Student Code: _____

20th INTERNATIONAL BIOLOGY OLYMPIAD

12th – 19th July, 2009

Tsukuba, JAPAN

PRACTICAL TEST 4

CELL PHYSIOLOGY

Total Points: 91

Duration: 90 minutes

Dear Participants, 敬愛的參賽同學

- In this test, you have been given the following 2 tasks:

  本測驗包含兩大題

  Task 1: Study on the cell cycle (61 points)

  第一大題：細胞週期的研究 (61 分)

  Task 2: Study on the motile mechanism of unicellular algae (30 points)

  第二大題：單細胞藻類運動機制的研究 (30 分)

- **You must write down your results and answers in the ANSWER SHEET. Answers written in the Question Paper will not be evaluated.**

  你必須將結果與答案回答於『答案紙』上，在試題卷上作答將不予計分。

- Please make sure that you have received all the materials and equipment listed for each task. If any of these items are missing, please raise your hand.

  請確定你每題的實驗設備與材料都是完整的。如果有缺少任何物品，請**『舉手』**。

- At the end of the test, put the Answer Sheet and Question Paper in the envelope. The supervisor will collect this envelope.
- 測驗結束後，請將答案紙與試題卷放入信封中，監試人員將會回收信封。

Good Luck!!

祝好運

Task 1 (61 points) 第一大題 (61 分)

Study on the cell cycle 細胞週期的研究

Introduction

In many unicellular organisms, gene duplication and segregation occur in a controlled manner as the cell body grows. When the environmental conditions in which cells are growing become less favorable or stressful, genetic exchange is often seen via cell conjugation (mating) between cells of different mating types. That phenomenon is essential for life and is controlled by both internal and external condition of the cells. To date, we have tried to reveal these mechanisms by studying mutants in several model organisms. For example, the investigation of mutants in the fission yeast, *Schizosaccharomyces pombe* has provided us with invaluable information. Wild-type *S. pombe* cells proliferate by repeated cell elongation followed by symmetric cell division. On the other hand, under stressful conditions such as starvation, cells undergo arrested growth at an appropriate stage of the cycle, and spore formation is induced via cell conjugation to overcome the stressful conditions.

The following task involves examining cell proliferation using *S. pombe*.

簡介

在許多的單細胞生物中，基因的複製與分離會受到細胞生長所控制。當外界環境改變時，例如不適細胞生長或逆境發生時，遺傳物質的互換會藉由不同交配型的細胞進行，如接合生殖 (cell conjugation [mating])。這些生命的基本現象會受到細胞本身與細胞外條件所控制。爲了瞭解這些現象，科學家會藉由許多模式生物的突變種來進行研究。關於細胞分裂的研究能藉由酵母菌 (*Schizosaccharomyces pombe*) 的突變種來進行。野生型的酵母菌，細胞分裂會藉由重複性的細胞增長而達成。另一方面，當細胞遭逢逆境時，如飢餓，會讓細胞停留在週期的某個階段。或者是當接合生殖完成後會有孢子形成。

接下來有關細胞增殖的實驗，都以酵母菌 (*S. pombe*) 作為實驗材料。

Materials and equipment 材料與設備	Quantity 數量
1. Fixed culture of wild-type strain; a 固定過的野生種； a	1
2. Fixed culture of wild-type strain; b 固定過的野生種； b	1
3. Fixed culture of wild-type strain; c 固定過的野生種； c	1
4. Fixed culture of wild-type strain; d 固定過的野生種； d	1
5. Micro tube stand 微量試管架	1
6. Microscope 顯微鏡	1
7. Disposable cell counter 細胞盤	1
8. Counter 計數器	1
9. 1.5ml microtube 1.5ml 微量吸管	3
10. Box of glass slides 載玻片盒	1
11. Box of coverslips 蓋玻片盒	1
12. Micropipette P-20 (capacity 2-20μL) 微量吸管 (2-20 μl)	1
13. Box containing micropipette tips 微量吸管頭盒	1
14. Fixed culture of wild-type strain incubated at 25° C; W25 已經固定過的 25°C 下野生種；W25	1

