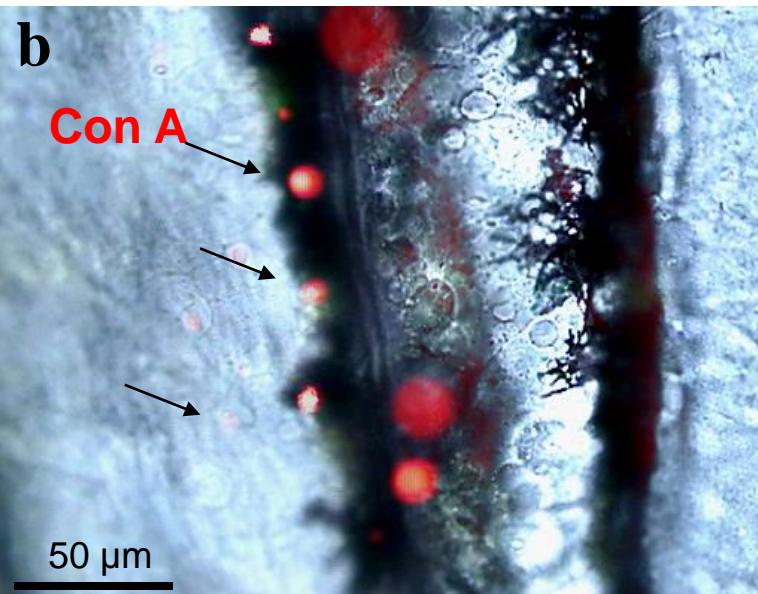
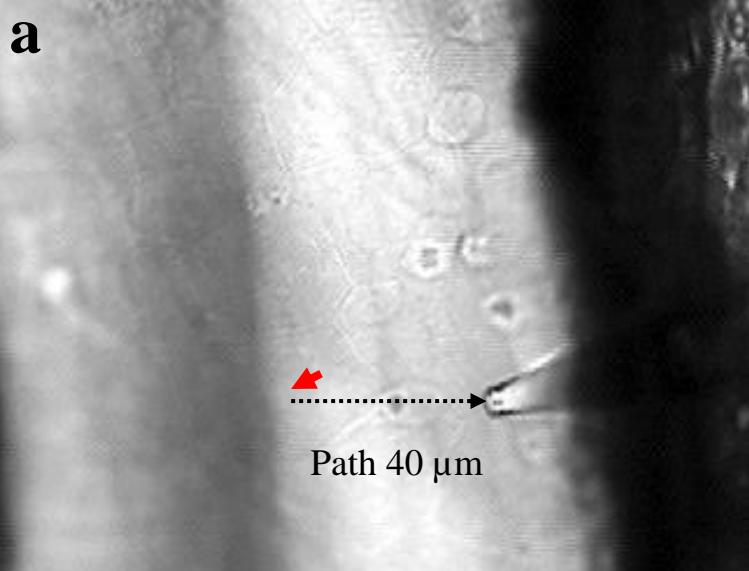
C



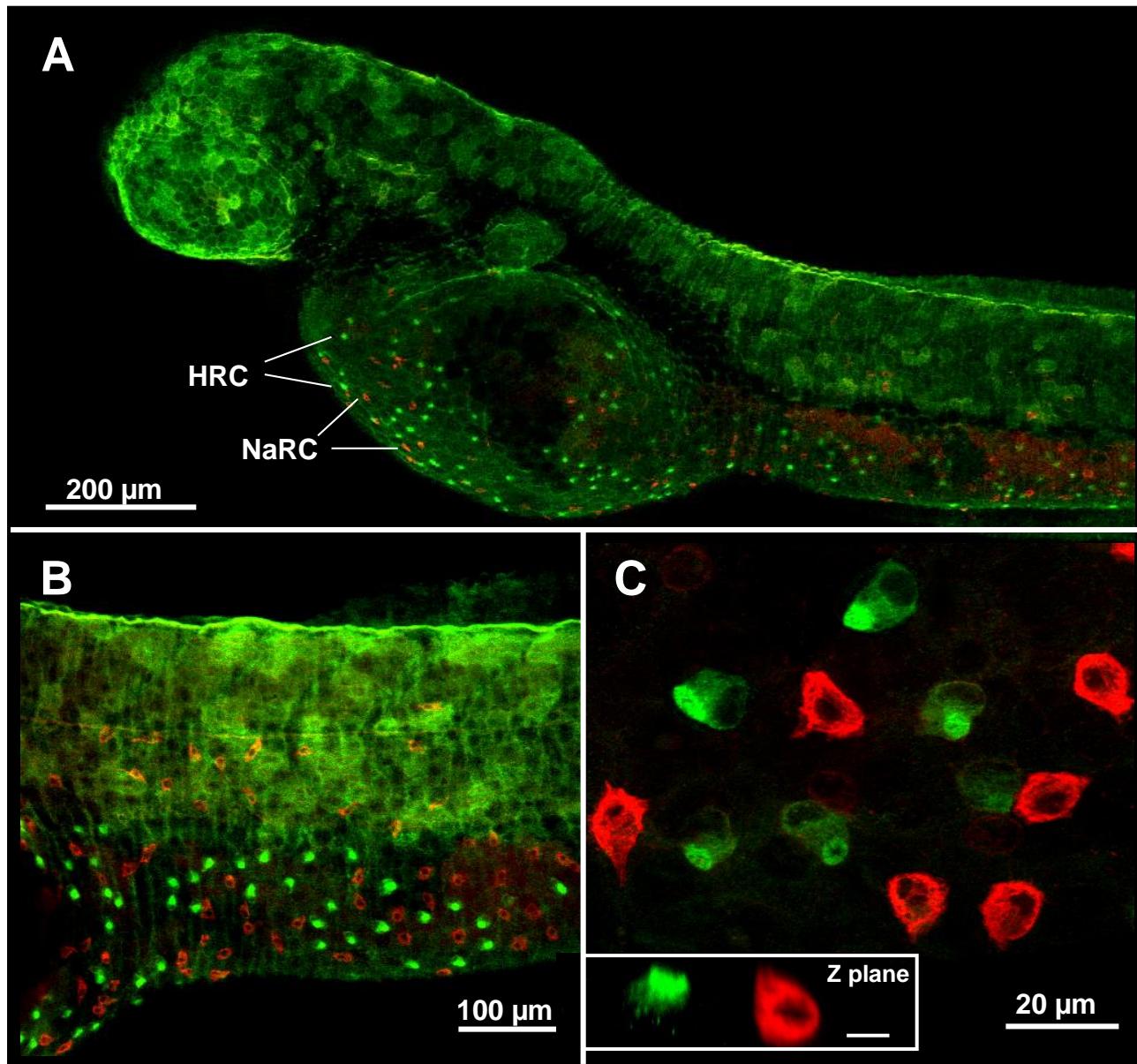
(Lin et al. 2006)

