參加日本發育生物學會第 51 屆年會心得

錢宗良

本年度日本發育生物學會(Japanese Society of Developmental Biology, JSDB)與日本細胞生物學會(Japanese Society of Cell Biology, JSCB) 聯合舉辦年會，訂於 6 月 5 日至 8 日在東京都江戶川區船堀舉行第 51 屆日本發育生物學會年會與第 70 屆日本細胞生物學會年會暨亞太發育生物學術網絡會議 (Asia Pacific Developmental Biology Network)。場地安排與去年相同就在船堀社區活動中心，距離都營新宿線船堀站非常近，對於外地來的學者而言在東京都內交通上方便許多(圖一)。今年負責主辦兩位 Organizers 分別為日本發育生物學會 Professor Shigeo Hayashi (RIKEN) 與日本細胞生物學會 Professor Akihiro Harada (Osaka Univ.) 均有相當人脈，配合亞太發育生物學術網絡會議，吸引許多亞太地區在發育生物學與細胞生物學領域的專家學者或研究生參加，除了來自臺灣以外，美國、澳洲、韓國、新加坡等大學研究機構均有發表學術研究成果。就國際參與度而言，本年與往年相似，臺灣學者參與最為積極，包括中研院、臺灣大學、陽明大學、國衛院等學者或研究生均參加學術研究成果發表，而本次受主辦單位邀演講的臺灣學者包括臺灣發育生物學會理事長李士傑教授與中研院陳俊安研究員等。筆者很高興也藉由參加本次大會，能與在 1999 年東京大學 Professor Hirokawa 實驗室共同研究的夥伴重聚，包括這次代表日本細胞生物學會主辦的大阪大學 Professor Akihiro Harada。

本次筆者指導實驗室碩士班郝貞明同學以”The expression pattern of neuronal intermediate filament α-internexin in the chicken developing pineal gland” 為研究主題，參加會議海報 (poster session)發表成果(圖三及圖四)。這項研究是針對雞松果體在發育過程中，探討神經元中間絲蛋白 α-internexin 的表達與分佈。利用共軛焦顯微鏡觀察發現雞松果體中光感受器樣細胞 (Photoreceptor-like cells) 同時具有神經元特化中間絲蛋白 α-internexin 和光感細胞特有蛋白 Visinin 共同存在。我們得出結論 α-internexin

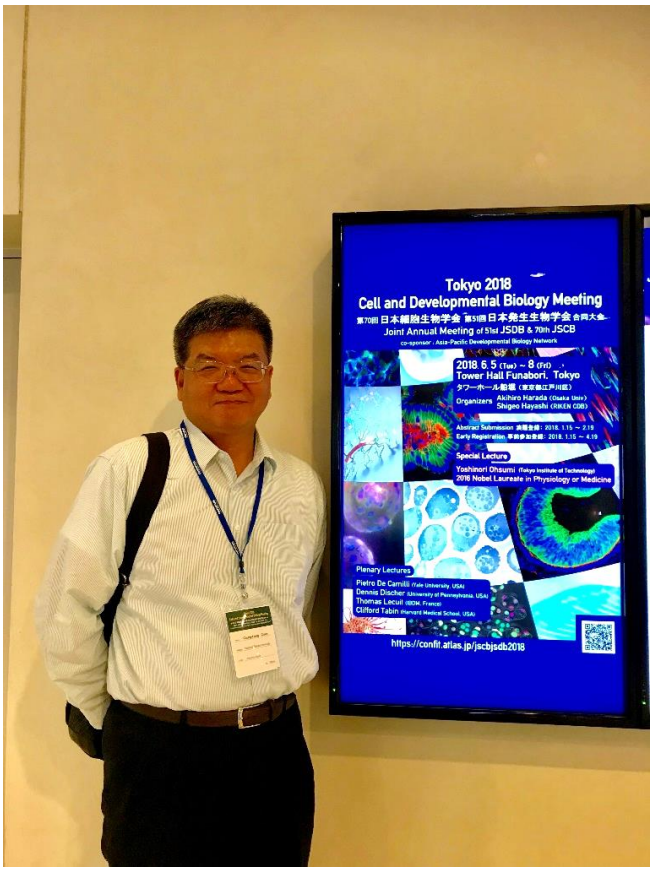
在雞松果體發育過程中可以做為是一個標識具有神經元特性的光感受器樣細胞；而在電子顯微鏡觀察發現成年雞松果體中的光感受器細胞有逐漸退化現象，因此我們推斷在雞成體松果體將失去光感官功能。郝貞明同學研究成果受到舉辦單位與亞太發育生物學術網絡會議委員推薦獲得本年度 Travel Fellowship 之獎勵。