15. Fixed culture of wild-type strain incubated at 36° C; W36 1

已經固定過的 36ºC 下野生種；W36

16. Fixed culture of *cdc25* mutant strain incubated at 25° C; M25 1

已經固定過的 25ºC 下 cdc25 突變種；M25

17. Fixed culture of *cdc25* mutant strain incubated at 36° C; M36 1

已經固定過的 36ºC 下 cdc25 突變種；M36

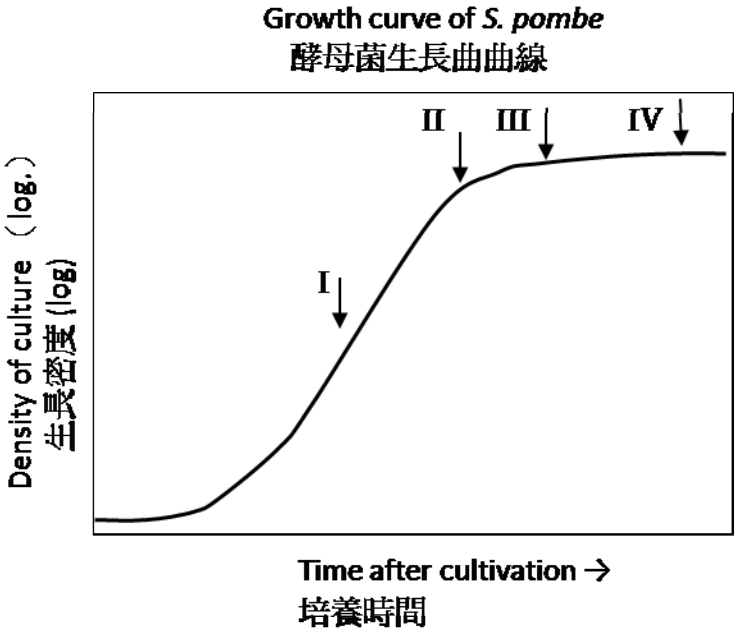
18. Photograph of cells stained with Calcofluor and DAPI 1

染過 Calcofluor 與 DAPI 的細胞照片。

Part A

The growth curve of *S. pombe* wild-type haploid (n=1) incubated at 25° C is shown below. Sampling of culture medium has been carried out at time points indicated by an arrow. Culture media a, b, c and d on the bench correspond to a sample of the culture taken at a certain time of cultivation I, II, III or IV. Observe each of the media with a microscope, and answer the following questions. Please stir the microtube just before observation.

酵母菌野生型單倍體 (n=1) 在 25ºC 下培養的生長曲線，如下圖。在箭頭所示的四個時間點，分別取出培養基。培養基 a, b, c 與 d，將分別與時間點 I, II, III 或 IV 進行配對，**但並非培養基 a 一定對應時間點 I，以下雷同。**利用顯微鏡觀察培養基，並回答下列各題。觀察前請將微量試管混合均勻。



Q.1.A.1. (2x2 points) Compare the cells in sample a with those in sample b. And answer the following questions

觀察並比較樣本 a 與 b。回答下列問題。 (2x2 = 4 分)

1 In which samples are the cells rounder?
何種樣本細胞較圓？

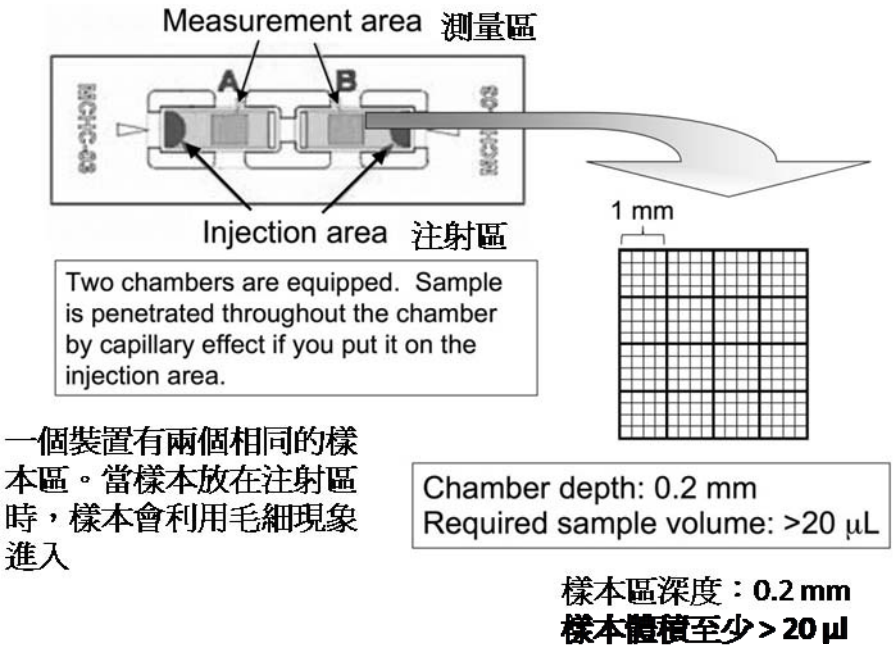
2 In which samples is there a higher population of cell undergoing cytokinesis?
何種樣本細胞有較高的細胞質分裂族群？

Cytokinesis is defined as the part of the cell cycle from initiation of septum formation to the separation of daughter cells.
細胞質分裂的定義自隔壁 (septa) 出現開始到分成兩個子細胞爲止的時間。

Q.1.A.2. (6 points) Measure the number of cells per 1 ml culture medium in sample a by using the cell counter as indicated below. Daughter cells that have not separated should be counted as a single cell. Write your Answer on the Answer Sheet. Notice that each student has received one cell counter but each counter has two counting chambers. You can make two measurements with this counter.

依照下列的指示，計算 樣本 a 中，每 ml 培養基中的細胞數目。未分裂完成的細胞只能計算爲一個。將答案寫在答案紙上。

注意：一位學生只有一個細胞計數盤，每個細胞計數盤有兩個樣本區，因此可以計算兩次。(6 分)



Q.1.A.3. (5 points) Measure the percentage of cells undergoing cytokinesis in the culture medium in sample a. You should count more than 100 cells in total by choosing several optical fields at random. You must write the percentage of cells undergoing cytokinesis AND the total number of cells you counted on the Answer Sheet.

