Physiology and Cultivation of Algae and Cyanobacteria

1.

Definition

- **Algae** formal tax. standing, polyphyletic origin, artificial assemblage of O_2 evolving photosynthetic organisms
- **Algae *vs.* Plants**

the same storage compounds, similar defence strategies against predators & parasites

Plants

•hi degree of differentiation
•repr. organs surrounded by jacket of sterile cells
•multicell. embryo remains developmentaly & nutrit. independent on parents
•meristems on root/shoot apices
•digenetic life cycles with alterations betw. hapl. gametophyte & dipl. sporophyte

Algae

•don't have roots, stems, leaves, not well defined vasc. tissue
•don't form embryo
•mono- & digenetic life cycles
•occur in dissimilar forms (micro algae, macro a. multicellular, colonies, branched,…)
•less complexity of the thalli
•hi diversity
  - 0.2 – 60m
  - ecology & habitats
  - reserve & structural polysacharides
  - evolutionary origin

•1 – 10 mil. species
•½ primary production in biosphaera

Klasification

- under constant revision

(Van Den Hoek *et* al. 1995)

TABLE 1.1
Classification Scheme of the Different Algal Groups

Kingdom	Division	Class
Prokaryota eubacteria	Cyanophyta	Cyanophyceae
	Prochlorophyta	Prochlorophyceae
Eukaryota	Glaucophyta	Glaucophyceae
	Rhodophyta	Bangiophyceae
		Florideophyceae
	Heterokontophyta	Chrysophyceae
		Xanthophyceae
		Eustigmatophyceae
		Bacillariophyceae
		Raphidophyceae
		Dictyochophyceae
		Phaeophyceae
	Haptophyta	Haptophyceae
	Cryptophyta	Cryptophyceae
	Dinophyta	Dinophyceae
	Euglenophyta	Euglenophyceae
	Chlorarachniophyta	Chlorarachniophyceae
	Chlorophyta	Prasinophyceae
		Chlorophyceae
		Ulvophyceae
		Cladophorophyceae
		Bryopsidophyceae
		Zygnematophyceae
		Trentepohliophyceae
		Klebsormidiophyceæ
		Charophyceae
		Dasycladophyceae

Occurrence & distribution

Aquatic

- almost everywhere (from freshwater spring to salt lakes)
- tolerance of wide range of pH, temp., turbidity, O_2 & CO_2 conc.
- **planctonic**
  - » unicellular, suspended throughout lighted regions of all water (inc. polar ice)
- **benthic**
  - » within sediments
  - » limited to shallow areas (because of rapid attenuation of light with depth)
  - » attached to stones – **epilithic**, on mud/sand – **epipelic**
  - » on oter algae/plants – **epiphytic**, on animals – **epizoic**
  - marine benthic – after habitat
    - **supralitoral** – above high-tide level within reach wave spray
    - **intertidal** – exposed to tidal cycles
    - **sublitoral** – from extreme low-water to cca 200m deep
- ocean – 71% of earth surface, more than 5000 spec. of planctonic algae
  - **phytoplancton**
    - » base of marine food chain
    - » produce 50% of O_2 we inhale - life
    - » death – blooms – too large populations (decrease water transparency, prod. toxins & poisons)
  - **kelps**
    - » giant algae – temperate **pelagic** marine environment, till 60m submerged forests
    - » also beneath polar ice sheet
    - » can survive at very low depth
  - record of 268m u.s.l. – dark blue red algae (blue-green ligh, 0.0005% of surface intensity)
    - » have accesory pigments, chanel the energy to chl *a*
- accesory & protective pigments – give algae wide variety of colors <> names

Occurrence & distribution

Freshwater fytoplancton & benthic algae

» base of aquatic food chain

» not exhibit size range of marine relatives

Subaerial

» life on land

» tree trunks, animal fur, snow, hot springs, desert rocks

» activity – convert rock > soil

– to minimize soil erosion & increase water retention & nutrient availability for plants

