Dr. Benjamin C. K. Lu 訪問報告

1. 訪問緣起與目的：

真菌種類繁多，具有豐富之多樣性，易於培養操作，為研究生物無性及有性生殖之型態分化調控之絕佳材料。加拿大 **University of Geulph** 名譽教授 **Dr. Benjamin C. K. Lu** 為國際知名之真菌細胞遺傳學專家，長久以來以模式真菌擔子菌墨水菇 *Coprinus cinereus* 進行有絲分裂、減數分裂及染色體之研究，撰寫相當多的綜論性文章及真菌專書，學有專精。數年前，**Dr. Benjamin C. K. Lu** 更發現真菌減數分裂細胞凋亡之現象。為使台灣大學對真菌研究有興趣之師生，有機會接觸真菌細胞凋亡之新知，並促進國內相關真菌領域之研究，及提昇相關技術之應用，特別邀請 **Dr. Benjamin C. K. Lu** 進行為期 20 天之學術訪問，相關學術活動包括短期授課、專題演講，並指導真菌細胞遺傳學研究之相關技術。

2. 訪問過程及效益：

A. 短期授課：

本次邀請 **Dr. Benjamin C. K. Lu** 主要目的為進行為期兩週之短期授課，課程為”現代分子真菌學(Current Topics in Molecular Mycology)”，授課內容為”真菌之細胞凋亡” 。課程資訊如下：

Introduction:

The goal of this course is to discuss the current researches in molecular mycology. This course is a one credit hour course and will meet two hours each time during the span of two weeks. Seniors or graduate level students who are interested in molecular mycology are encouraged to participate in this class. Students are preferred to have basic knowledge of mycology and molecular genetics. The course focus for this semester is “**Programmed Cell Death in Fungi**” and the topics covered are listed below. The course will be lectured and students are encouraged to read the reference before each class and are expected to participate in course discussion. The grade will be determined by the final examination and class participation.

Date	Lecture	Hour
May 19	Introduction: discovery of genes in higher eukaryotes	1
May 21	Apoptosis, the type I programmed cell death	3
May 23	Apoptosis, triggered by genetic defects	2
May 26	Genes that regulate apoptosis	2
May 28	Mitochondria and apoptosis	2
May 30	Autophagy, the type II programmed cell death	1
May 30	Meiotic apoptosis	1

June	Examination	

Reference:

I. Introduction

1. Adachi, M, Cossman, J, Longo, D, Croche, CM, and Tsujimoto, Y (1989) Variant translocation of the bcl-2 gene to immunoglobulin λ light chain gene in chronic lymphocytic leukemia. Proc. Natl. Acad. Sci. USA 86: 2771-2774.
2. Ellis, HM and Horvitz, HR (1986) Genetic control of programmed cell death in the nematode *C. elegans*. Cell, 44: 817-829.
3. Yan, N, Chai, J, Lee, ES, Gu, L, Liu, Q, He, J, Wu, JW, Kokei, D, Li, H, Hao, Q, Xue, D and Shi, Y (2005) Structure of the CED-4-CED-9 complex provides insights into programmed cell death in *Caenorhabditis elegans*. Nature, 837: 831-837.

IIa. Apoptosis induced by environmental stimuli

1. Madeo, F, Frohlich, E, Ligr, M, Grey, M, Sigrist SJ, Wolf, DH, and Frohlich K-U (1999) Oxygen stress: a regulator of apoptosis in yeast. J. Cell Biol. 145: 757-767.
2. Ludovico, P, Sousa, MJ, Silva, MT, Leao, C, and Corte-Real, M (2001) *Saccharomyces cerevisiae* commits to a programmed cell death process in response to acetic acid. Microbiology 147: 2409-2415.
3. Chen, SR, Dungan, DD, and Dickman, MB (2003) Bcl-2 family members inhibit oxidative stress-induced programmed cell death *in Saccharomyces cerevisiae*. Free Rad. Biol. & Medicine, 34: 1315-1325.
4. Eisler, H, Frohlich, K-U, and Heidenreich, E (2004) Starvation for an essential amino acid induces apoptosis and oxidative stress in yeast. Exp. Cell Res. 300: 345-353.
5. Phillips, AJ, Sudbery, I and Ramsdale, M (2003) Apoptosis induced by environmental stresses and amphotericin B in *Candida albicans*. PNAS 100: 14327-14332.
6. Kitagaki, H, Araki, Y, Funato, K and Shimoi, H (2007) Ethanol-induced death in yeast exhibits features of apoptosis mediated by mitochondrial fission pathway. FEBS Lett 581: 2935-2942.
7. Herker E, Jungwirth H, Lehmann KA, Maldener C, Frohlich K-U, Wissing S, Buttner A, Fehr M, Sigrist S, Madeo F (2004). Chronological aging leads to apoptosis in yeast. J Cell Biol 164: 501-507.