細胞質分裂的定義自隔壁 (septa) 出現開始到分成兩個子細胞爲止的時間，計算 樣本 a 的細胞質分裂百分率。在視野下，至少要計算超過 100 個細胞，並將計算的細胞總數與細胞質分裂的百分率寫在答案紙上。

Q.1.A.4. (4 points) Estimate the time period required for one round of the cell cycle of cells in logarithm of sample a, provided that it takes 25 min from the beginning of septum formation to the separation of the daughter cells. Enter both the formula and your answer in the Answer Sheet.

估算 樣本 a 在對數期時，完成一個細胞週期所需的時間。
若自隔壁 (septa) 形成開始到完全分裂成兩個子細胞所需的時間爲 25 分鐘，請估算 樣本 a 的細胞週期時間爲何？請將計算公式與答案寫於答案紙上。

Q.1.A.5. (3 points) What applies to the cells in culture medium c?

樣本 c 爲下列何種時期？

A vigorously growing 快速生長期

B forming spores 孢子形成期

C conjugating 接合生殖期

D most of cells are dead 大多數的細胞已經死亡

E undergoing meiosis 進行減數分裂

Q.1.A.6. (8 points) Which culture medium (I, II, III, or IV.) corresponds to a, b, c and d, respectively?

請將培養基 a, b, c 與 d，將分別與時間點 I, II, III 或 IV 進行配對。將正確的答案填於答案紙上。(8 分)

Part B

Both wild-type and *cdc25*-mutant strains were incubated at 36° C for 4 hrs after logarithmic growth at 25° C.

分別將培養於 25°C 下對數期生長的野生型與 *cdc 25* 突變種移到 36°C 下培養 4 小時。

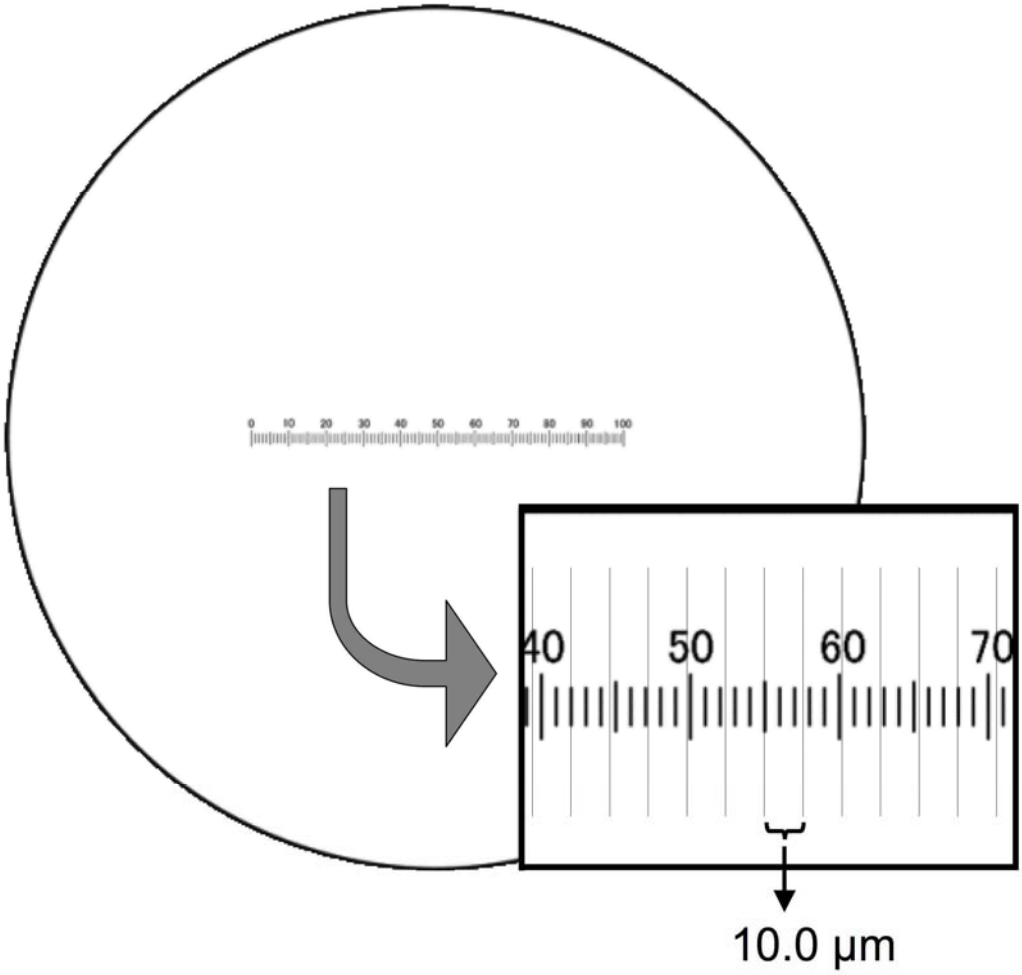
Q.1.B.1.(3 points) By observing the phenotypes of the cultures W25, W36, M25 and M36, what can we conclude?