氫離子排放細胞的發現證據

(Lin et al. 2006)

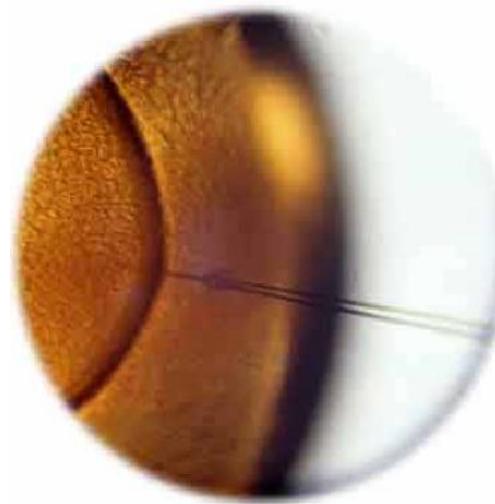
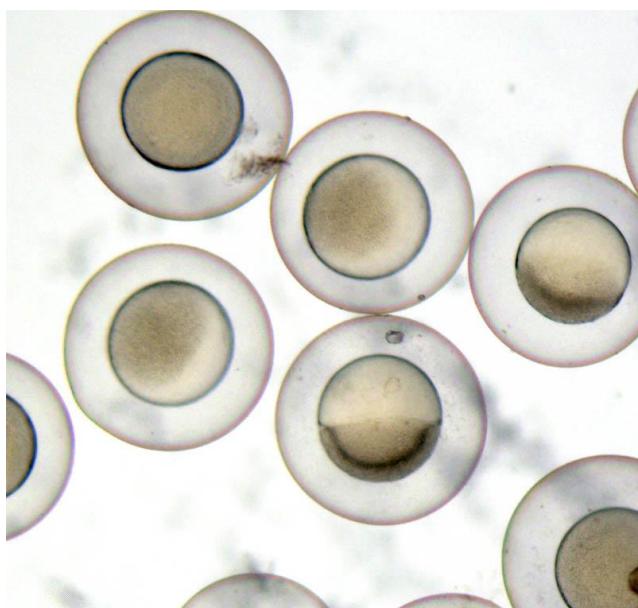
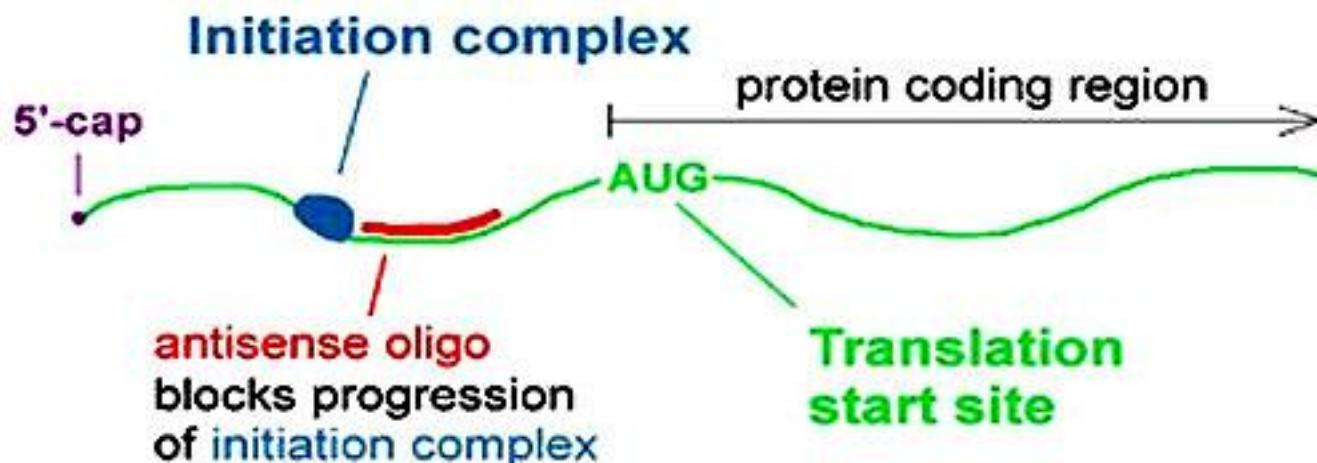