IIb. Apoptosis induced by heterologous genes

1. Ink, B, Zornig, M, Baum, B, Hajibagheri, N, James, C, Chittenden, T and Evan, G (1997) Human Bak induces cell death in *Schizosaccharomyces pombe* with morphological changes similar to those with apoptosis in mammalian cells. Mol. Cell. Biol. 17: 2468-2474.
2. Ligr, M, Madeo, F, Frohlich, E, Hilt, W, Frohlich K-U, and Wolf, DH (1998) Mammalian bax triggers apoptotic changes in yeast. FEBS Lett 438: 61-65.
3. James C, Gschmeissner S, Fraser A and Evan GI (1997). CED-4 induces chromatin condensation in *Schizosaccharomyces pombe* and is inhibited by physical association with CED-9. Curr Biol 7: 246-252.
4. Wu D, Chen P-J, chen S, Hu Y, Nunez G and Ellis RE (1999). *C. elegans* MAC-1, an essential member of the AAA family of ATPases, can bind CED-4 and prevent cell death. Development 126: 2021-2031.

III. Apoptosis triggered by genetic defects

1. Qi H, Li T-K, Kuo D, Nur-E-Kamal A and Liu LF (2003) Inactivation of Cdc13p triggers MEC1-dependent apoptotic signals in yeast. J. Biol. Chem.278: 15136-15141.
2. Baek Y-U, Kim Y-R, Yim H-S and Kang S-O (2004) Disruption of g-glutamylcysteine synthetase results in absolute glutathione auxotrophy and apoptosis in *Candida albicans*. FEBS Letters 556: 47-52.
3. Weinberger M, Ramachandran L, Feng L, Sharma K, Sun X, Marchetti M, Huberman JA, and Burhans WC (2005) Apoptosis in budding yeast caused by defects in initiation of DNA replication. J Cell Sci. 118:3543-3553.

IVa. Genes that regulate apoptosis: Bcl-2 family

1. Zha H, Fisk HA, Yafee MP, Mahajan N, Herman B and Reed JC (1996) Structure-function comparisons of the proapoptotic protein Bax in yeast and mammalian cells. Mol Cell Biol. 16: 6494-6508.
2. Priault M, Camougrand N, Chaudhuri B, Schaeffer J and Manon S (1999) Comparison of the effects of bax-expression in yeast under fermentative and respiratoryu conditions: investigation of the role of adenine nucleotides carrier and cytochrome c. FEBS Letters 456: 232-238.
3. Priault M, Camougrand N, Chaudhuri B and Manon S (1999) Role of the C-terminal domain of Bax and Bcl-xL in their localization and function in yeast cells. FEBS Letters 443: 225-228.

4. Oliver L, Priault M, Tremblais K, LeCabellec M-T, Meflah K, Manon S and Vallette FM (2000) The substitution of the C-terminus of bax by that of bcl-xL does not affect its subcellular localization but abrogates its pro-apoptotic properties. FEBS 487: 161-165.
5. Huang DCS and Straser A (2000) BH3-only proteins—essential initiators of apoptotic cell death. Cell 103: 839-842.
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IVb. Genes that regulate apoptosis: yeast genes

1. Wissing S, Ludovico P, Herker E, Buttner S, Engelhardt SM, Decker, T, et al. (2004) An AIF orthologue regulates apoptosis in yeast. J Cell Biol. 166: 969-974.
2. Cande C, Vahsen N, kouranti I, Schmitt E, Daugas E, Spahr C, Luban J, Kroemer RT, Ciordanetto F, Garrido C, Penninger JM and Kroemer G (2004) AIF and cyclophilin A cooperate in apoptosis-associated chromatinolysis. Oncogene 23: 1514-1521.
3. Fahrenkrog B, Sauder U and Aebi U (2004). The S. cerevisiae HtrA-like protein Nma111p is a nclear serine protease that mediates yeast apoptosis. J Cell Sci 117: 115-126.
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5. Madeo F, Herker E, Maldener C, Wissing S, Lachelt S, Herlan M, Fehr M, Lauber K, Sigrist SJ, Wesselborg S and Frohlich K-U (2002). A caspase-related protease regulates apoptosis in yeast. Mol Cell 9: 911-917.
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7. Ahn S-H, Cheung WL, Hsu J-Y, Diaz RL, Smith MM, and Allis CD (2005). Sterile 20 kinase phosphorylates histone H2B at serine 10 during hydrogen peroxide-induced apoptosis in *S. cerevisiae*. Cell 120: 25-36.