觀察 W25, W36, M25 與 M36 表現型後，可以得到下列何種結論。(3 分)

	Condition 條件	Most of *cdc25* mutant cells 大部分的 *cdc 25* 突變種	Wild type cells 野生型
A	25° C	Do not undergo cytokinesis 未進行細胞質分裂	Undergo cytokinesis 進行細胞質分裂
B	25° C	Undergo cytokinesis 進行細胞質分裂	Do not undergo cytokinesis 未進行細胞質分裂
C	36° C	Do not undergo cytokinesis 未進行細胞質分裂	Undergo cytokinesis 進行細胞質分裂
D	36° C	Undergo cytokinesis 進行細胞質分裂	Do not undergo cytokinesis 未進行細胞質分裂
E	25° C and 36° C	No significant difference in cytokinesis between *cdc25* mutant and wild type cells 兩者無明顯差異	

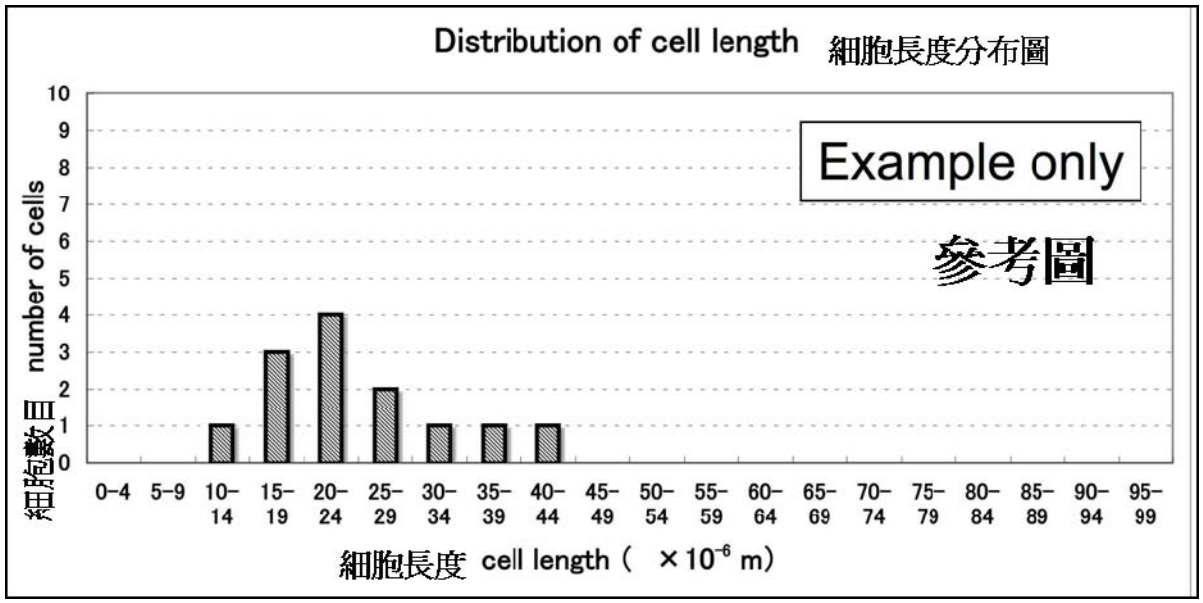
Q.1.B.2.(4 points) To measure cell length, your microscope is equipped with a micrometer in the eyepiece lens. In order to calibrate the eyepiece micrometer, a second micrometer, called the stage micrometer, is place on the stage of the microscope. The distance between any two adjacent lines on the stage micrometer is known to be 10.0μm. By matching the lines on both micrometers, we can determine the distance between two adjacent lines of the eyepiece micrometer. Determine this distance in μm to two decimal places using the figure shown below.

爲了測量細胞長度，目鏡中已被置入目鏡測微器 (micrometer)。爲了要校正目鏡測微器， 須將載物台測微器放在載物台上，載物台測微器每小格的距離爲 10 μm。當兩種測微器的線條相重疊後，便能知道目鏡測微器的每格長度。參考下圖的量測結果。請以 μm 爲單位，進行目鏡測微器的校正，計算目鏡測微器每小個的長度到小數點後兩位。(4 分)



Q.1.B.3.(12 points) Measure the longitudinal length of more than 10 cells selected at random in culture media of M36. Graph your results in the Answer Sheet according to the example indicated below. The scale of your eyepiece micrometer is 4μm. Do not forget to indicate the unit of length.

量測 M36 中細胞長度，至少量測 10 個細胞以上。依照下列參考圖作法，將你的量測結果於答案紙中作圖。目鏡測微器每格長度爲 4μm。記得要標記單位。(12 分)



Q.1.B.4.(2 points) What can you conclude from your observations of each culture?

cdc25 cells are longer than wild-type cells at:

在觀察完所有樣本後， *cdc25* 突變種的細胞較野生型細胞爲長，會出現於下列何種情形？(2 分)

A both 25° C and 36° C.

B 36° C but not 25° C.

C 25° C but not 36° C.

D There is no significant difference in cell length between wild-type and *cdc25* cells at both 25° C and 36° C. 無顯著差異存在

Part C

The following experiment was done using wild-type cells and 5 mutant strains (A-E). These mutant strains grow at 25° C as well as wild-type cells but are not able to grow at 36° C.