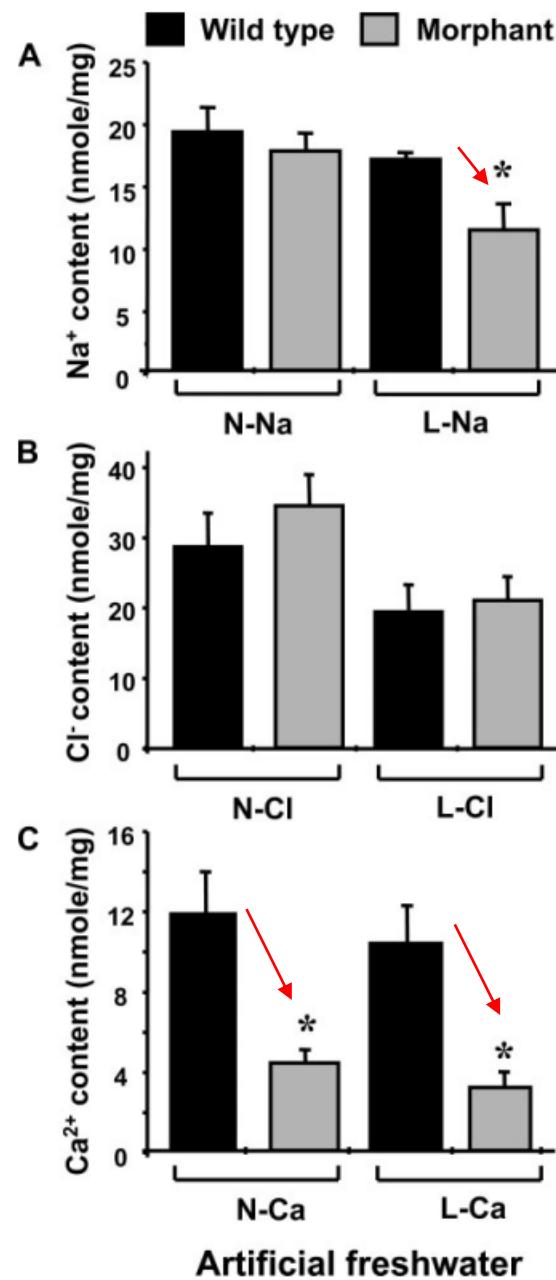
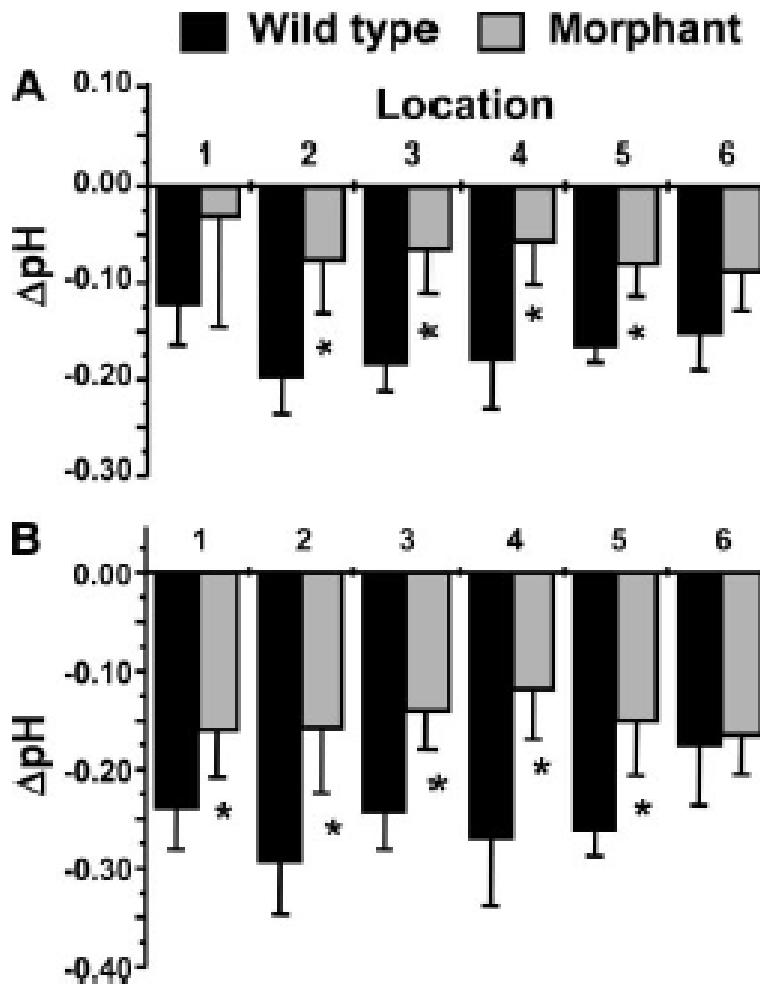
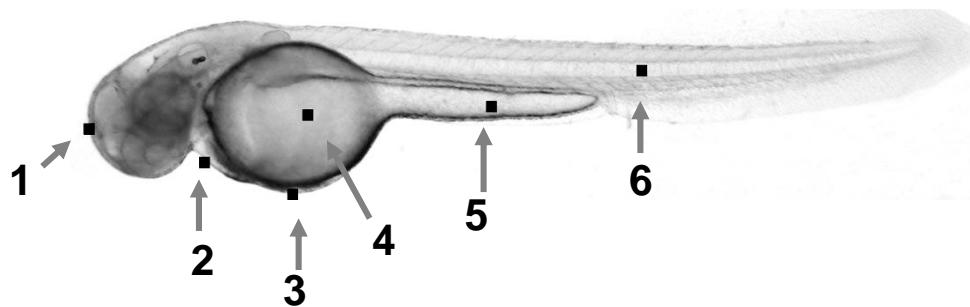
氫離子排放細胞的免疫標定



Na/K-ATPase rich cells (NaRC)

Morpholino knockdown of H⁺-ATPase





斑馬魚排氨細胞的發現

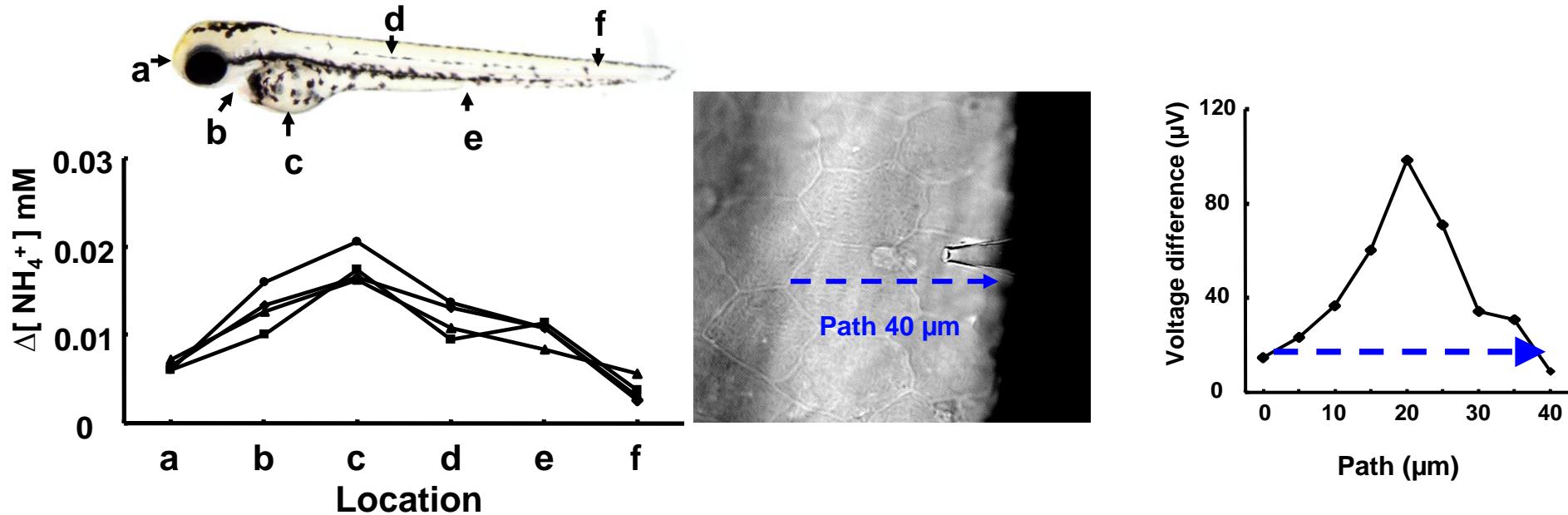
Am J Physiol Cell Physiol 295: C1625–C1632, 2008.
First published September 24, 2008; doi:10.1152/ajpcell.00255.2008.