本年度由兩個日本學會共同合辦年會，在議程上比往年增加許多國際學者受邀演講，其中特別邀請日本在 2016 年獲得諾貝爾生理醫學獎得主 Professor Yoshinori Ohsumi (Tokyo institute of Technology)介紹其主要研究 “Looking back on my 30 years of autophagy research”， Professor Ohsumi 精彩的演講得知大師之畢生努力，足可成為年輕一輩崇敬之典範。

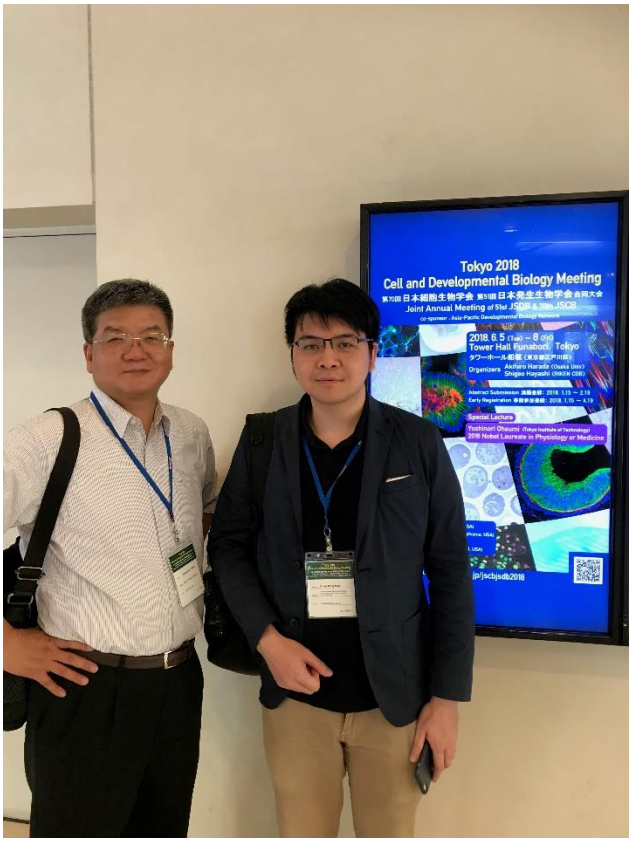
6 月 6 日第一天特別演講部分邀請由美國賓州大學 Professor Dennis Discher 介紹 “From matrix rigidity to nuclear mechanics in development and disease”對於染色體上之 DNA 受損及修復機制做深入的介紹。此外，亦邀請法國 CNRS & Aix-Marseille University 之 Professor Thomas Lecuit 介紹演講 “Control and self-organization of cell mechanics during tissue morphogenesis”，以果蠅胚胎發育之模式探討細胞組織間之物理特性，以光學原理技術呈現出非常有趣之細胞動態影像。緊接著的受邀外賓演講為來自 Yale University 的 Professor Pietro De Camilli，其演講主題為 “Intracellular Membrane Contact Sites and Lipid Dynamics”，介紹目前細胞膜結構從細胞內質網(ER)到其他細胞膜性的胞器生理功能，及因功能之缺損造成可能衍生之疾病模式。第一天最後壓軸之外賓演講是邀請到 Harvard Medical School 的 Professor Clifford Tabin，特別是針對 “Organizing the radial axis of the vertebrate gut”做系列研究之介紹，Professor Tabin 在 Nature 2011 發表之“On the growth and form of the gut” 及 Science 2013 年發表之 “Villification: how the gut get its villi”，均為發育生物學之經典貢獻研究，而本次演講內容主要發表在 PNAS 之“BMP signaling controls buckling forces to modulate looping morphogenesis of the gut”。進一步探討腸道發育過程的迴轉有趣現象，瞭解 BMP 訊息傳遞的重要角色。

筆者本次參加日本發育生物學會與日本細胞生物學會聯合舉辦年會之學術研討會，可深刻感受到日本學術界與國際接軌之完整，邀請到世界各國在發育生物與細胞生物學領域頂尖傑出之學者與會，足見日本之學術國際化程度。筆者特別感謝科技部計畫補助及舉辦單位與亞太發育生物學術網絡會議委員獎勵學生參加此盛會，相信同學藉由此參加國際學術研討會的機會，可以學習到許多不同領域研究學者對於學術研究之態度與用心。最後感謝日本發育生物學會與日本細胞生物學會，能夠持續藉由亞太發育生物學術網絡會議之平台，邀請臺灣相關領域的學者參加在日本舉辦的研討會。對於臺灣與日本在發育生物學會與細胞生物學學術領域交流之長期發展，將具有顯著的貢獻。