All cells undergoing logarithmic growth at 25° C were then incubated at 36° C for an additional 4 hrs before chemical fixation. Fixed cells were stained with both Calcofluor (stains septa) and DAPI (stains DNA) for observation using fluorescence microscopy (as seen in the photograph provided on the bench).

以下的實驗將使用野生型與 5 種突變種 (A-E)進行。所有的突變種均可生長於 25° C，並且與野生型無差異，但是無法生長於 36° C。所有的細胞都先培養於 25°C 下達到對數期生長，再移到 36°C 下培養 4 小時後，進行固定。固定後的細胞，將以 Calcofluro (染色對象爲隔壁 [septa])與 DAPI (染色對象爲 DNA)，並經由螢光顯微鏡觀察後，拍照如實驗桌上的圖所示。

Q.1.C.1.(10 points) The following statements describe the phenotype of the mutants incubated at 36° C. Identify the descriptions that correspond with each of the mutant strains (A-E), respectively.

有關突變種在 36°C 下培養後的型態描述，請將下列的描述與突變種 A-E 進行配對。(10 分)

1. Cytokinesis is repeated independently of progression of the cell cycle.
   細胞質分裂重複且獨立進行中
2. Cell cycle progresses but cytokinesis has not begun.
   細胞週期持續但缺乏細胞質分裂
3. Cell cycle is arrested at interphase.
   細胞週期被限制在間期
4. Karyokinesis is severely defective.
   缺少核分裂
5. Completion of cytokinesis is suppressed.
   細胞質分裂受阻

Task 2 (30 points) 第二大題 (30 分)

Study on the motile mechanism of unicellular algae

單細胞藻類運動機制的研究

Introduction

Some unicellular algae and zygotes of multicellular algae swim actively. This behavior is important for migration to appropriate conditions for growth and sexual reproduction. *Chlamydomonas reinhardtii*, an unicellular green alga, swims using flagella movement. Flagella often fall out when in contact with some stimuli, and some are absorbed into the cell body at a specific stage of the cell cycle.

This task concerns the machinery of flagella movement and flagella regeneration in *C. reinhardtii*.

簡介

許多單細胞藻類與多細胞藻類的合子都具有游泳的能力。這種行爲對於移動到適當的環境生長與有性生殖是很重要的。*Chlamydomonas reinhardtii* 爲一種單細胞綠藻，會利用鞭毛進行游泳。鞭毛會因爲某些刺激而掉落，有時會在特定的細胞週期時縮入細胞內。

本實驗將利用 *C. reinhardtii* 爲材料進行鞭毛運動與再生的機轉研究。

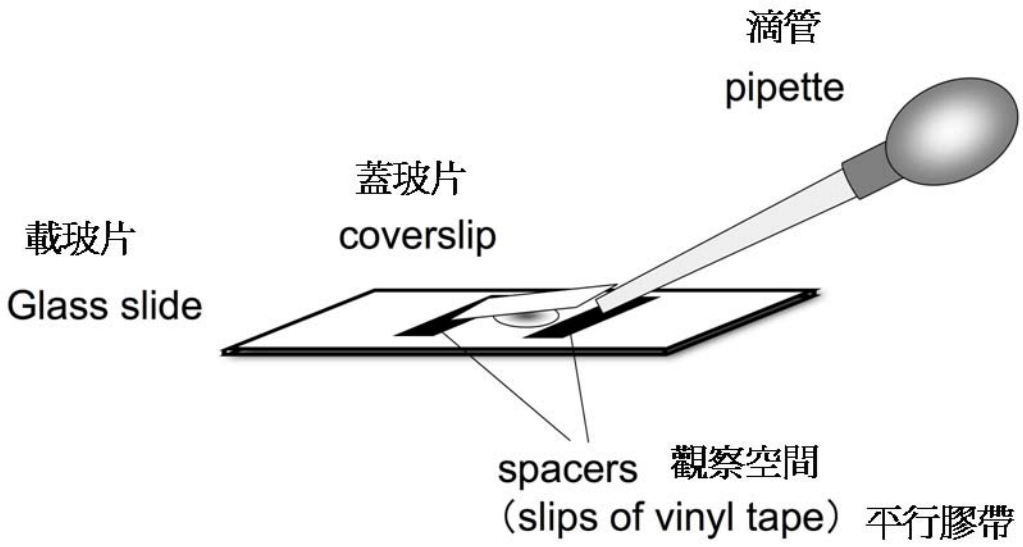
Materials and equipment 材料與設備	Quantity 數量
1. *C. reinhardtii* wild-type cells (wt) 野生種 (wt)	1
2. *C. reinhardtii oda1*mutant (oda) *oda1* 突變種 (oda)	1
3. *C. reinhardtii pf17* mutant (pf) *pf17* 突變種 (pf)	1
4. Microscope 顯微鏡	1
5. Box of glass slides 載玻片盒	1
6. Box of glass coverslips 蓋玻片盒	1
7. Acetic acid solution (A) 醋酸溶液 (A)	1
8. Neutralizing solution (N) 中和溶液 (N)	1
9. Disposable pipette（1 ml） 拋棄式滴管 (1 ml)	10
10. 1.5 ml microtube 微量試管	5
11. Vinyl tape 膠帶	1
12. Scissors 剪刀	1

Caution

C. reinhardtii flagella frequently stick to glass slides. As a result, the swimming ability of the cell is hindered. Therefore, cells immobilized on a glass slide should be excluded from observations for cell movement. It is recommended to make a chamber as indicated below for the observation. Slips of vinyl tape are stuck on a glass slide in parallel, and a coverslip is mounted on the slips after the samples are loaded by pipette. This chamber will provide a space for the cells to swim.