Ammonia excretion by the skin of zebrafish (*Danio rerio*) larvae

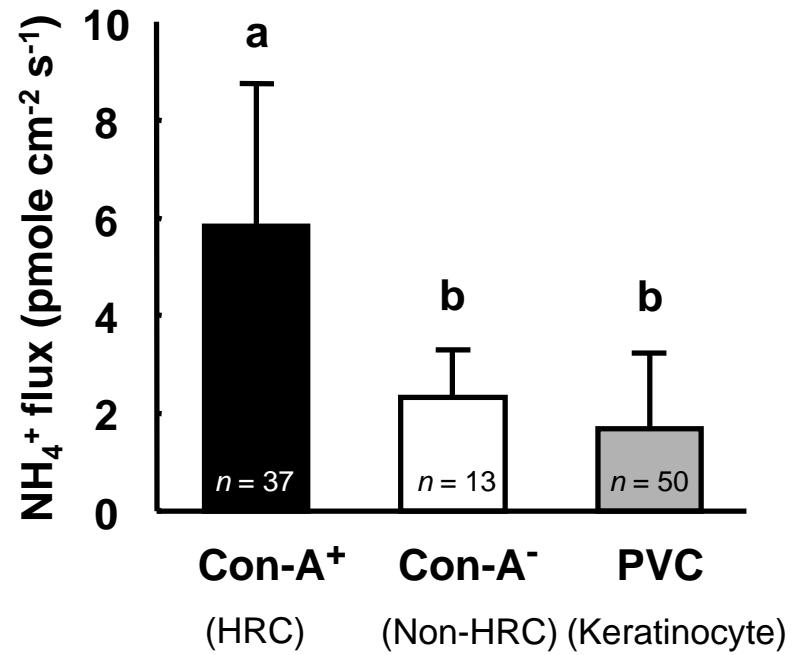
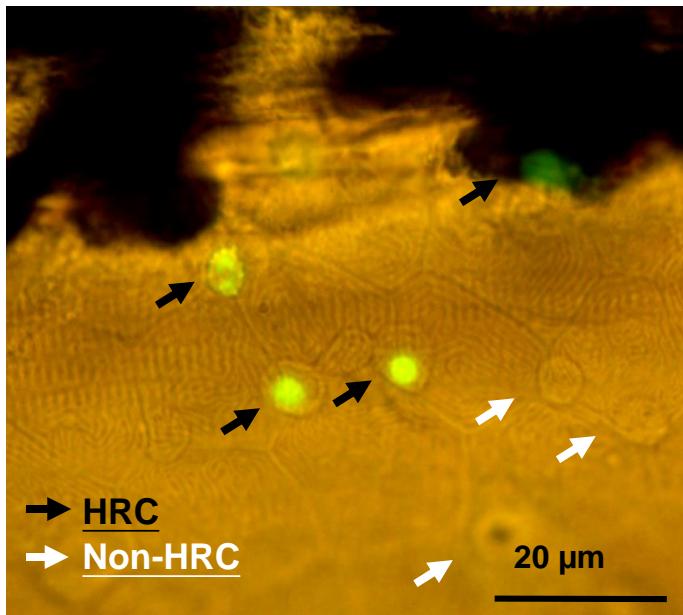
Tin-Han Shih,¹ Jiun-Lin Horng,² Pung-Pung Hwang,² and Li-Yih Lin¹

¹Department of Life Science, National Taiwan Normal University, Taipei, Taiwan, Republic of China; and ²Institute of Cellular and Organismic Biology, Academia Sinica, Taipei, Taiwan, Republic of China

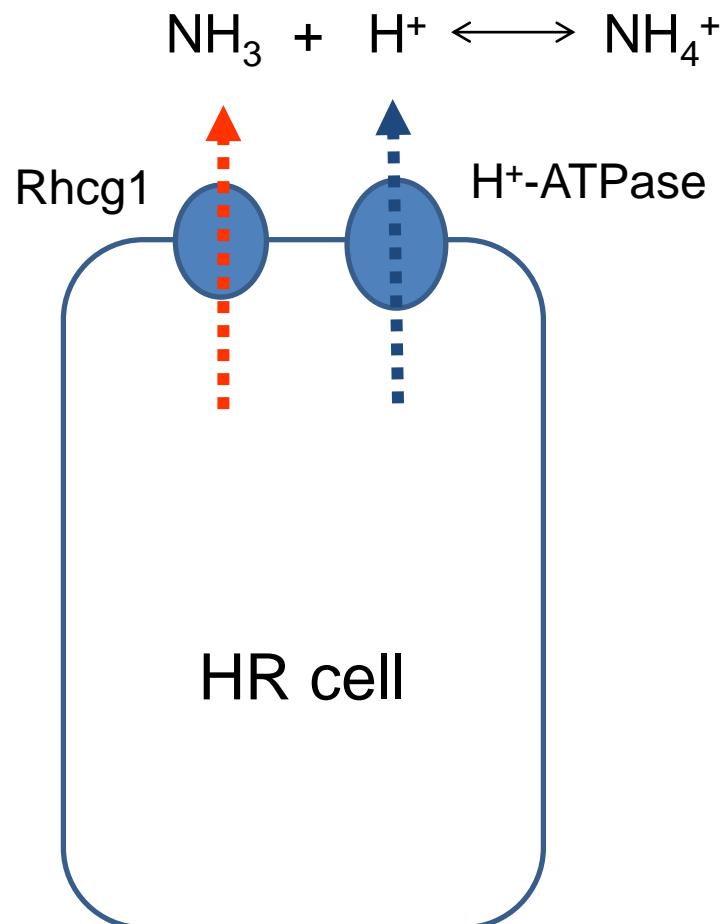
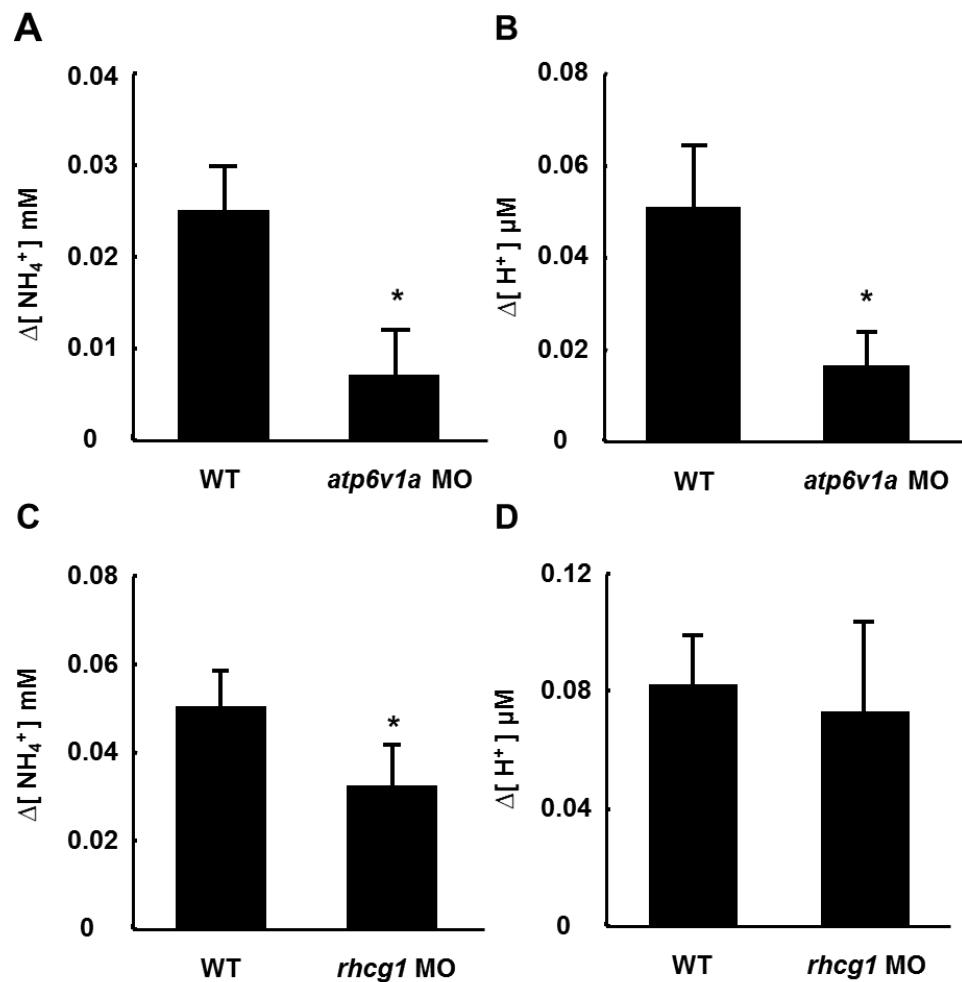
Submitted 14 May 2008; accepted in final form 22 September 2008