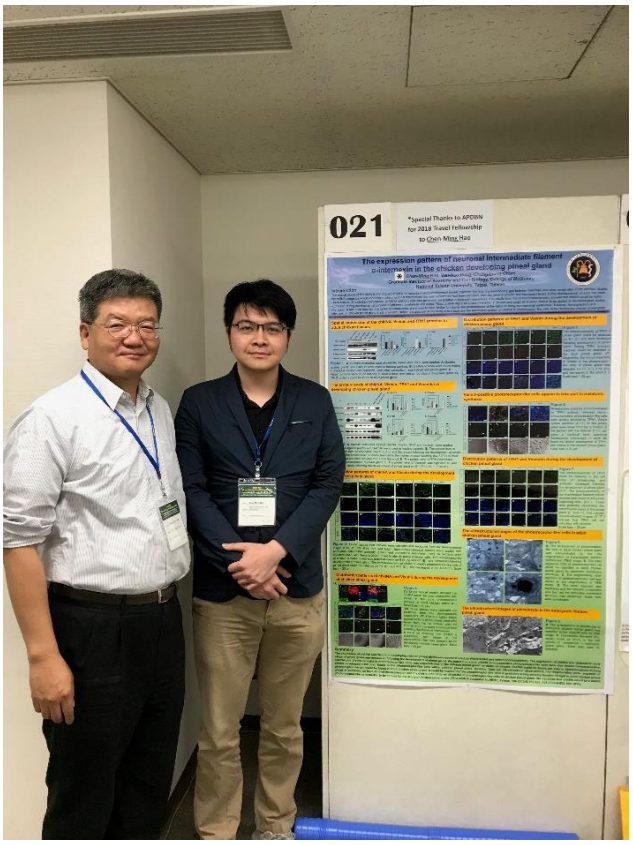
圖一、開會地點社區活動中心緊臨都營新宿線船堀站



圖二、筆者 6 月 6 日參加日本發育生物學會第 51 屆年會



圖三、筆者與郝貞明同學 6 月 8 日參加會議現場合影



圖四、筆者與郝貞明同學 6 月 8 日於海報論文發表成果前留影

The expression pattern of neuronal intermediate filament α-internexin in the chicken developing pineal gland

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Introduction

The animal model of this study is the chicken which is an important neurodevelopment model organism that links the evolutionary gap between mammals and other vertebrates. From previous studies, the mRNA sequence which encoding chicken neuronal intermediate filament α-internexin had been identified, also the gene structures and expression levels during developmental process had been characterized. The chicken α-internexin (chkINA) antibody was also generated via putative α-internexin sequences. The results from the immunohistochemistry showed that chkINA could be highly expressed during developmental process of chicken's cerebellum and retina. Thus, chkINA might be a neuron-specific IF protein and could be a useful marker to be applied to neurobiological studies in chicken. It has been reported that some of pinealocytes are shown to have similar function to the photoreceptor of the retina in many non-mammal vertebrates. However, the expression of α-internexin in chicken pineal gland is still unknown. Hence, the purpose of this study is to investigate the expression pattern of chkINA in photoreceptor-like cells of the developing chicken pineal gland.

Spatial expression of the chkINA, Visinin and TPH1 proteins in adult chicken tissues

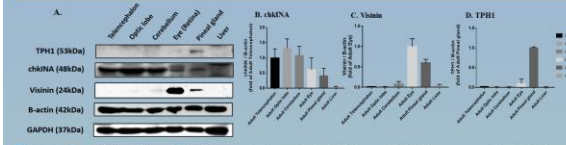

Figure 1. **A.** Specific antibodies against chkINA, Visinin and TPH1 were applied. Antibodies against β-actin and GAPDH were used as loading controls. **B.** chkINA proteins were found mainly in the adult chicken telencephalon, optic lobe, cerebellum, eye (retina) and pineal gland. **C.** Visinin proteins were found not only in adult chicken eye (retina), but also in the pineal gland. **D.** TPH1 proteins could be found in adult pineal gland.