注意

C. reinhardtii 的鞭毛很容易黏在載玻片，這樣就很容易影響到游泳能力的觀察。因此，在載玻片上無法運動的細胞應該不列入計算。因此建議在此製造一個觀察室 (如下圖) 進行實驗。作法是將一小片的膠帶平行地貼在載玻片上，蓋玻片可以在添加樣本後，再蓋在膠帶上。因此將會創造出一個空間進行游泳行爲觀察。



Part A

Microscopically compare the wild-type (wt) and *pf17* mutant (pf) cells. This mutant has a normal shape and cellular structure but lacks a component of the radial spoke head in its flagella.

在顯微鏡下比較觀察野生型 (wt) 與 *pf17* 突變種 (pf) 的細胞，突變種細胞具有正常的形狀與細胞構造，唯獨在鞭毛上缺乏環狀幅射頭 (radial spoke head)。

Q.2.A.1. (6 points) In comparison to wild-type cells, *pf17* mutant cells:

與野生型比較，*pf17* 突變種具有：(6 分)

A swim in the same manner 相同的游泳模式

B swim but more slowly 游的比較慢

C swim but more rapidly 游的比較快

D do not swim at all 根本不會游泳

Q.2.A.2. (2 points) What can you conclude about the function of the radial spoke head?

請問環狀幅射頭 (radial spoke head) 的功能爲何？(4 分)

A essential for flagella movement 對鞭毛的運動很重要

B no effect on flagella movement 與鞭毛的運動無關

C suppresses flagella movement 抑制鞭毛的運動

D coordinates flagella movement 協調鞭毛的運動

Part B

Microscopically compare the wild-type (wt) and *oda1* mutant (od). This mutant lacks a kind of dynein in flagella whereas the shape and other cellular structures are normal.
在顯微鏡下比較觀察野生型 (wt) 與 *oda1* 突變種 (od)，突變種具有正常的形狀與細胞構造，唯獨缺乏鞭毛上的一種動力蛋白 (dynein)。

Q.2.B.1. (6 points)

In comparison to wild-type cells, *oda1* mutant cells swim:
與野生型比較，*oda1* 突變種的游泳模式爲：(6 分)

A in the same manner 與野生型相同

B more slowly and smoothly 較緩慢且平順

C more slowly and jerkily 較緩慢且抽動

D more rapidly and smoothly 較快速且平順

E rapidly and jerkily 快速且抽動

Q.2.B.2. (2 points)

What can you conclude about the function of the dynein lost in the *oda1* mutant?
請問動力蛋白 (dynein) 的功能爲何？(4 分)

A essential for flagella movement 對鞭毛的運動非常重要

B no effect on flagella movement 與鞭毛的運動無關

C increase flagella movement 增加鞭毛的運動

D coordinates flagella movement 協調鞭毛的運動

Part C

Study the effect of acetic acid on flagella as follows.

(i) Measure the percentage (A) of wild type cells with flagella in 20 cells.

(ii) Transfer about 1 ml of the culture selected in (i) into a 1.5 ml microtube by disposable pipette, and add one drop of acetic acid solution

(iii) Add one drop of neutralizing solution after 30 seconds

(iv) Measure the percentage (B) of cells containing flagella in 20 cells after the treatment

醋酸對鞭毛影響的研究 操作步驟

(i) 選取 20 個野生型細胞，計算其中具有鞭毛的細胞百分率 (A)。

(ii) 用拋棄式滴管由 (i) 中吸取 1ml 的細胞培養液，置入 1.5 ml 的微量試管中，並滴入一滴醋酸溶液。

(iii) 靜置 30 秒，加入一滴中和溶液。

(iv) 經上述處理後，任選 20 個細胞，計算其中具有鞭毛的細胞百分率 (B)。

Q.2.C.1.((4 points x 2)=8 points) Calculate the percentage of cells containing flagella in the pretreatment (A) and posttreatment (B) samples.