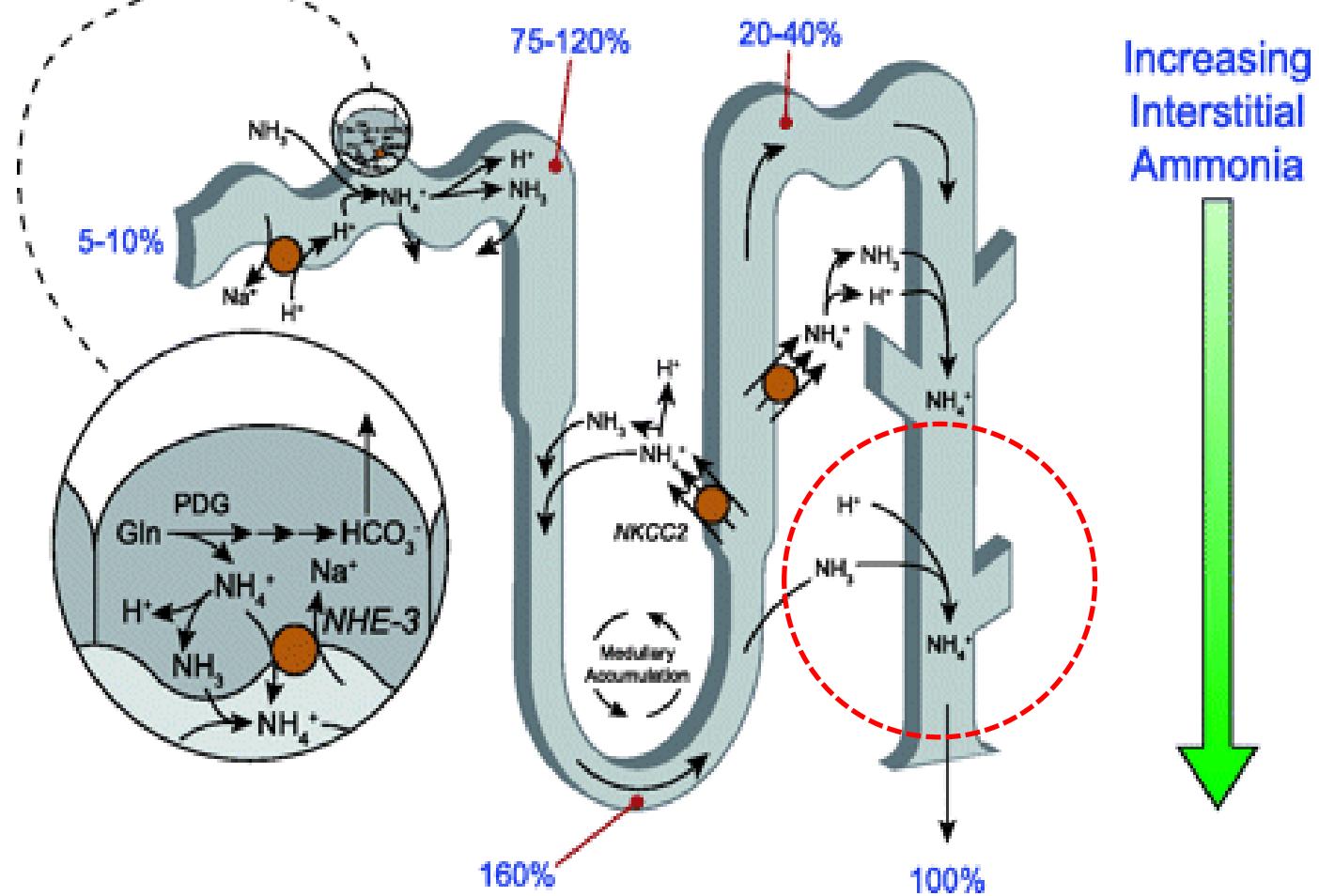
排酸細胞同時也是排氮細胞



排氮細胞的分子機制



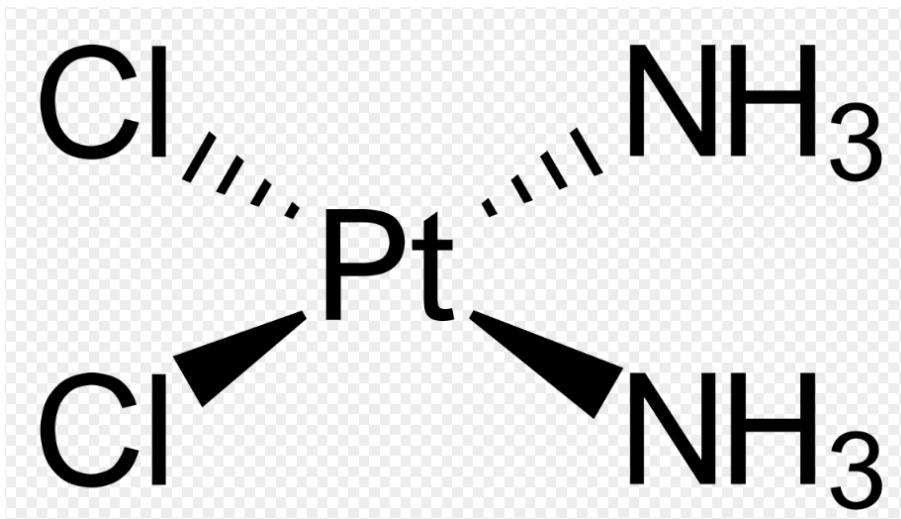
斑馬魚排酸(排氨)細胞與人類腎臟細胞相似



(Weiner and Verlander 2010)