V. Mitochondria and apoptosis

1. Fannjiang Y, Cheng W-C, Lee SJ, Qi B, Pevsner J, McCaffery JM, Hill RB, Basanez G, and Hardwick JM (2004). Mitochondrial fission proteins regulate programmed cell death in yeast. Genes Dev 18: 2785-2797.
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6. Ludovico P, Rodrigues F, Almeida A, Silva MT, Barrientos A, and Corte-Real M (2002). Cytochrome c release and mitochondrial involvement in programmed cell death induced by acetic acid in *Saccharomyces cerevisiae*. Mol Biol Cell 13: 2598-2606.
7. Kuwana T, Mackey MR, Perkins G, Ellisman MH, Latterich M, Schneiter R, Green DR and Newmeyer DD (2002). Bid, Bax and lipids cooperate to form supramolecular openings in the outer mitochondrial membrane. Cell 111: 331-342.
8. Polcic P and Rorte M (2003). Response of yeast to the regulated expression of proteins in the Bcl-2 family. Biochem J 374: 393-402.
9. Braun R, Zischka H, Madeo F, Eisenberg T, Wissing S, Buttner S, Engelhardt SM, Buringer D, and Ueffing M (2006). Crucial mitochondrial impairment upon CDC48 mutation in apoptotic yeast. J Biol Chem 281: 25757-25767.
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VI. Autophagy, the type II programmed cell death

1. Inbal B, Bialik S, Sabanay I, Shani G, and Kimchi A (2002). DAP kinase and DRP-1 mediate membrane blebbing and the formation of autophagic vesicles during programmed cell death. J Cell Biol. 157: 455-468.
2. Huang W-P and Klionsky DJ (2002). Autophagy in yeast: a review of the molecular machinery. Cell Structure Function 27: 409-420.
3. Suriapranata I, Epple UD, Bernreuther D, Bredschneider M, Sovarasteanu K and Thumm M (2000). The breakdown of autophagic vesicles inside the vacuole depends on Aut4p. J Cell Sci 113: 4025-4033.
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5. Kissova I, Plamondon L-T, Brisson L, Priaualt M, Renouf V, Schaeffer J, Camougrand N, and Manon S (2006). Evaluation of the roles of apoptosis, autophagy, and mitophagy in the loss of plating efficiency induced by Bax expression in yeast. J Biol Chem 281: 36187-36197.

VII. Meiotic apoptosis

1. Lu, BC, Gallo N, Kues U (2003). White-cap mutants and meiotic apoptosis in the basidiomycete *Coprinus cinereus*. Fungal Genet Biol 39: 82-93.
2. Celerin M, Merino ST, Stone JE, Menzle AM, and Zolan ME (2000). Multiple roles of Spo11 in meiotic chromosomes behavior. EMBO J 19: 2739-2750.
3. Yang H, Ren Q, and Zhang Z (2006). Chromosome or chromatin condensation leads to meiosis or apoptosis in stationary yeast (*Saccharomyces cerevisiae*) cells. FEMS Yeast Res 6: 1254-1263.

真菌細胞凋亡，為一相當新的研究領域，發現至今約十年左右，並且近年累積相當多的研究結果。**Dr. Benjamin C. K. Lu** 應此次邀請，特別準備相關最新研究資訊，討論原始研究報告，其深入淺出的介紹，課程亦介紹許多研究歷史，相關研究歷程及其科學思維，對本系教師與同學在科學研究上，有很大的啟發與鼓舞。

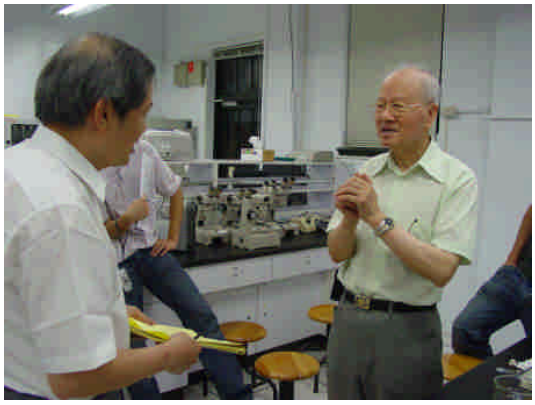
現代分子真菌學上課時與本系師生討論”真菌細胞凋亡”之最新研究新知資訊，引領學生對科學研究之興趣及獨立思考。