The protein levels of chkINA, Visinin, TPH1 and Vimentin in developing chicken pineal gland

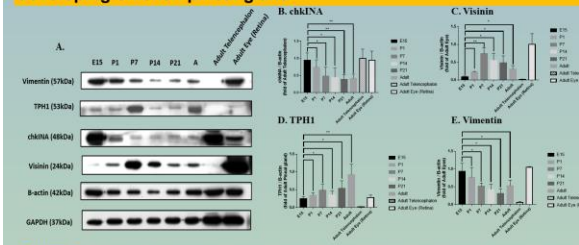

Figure 2. **A.** Specific antibodies against chkINA, Visinin, TPH1 and Vimentin were applied. Antibodies against β-actin and GAPDH were used as loading controls. **B.** The protein level of chkINA was high on embryonic day 15 (E15), and decreased following the development of pineal gland. **C.** The protein level of Visinin reached to the highest in post-hatching day 7 (P7) of chick pineal gland and then reduced from P7 to the adult. **D.** The protein level of TPH1 increased during the development of pineal gland. **E.** The protein level of Vimentin was high on E15, and decreased slowly following the development of pineal gland (n=5; *, P<0.05; **, P<0.01).

Distribution patterns of chkINA and Visinin during the development of chicken pineal gland

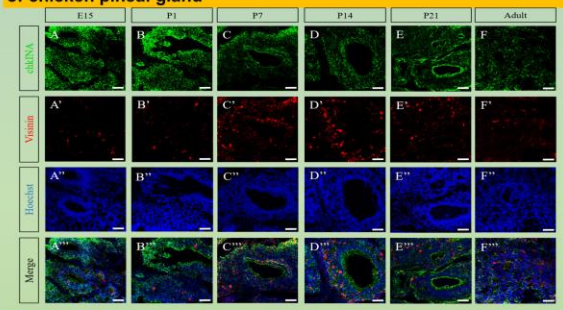

Figure 3. Pineal tissues from chicken were collected and sectioned from six developmental stages (E15, P1, P7, P14, P21 and Adult). Immunohistochemical staining were applied with polyclonal anti-chkINA antibody (green) and monoclonal anti-Visinin (red). All sections were counterstained with Hoechst 33342 (blue) to identify nuclei of pineal cells. The immunoreactivity of chkINA in pineal gland was detected easily on E15 (A) and P1 (B), and decreased following development of pineal gland. The immunoreactivity of Visinin to identify photoreceptor-like cells of pineal gland could be detected on P7 (C') and P14 (D'), and decreased in the adult (F'). Scale bars = 25 μm.

Distribution patterns of chkINA and Visinin during the development of chicken pineal gland

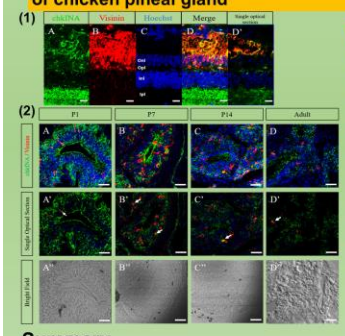

Figure 4.
(1) Single optical section showed that chkINA could be also colocalized with Visinin in the cone photoreceptors (arrows) at adult chicken retina (D'). Scale bars = 10 μm.
(2) Pineal tissues were collected and sectioned from four developmental stages (P1, P7, P14 and Adult). Single optical sections of the pineal gland were taken from top to bottom with the interval of 0.5 μm using a confocal laser scanning fluorescence microscope. It could be observed that chkINA is colocalized with Visinin in the photoreceptor-like cells (arrows) of the developing chicken pineal gland. Scale bars = 25 μm.

Distribution patterns of TPH1 and Visinin during the development of chicken pineal gland

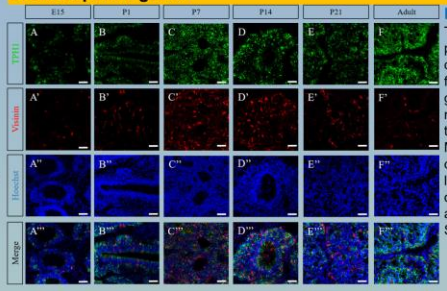

Figure 5. The immunoreactivity of TPH1 in pineal gland could be detected on P7 (C) and then increased following development of pineal gland. Strong TPH1 immunoreactivity could be detected in the adult pineal gland (F). Meanwhile, the immunoreactivity of Visinin in the photoreceptor-like cells of pineal gland was detected on P7 (C'), P14 (D'), and decreased in the adult (F'). Scale bars = 25 μm.