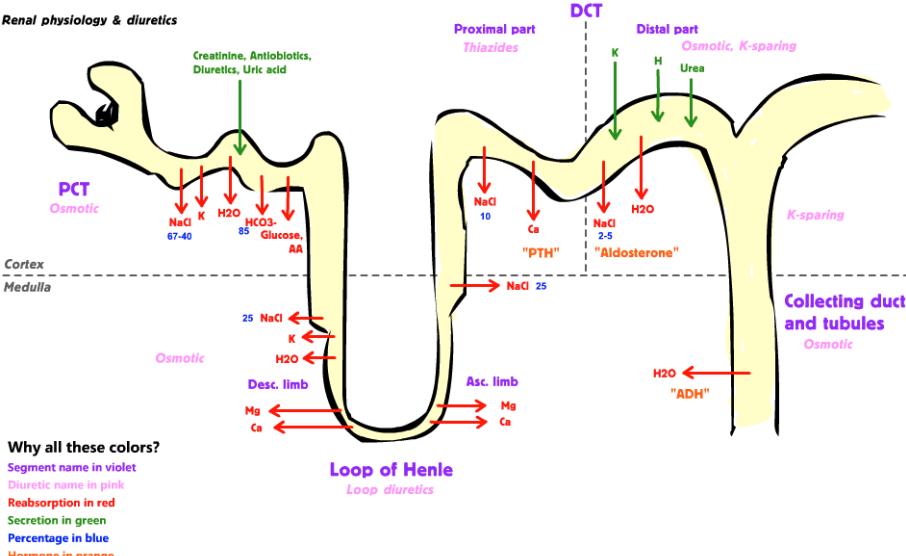
Cisplatin(順鉑) 對離子細胞的毒性分析

- 分子量: 300.01 g/mol
- 化學式: Cl₂H₆N₂Pt
- 是一種使用最廣泛的鉑(Pt)類化療藥物。
- 治療非小細胞肺癌、睪丸癌、頭頸癌、婦癌等癌症(Chen et al., 2013; Dilruba and Kalayda, 2016)。

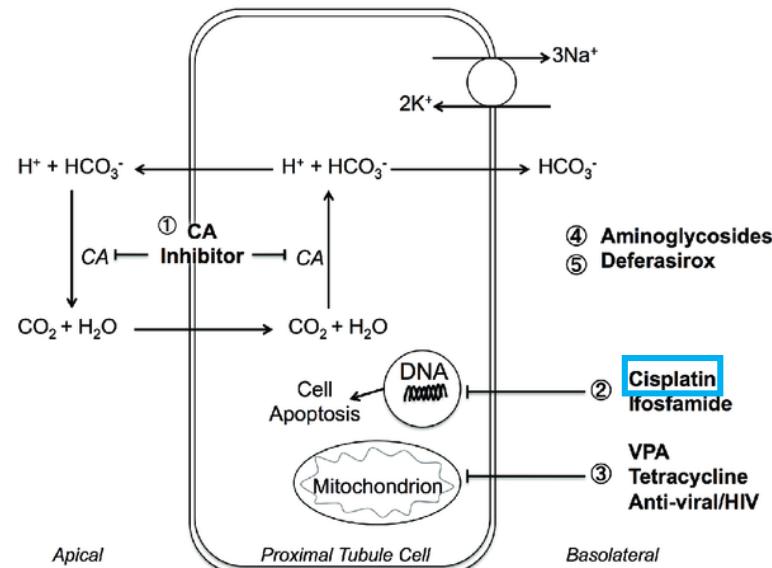


Cisplatin造成腎小管細胞發炎死亡

Drug-induced Fanconi syndrome:
Acidosis: decreased bicarbonate reabsorption
Sodium and potassium wasting



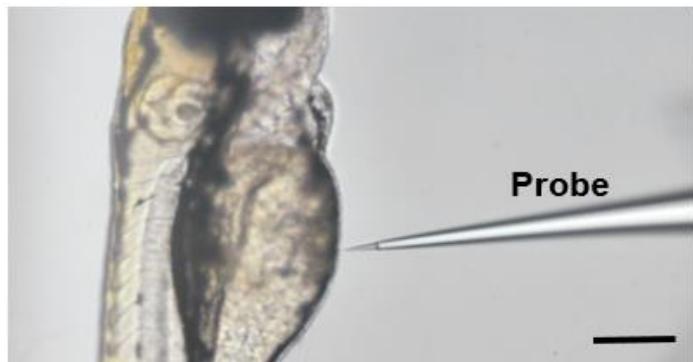
https://en.wikipedia.org/wiki/Renal_physiology#/media/File:Renal_Diuretics.gif



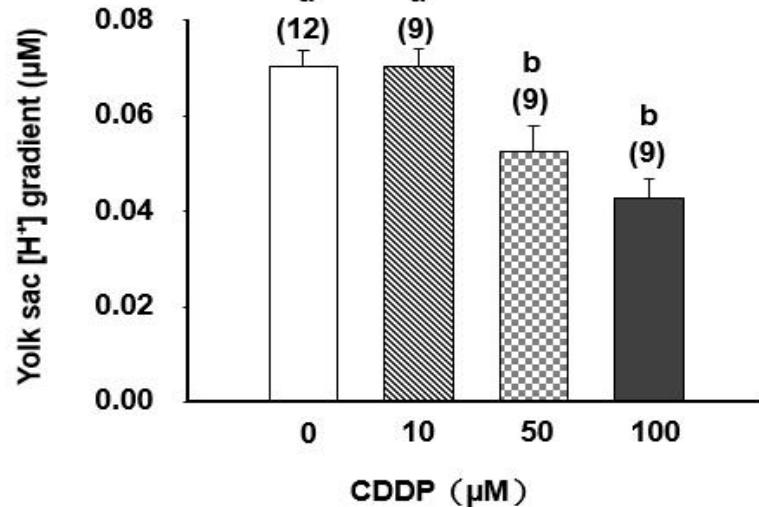
近端腎小管細胞

Cisplatin造成斑馬魚離子細胞死亡

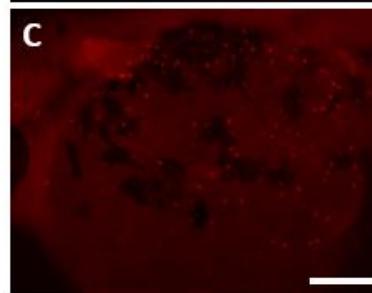
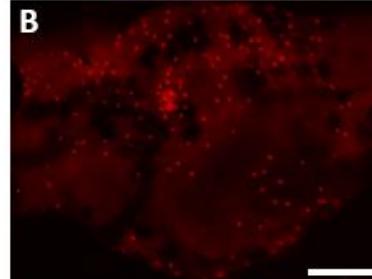
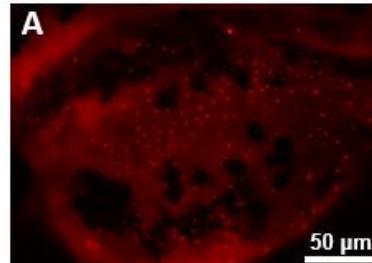
A