Dr. Benjamin C. K. Lu (前排中)、本系劉瑞芬老師(前排左)、沈偉強老師(前排右)，與現代分子真菌學修課學生合影。

B. 指導真菌染色體研究相關技術：

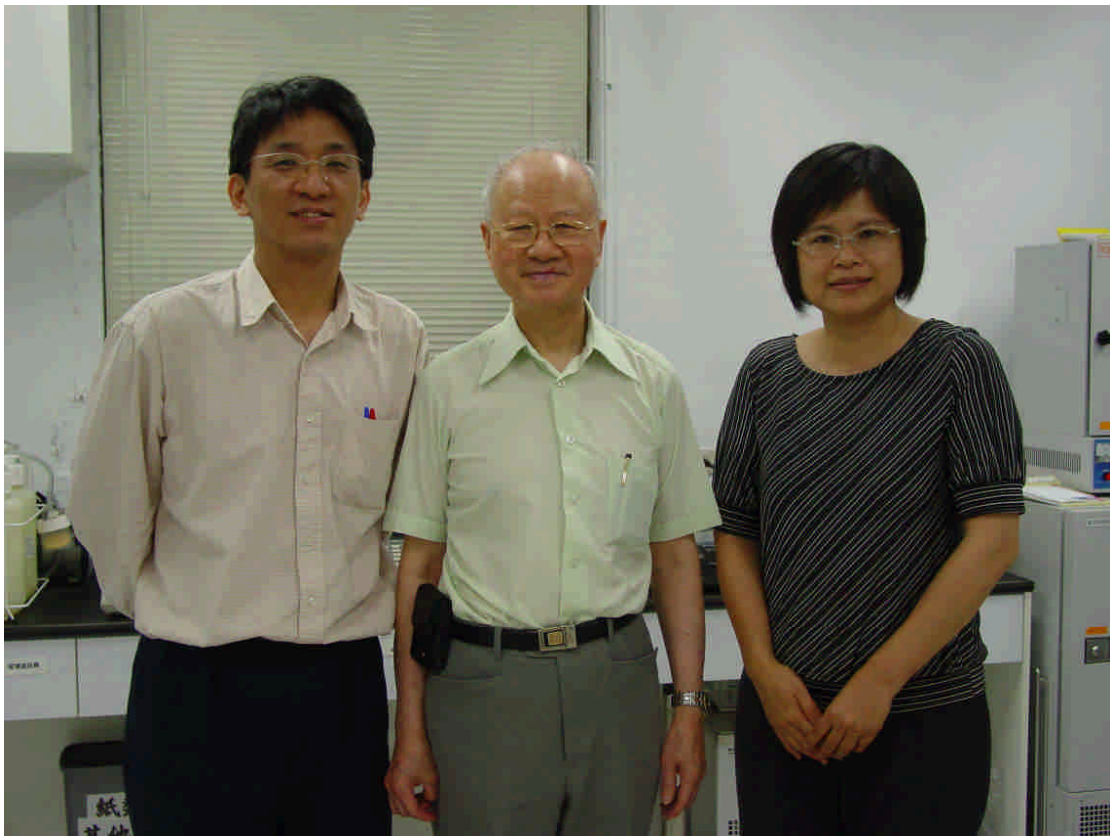
Dr. Benjamin C. K. Lu 為國際知名之真菌細胞遺傳學專家，其碩士研究期間之成果，即發表論文於Nature期刊。長久以來，以模式真菌擔子菌墨水菇 *Coprinus cinereus* 進行減數分裂及染色體之研究，其許多重要發現接收錄於真菌之教科書中，並撰寫相當多的綜論性文章及真菌專書，學有專精。此次訪問期間，特別於

本系植物分子檢測實驗室，示範菇類染色體樣品之製備與觀察，指導本系師生操作相關技術，本系師生獲益良多。



Dr. Benjamin C. K. Lu 為國際知名之真菌細胞遺傳學專家，訪問期間於本系植物分子檢測實驗室，示範菇類染色體樣品之製備與觀察，指導本系師生操作相關技術，並與曾顯雄教授進行意見交換。

Dr. Benjamin C. K. Lu 為國際知名之真菌細胞遺傳學專家，訪問期間於本系植物分子檢測實驗室，示範菇類染色體樣品之製備與觀察，指導本系師生操作相關技術。



Dr. Benjamin C. K. Lu 與本系陳昭瑩主任及沈偉強老師於本系植物分子檢測實驗室合影。

C. 專題演講：

Dr. Benjamin C. K. Lu 於 5 月 29 日上午於本系植微講座專題演講，講題為「A mushroom from horse manure: from chromosomes and genetics to photobiology and meiotic apoptosis」。**Dr. Benjamin C. K. Lu** 很會說故事，其在真菌細胞遺傳過程之研究上，充滿了細膩之觀察及思考，對擔子菌 Coprinus 之染色體有很仔細的研究，其研究路程及思維對本系教師同學在科學上的研究有很大的啟發與鼓勵，本系師生獲益良多。

Dr. Benjamin C. K. Lu 於 5 月 29 日在本系進行專題演講”’”，陳昭瑩主任致贈感謝狀，並合影留念。