

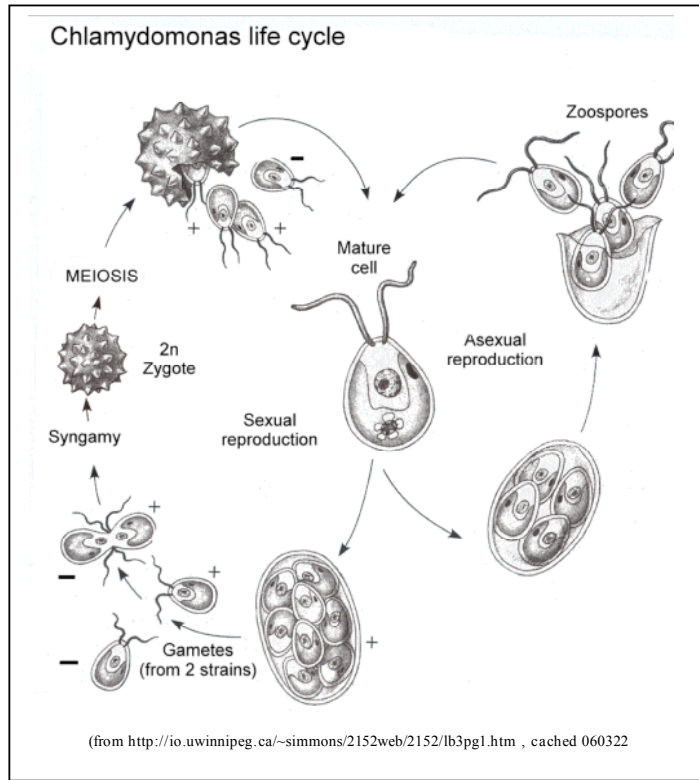
Modeling the Organism: The Cell in Development

Laboratory 3: Cell-cell interactions and mating in *Chlamydomonas*

Introduction

Cells of the green alga *Chlamydomonas reinhardtii* come in two mating types, plus (mt+) and minus (mt-). During vegetative growth (the “asexual reproduction” cycle on the right half of the figure), haploid cells increase in mass during the day (when they are photosynthetically active) and then divide at night. A single cell undergoes multiple mitoses, resulting in 2^1 (2), 2^2 (4), or 2^3 (8) daughters, depending upon its initial size at dusk.

When starved for nitrogen, vegetative cells differentiate into reproductively competent gametes (the “sexual reproduction” cycle on the left half of the figure), initiating a genetic differentiation program that involves the expression of plus or minus agglutinins (defined by the cells mating type) and a fusion apparatus. Gametes do not grow or divide but seek out cells of the opposite mating type. Mating type identity is established by regulatory proteins encoded in the mating type locus, a large stretch of DNA that is inherited in a simple Mendelian fashion. Plus *or* minus agglutinins expressed by the gamete are arrayed on the surface of its flagella and can be thought of as molecular Velcro. Plus agglutinins stick (or agglutinate) with minus agglutinins, but plus does not stick to plus and minus does not stick to minus. Thus, gametes of a single mating type do not interact, but when gametes of opposite mating types are mixed, we see the *Chlamydomonas* equivalent of a mosh pit (to cast it in technical lingo).



Mating of plus and minus gametes occurs following a complex choreography that begins with mass agglutination, followed by pair formation, cell wall shedding and cell fusion. Cellular events are orchestrated by an agglutination-triggered rise in cytoplasmic cAMP that triggers local actin polymerization near the base of the plus gamete's flagella to generate a mating structure that extends outward and contacts the surface of the minus gamete's membrane. Cell union occurs as the mating structures fuse, resulting in quadriflagellate (temporarily diploid) zygotes. These zygotes then swim to a surface where they anchor themselves, generate a thick spore-like wall and form a zygospor. The zygospor then becomes dormant until nitrogen again becomes available in the environment, at which point it completes meiosis, resulting in a tetrad of meiotic products (2 mt+ and 2 mt-), each of which germinates as a haploid cell to reenter the vegetative cycle.

Protocol:

Each table will be provided with several cultures of gametes designated by a culture number. One of these will also be marked with a “+” designation.

1. Select one of the gametic cultures and prepare a slide for examination under the microscope. Transfer 25 ul of the culture to the left half of a cleaned microscope slide (use a little 70% ethanol and a Kimwipe to clean the surface) and then gently cover with a coverslip. Locate the cells using the 10x (Ph1 condenser setting) or 20x (Ph2) objective and then increase to 40x (Ph3). Record your observations of your living cells in your notebook. Note if the cells are interacting with each other.
2. Next, prepare a fixed sample of cells by transferring 25 ul of pre-diluted Lugol’s solution (1:10) to a microfuge tube and then (with a fresh tip!) adding an equal volume of your culture. Lugol’s fixative is an iodine solution; it will permanently stain skin and clothes a dark brown (so handle carefully!).
3. Using the same slide as before, place 25 ul of the fixed cells on the right half of the slide and coverslip, being careful that the two specimens remain separate. Replace the slide on the microscope and examine the fixed culture. What is the effect of the Lugol’s solution on the cells? Count 50 cells, noting whether cells are singlets, doublets or clumps (>2 cells).
4. You will now set up a series of matings involving all the pair-wise combinations of gametes available at your bench. Rinse a 15 ml centrifuge tube with purified water (mating cells are very sensitive to contaminants) and transfer 250 ul of your culture into the washed tube. Next add 250 ul of a different culture (remember to mark each “mating” with the culture numbers and time of introduction). Mix gently by tapping the tube with your finger and then stand upright in a test tube rack. Prepare a slide and examine the live “mating” mixture for any change in motility (save the tube for later observations). Record your observations. Fix a 25 ul aliquot of the mating mixture with Lugol’s solution, and look for cell pairs/fusion (noting the time since cell mixing).
5. Repeat your observations of the mating mixtures at 30 min and 1 hour after the initial mating, examining the behavior of live cells as well as looking at fixed cells to survey for quadriflagellate zygotes (bearing two pyrenoids and two pairs of flagella). Based on your observations (agglutination, wall shedding, cell fusion), assign a mating type (relative to the designated mt+ tester strain) for each culture.
6. At the one hour timepoint, count 50 cells for each mating, selected at random (just move the stage right/left or up/down in a search pattern), as either biflagellate gametes (GAM) or quadriflagellate zygotes (QFZ) and calculate a mating efficiency using the following formula:

$$\text{Mating efficiency} = 2\text{QFZ}/(2\text{QFZ} + \text{GAM})$$

(Why is there a factor of “2” for the QFZ numbers?)

7. Compare your results with the other groups at your bench and discuss any differences in your observations and results.

Lab report:

You are responsible for submitting a one page lab “brief” describing your results from this experimental module (each student should do this for their own results; you are encouraged to discuss things with your partner and others in the lab but compose your own report). Please write a short paragraph introducing your data, present your results (using either a table or graph) and then close with a short summary of what you learned. This “brief” will be due next week at the start of lab.