計算處理前之樣本 (A) 及處理後之樣本 (B)，其中所含具有鞭毛的細胞百分率

Part D

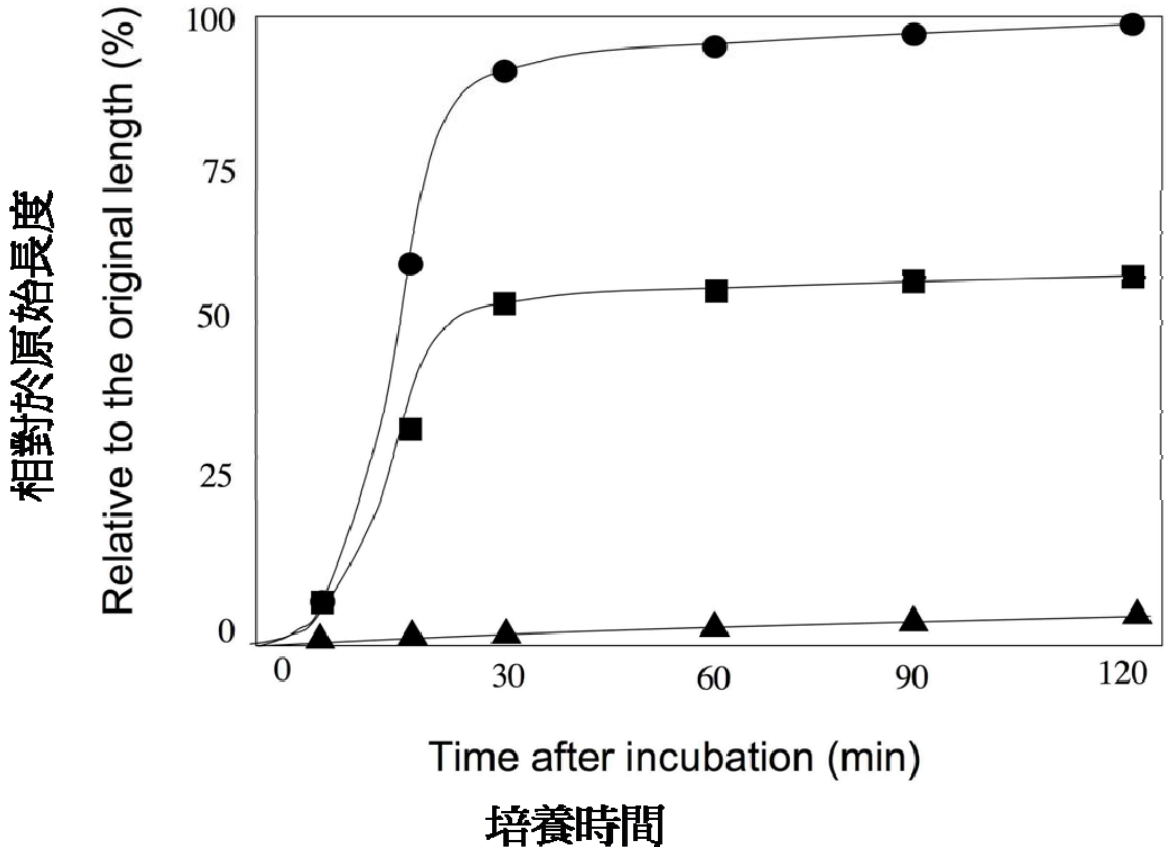
Wild-type cells with their flagella removed were incubated under different conditions (i, ii or iii). The following graph indicates the flagella length relative to its original length at different time points.

去除鞭毛後的野生型細胞培養於不同環境 (i, ii 或 iii) 中。下圖爲不同條件下，不同時間，相對於原始鞭毛長度的百分率圖。

(i) control (incubated without inhibitors)（●）
對照組 (未添加抑制劑)

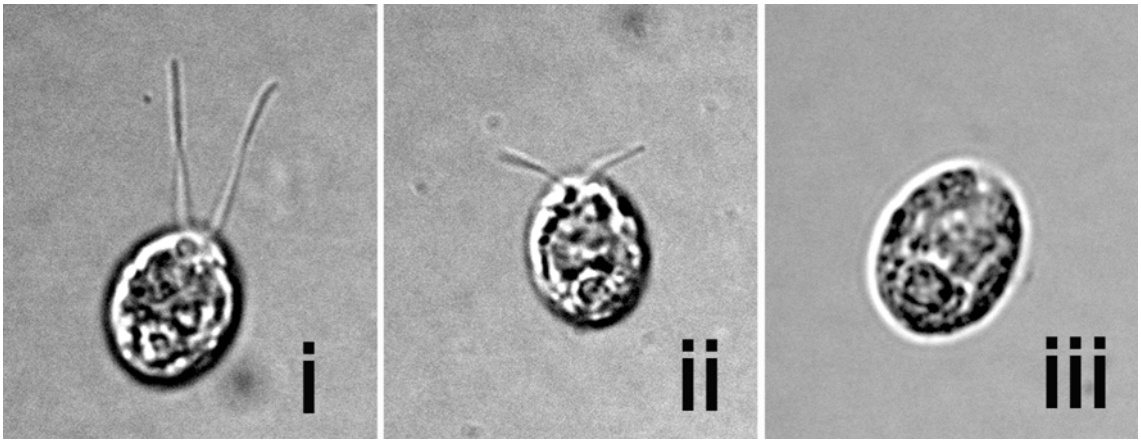
(ii) incubated with a high cycloheximide, an inhibitor of protein synthesis（■）
添加放線菌酮 (cycloheximide) (一種蛋白質合成抑制劑)

(iii) incubated with colchicine, an inhibitor of microtubule formation （▲）
添加秋水仙素 (colchcicine) (一種阻止微小管形成藥物)



In addition, photographs of cells after incubation for 120 min are shown.

經過 120 分鐘培養後，分別拍攝細胞型態如下圖：



Q.2.D.1.(4 points) Are the following statements supported by observing the results of cells incubated with cycloheximide? Put a cross mark (x) in the appropriate boxes in the answer sheet.