Symbiosis

- lichens, corals

» to survive in environments that they could not alone

TABLE 1.2
Distribution of Algal Divisions

Division	Common Name	Habitat			
		Marine	Freshwater	Terrestrial	Symbiotic
Cyanophyta	Blue-green algae	Yes	Yes	Yes	Yes
Prochlorophyta	n.a.	Yes	n.d.	n.d.	Yes
Glaucophyta	n.a.	n.d.	Yes	Yes	Yes
Rhodophyta	Red algae	Yes	Yes	Yes	Yes
Heterokontophyta	Golden algae Yellow-green algae Diatoms Brown algae	Yes	Yes	Yes	Yes
Haptophyta	Coccolithophorids	Yes	Yes	Yes	Yes
Cryptophyta	Cryptomonads	Yes	Yes	n.d.	Yes
Chlorarachniophyta	n.a.	Yes	n.d.	n.d.	Yes
Dinophyta	Dinoflagellates	Yes	Yes	n.d.	Yes
Euglenophyta	Euglenoids	Yes	Yes	Yes	Yes
Chlorophyta	Green algae	Yes	Yes	Yes	Yes

Note: n.a., not available; n.d., not detected.

Structure o thallus

Unicells & unicell colonial algae

- solitary cells, unicells with/w-out flagela, motile (*Ochromonas*)/non-motile (*Nannochloris*)

- **colony**
  - aggregates of several single cells held together ±organized
  - grow – cell division
  - each cell can survive solely
- **coenobium**
  - colony with number of cells & arrangement determined at the time of origin (*e.g. Volvox* – motile, *Pediastrum* – non-motile)

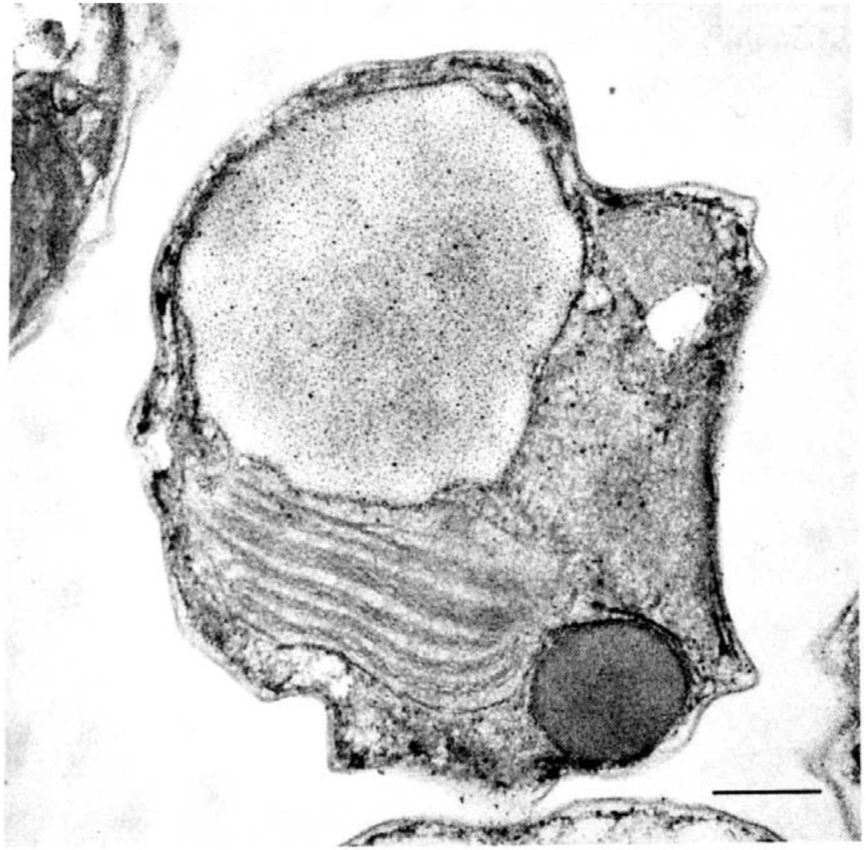


FIGURE 1.1 Transmission electron micrograph of *Nannochloropsis* sp., non-motile unicell. (Bar: 0.5 μm.)