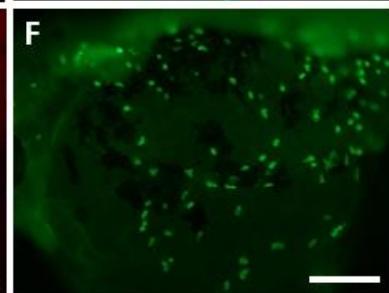
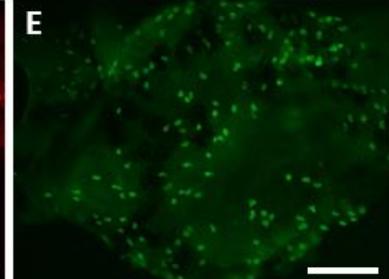
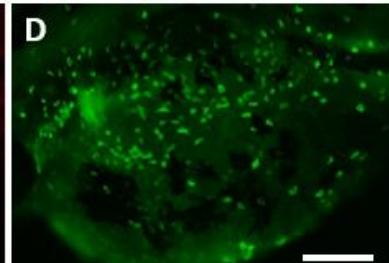
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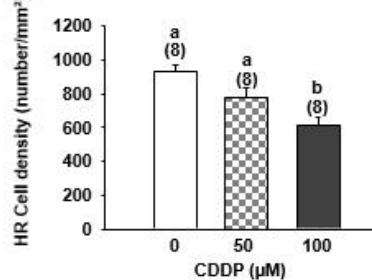
HR cells



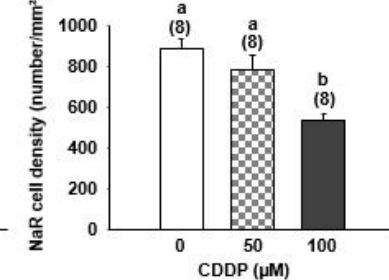
NaR cells



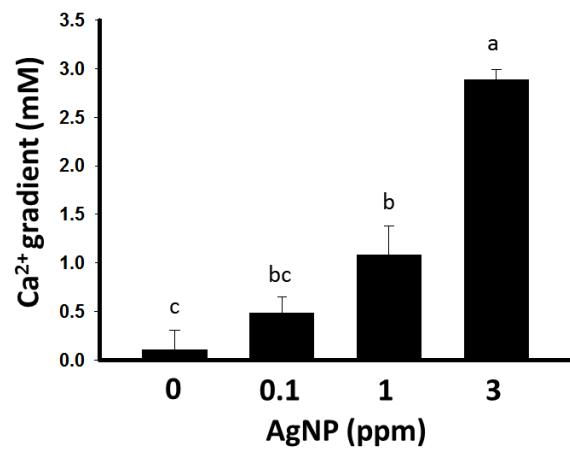
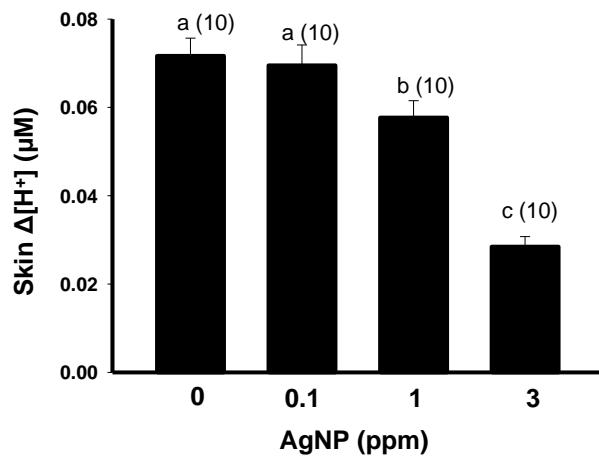
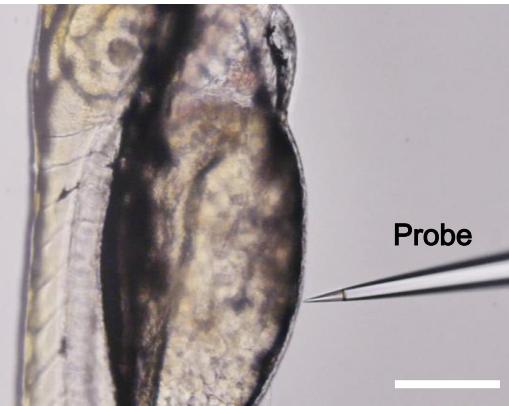
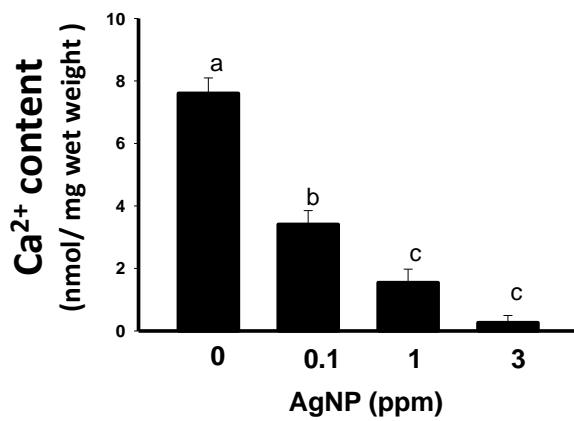
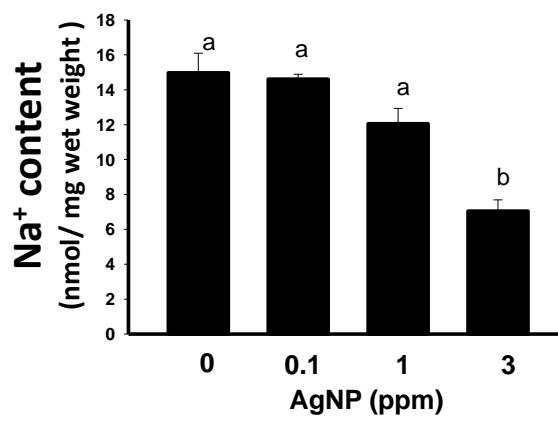
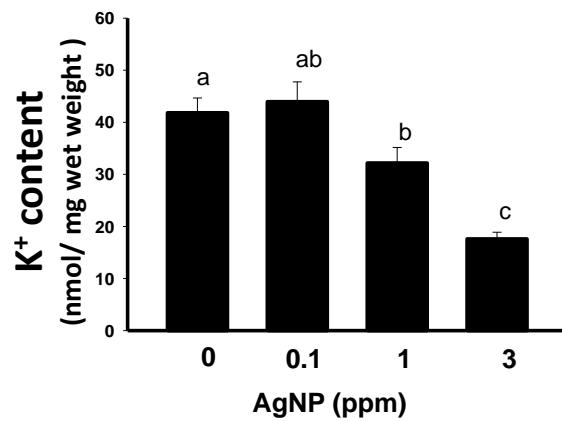
G