下列答案何者爲細胞培養於放線菌酮 (cycloheximide) 中的結果。請在 **適當** 的答案處打『X』。(5 分)

1 All proteins incorporated in regenerated flagella are synthesized *de novo*
所有參與鞭毛再生的蛋白質均爲重新合成 (*de novo*)

2 Regenerated flagella show no motility because of a lack of dynein
所有再生的鞭毛均不具運動能力，因爲缺乏動力蛋白 (dynein)

3 *De novo* synthesis of protein is essential for the complete regeneration of flagella.
蛋白質重新合成對於鞭毛的再生是必須的

4 *De novo* synthesis of protein is essential for the formation of the basal body of flagella
蛋白質重新合成對於形成鞭毛的基體 (basal body) 是必須的

Q.2.D.2.(2 points) Based on your observations of cells incubated with colchicines, what is required for the regeneration of flagella?

根據你的觀察，培養在秋水仙素 (colchicines) 中的細胞，下列何者與鞭毛再生有關？(2分)

A Polymerization of tubulin 微管蛋白的聚合作用

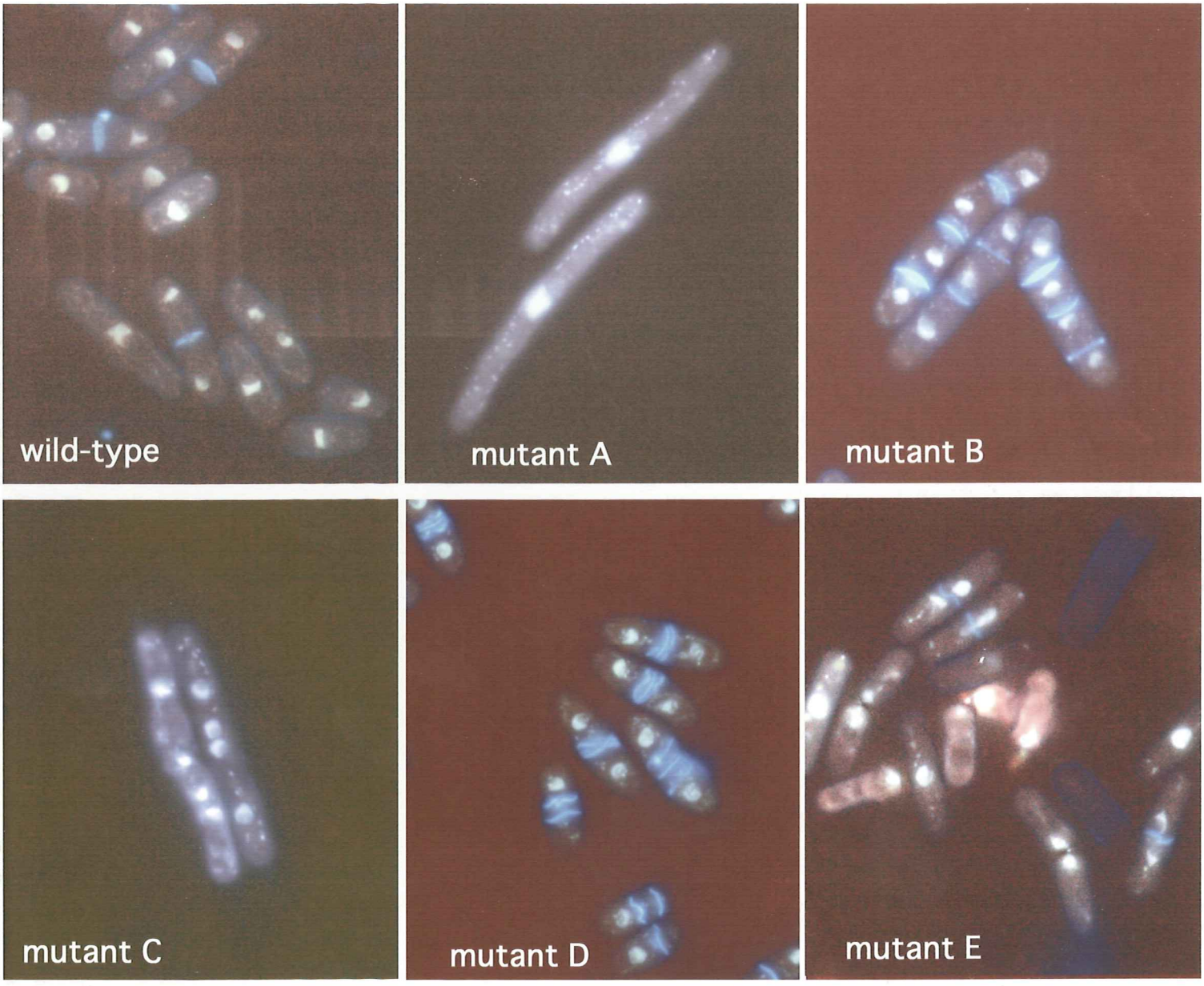
B Polymerization of actin 肌動蛋白的聚合作用

C Polymerization of keratin 角蛋白的聚合作用

D Depolymerization of tubulin 微管蛋白的去聚合作用

E Depolymerization of actin 肌動蛋白的去聚合作用

F Depolymerization of keratin 角蛋白的去聚合作用


wild-type
mutant A
mutant B
mutant C
mutant D
mutant E