FIGURE 1.2 *Ochromonas* sp., motile unicell. (Bar: 4 μm.)

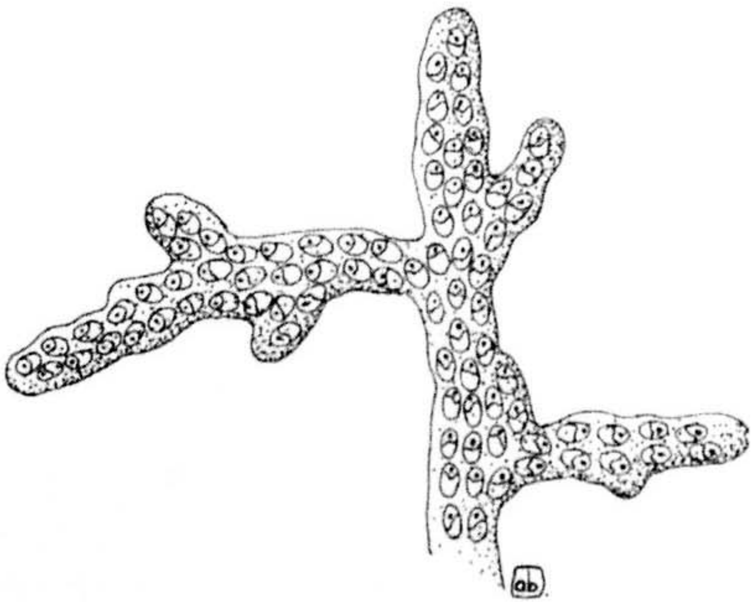


FIGURE 1.3 Non-motile colony of *Hydrurus foetidus.*