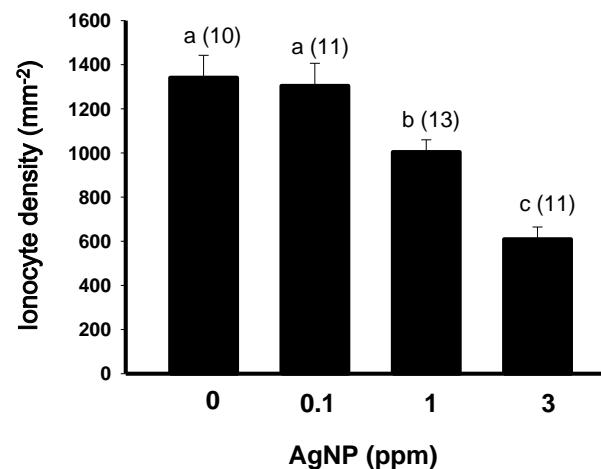
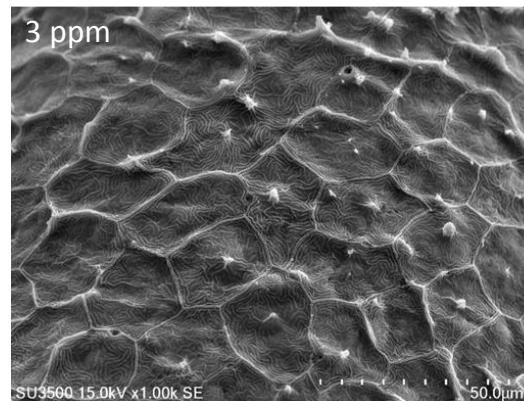
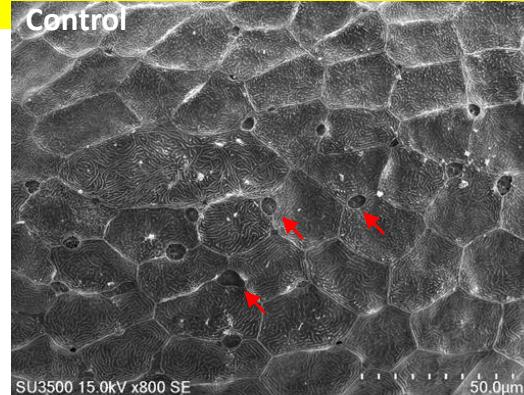
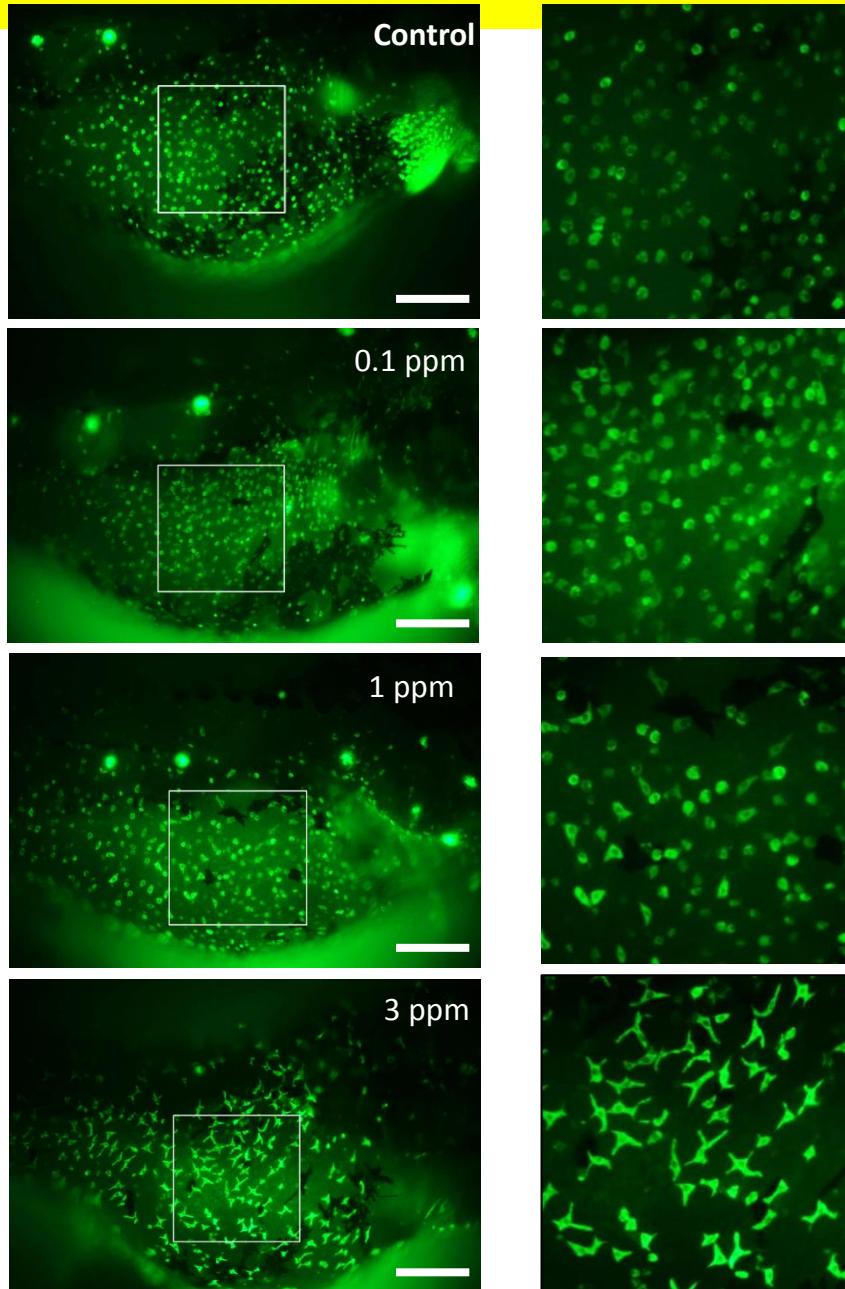
H



環境毒物對離子細胞的損傷 (以AaNP為例)



AgNP 造成斑馬魚離子細胞死亡



總結

1. 斑馬魚胚胎是很好研究生理、藥理、毒理的模式動物。
2. 非損傷微側系統能夠有效率且精確分析斑馬魚胚胎的生理功能，以及反映出藥物、毒物所產生的影響。
3. 非損傷微側系統在斑馬魚研究上的應用很大的仍有發展空間。

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