Visinin-positive photoreceptor-like cells appear to take part in melatonin synthesis

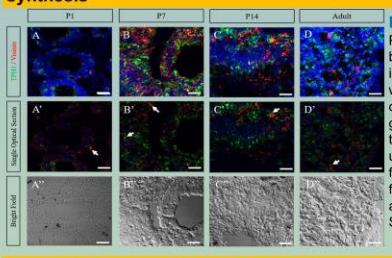

Figure 6. Pinealocytes could be immuno-labelled by TPH1 antibody, whereas visinin-immunopositive photoreceptor-like cells were weakly labelled by TPH1. Single optical sections (A'-D') of the pineal gland were taken from top to bottom of the section with the interval of 0.5 μm using a confocal laser scanning fluorescence microscope. It could be found the limited coexistence of TPH1 and Visinin in the chicken pineal gland. Scale bars = 25 μm.

Distribution patterns of TPH1 and Vimentin during the development of chicken pineal gland

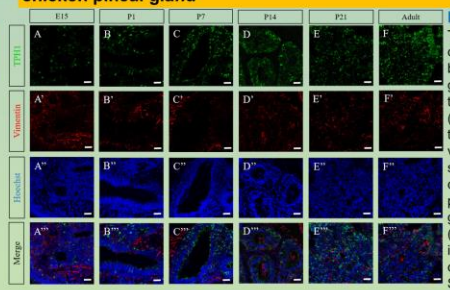

Figure 7. The immunoreactivity of TPH1 could be detected in the cell body of pinealocytes and gradually increased following the development of pineal gland (A-F). The immunoreactivity of the intermediate filament protein vimentin was found in the pineal supporting-cells (A'-F'). These cells gradually penetrated into parenchymal areas of the pineal gland (E' and F'). The merged (double-labeled) images (A'''-F''') indicate that TPH1 did not colocalize with vimentin. Scale bars = 25 μm.

The ultrastructural images of the photoreceptor-like cells in adult chicken pineal gland

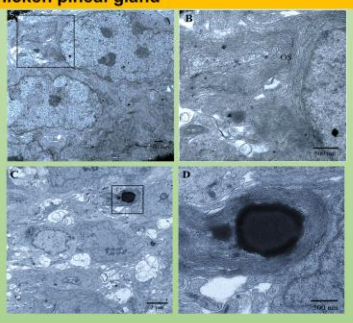

Figure 8.
A. The arrangement of photoreceptor-like cells in adult chicken pineal gland was demonstrated by the low magnification of transmission electron microscopic (TEM) image. **B.** The outer segments (OS) of photorecptor-like cell could be identified in adult chicken pineal gland by laminated membrane structures. **C.** The degenerating outer segment of photoreceptor-like cell was shown in low magnification of TEM image. **D.** The outer segment was regressed and the cytosolic component was lost, yet the laminated membrane structure was preserved. Scale bars were as indicated.

The ultrastructural images of pinealocyte in the embryonic chicken pineal gland

Figure 9.
A. The arrangement of pinealocyte in embryonic chicken pineal gland was revealed at low magnification of TEM image. **B.** Intermediate filaments (IFs, arrow) could be found in the cell pedicle of pinealocyte in embryonic pineal gland. Scale bars were as indicated.

Summary

The expression of chkINA and Visinin in developing chicken pineal gland were demonstrated by Western blot and immunohistochemistry. The expression of chkINA was detected in early stage of pineal gland and decreased following the development of pineal gland. We found that some chkINA immunopositive photoreceptor-like cells were also Visinin immunopositive. chkINA and Visinin co-exist in photoreceptor-like cells was demonstrated in the chicken pineal gland by confocal images. chkINA could be found not only in photoreceptors durung retinal development but also found in the photoreceptor-like cells within chicken pineal gland. Besides, from our ultrastructural observations, the degenerating outer segment of photoreceptor-like cell could be found in adult chicken pineal gland. It could be implied that the photoreceptor-like cells is gradually losing sensory function of light in adult chicken pineal gland. In summary, we have studied the expression and the distribution patterns of chkINA in photoreceptor-like cells of chicken pineal gland. We conclude that chkINA could be a useful photoreceptor-like cells marker to be applied for the study of chicken pineal gland. **(This work is supported by MOST, Taiwan, 106-2312-B-002-003; 105-2320-B-002 -008 -MY3)**