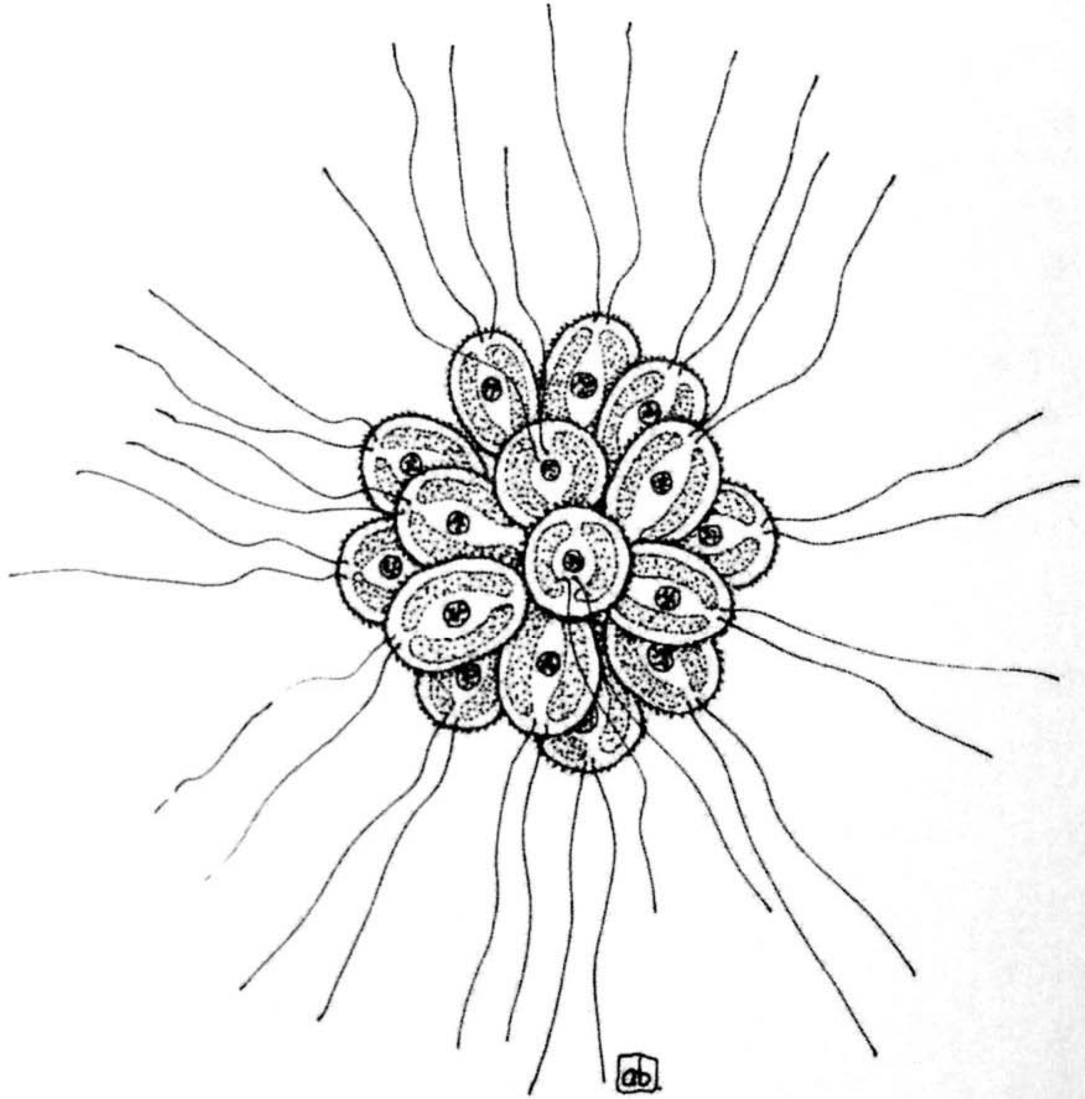


FIGURE 1.4 Free-swimming colony of *Synura uvella.*

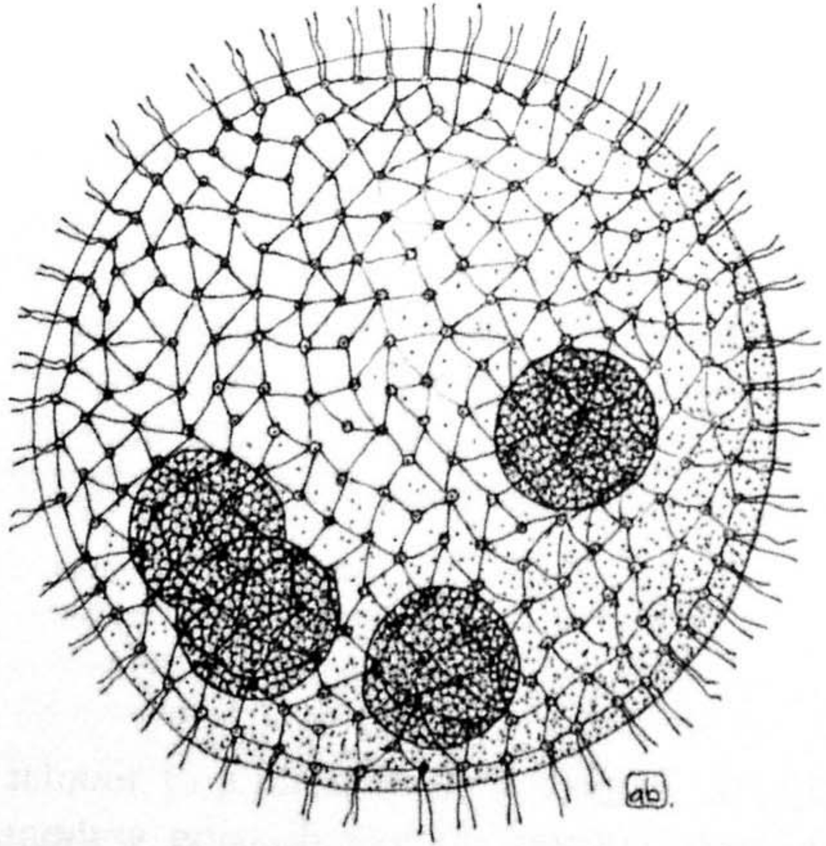


FIGURE 1.5 Motile coenobium of *Volvox aureus.*

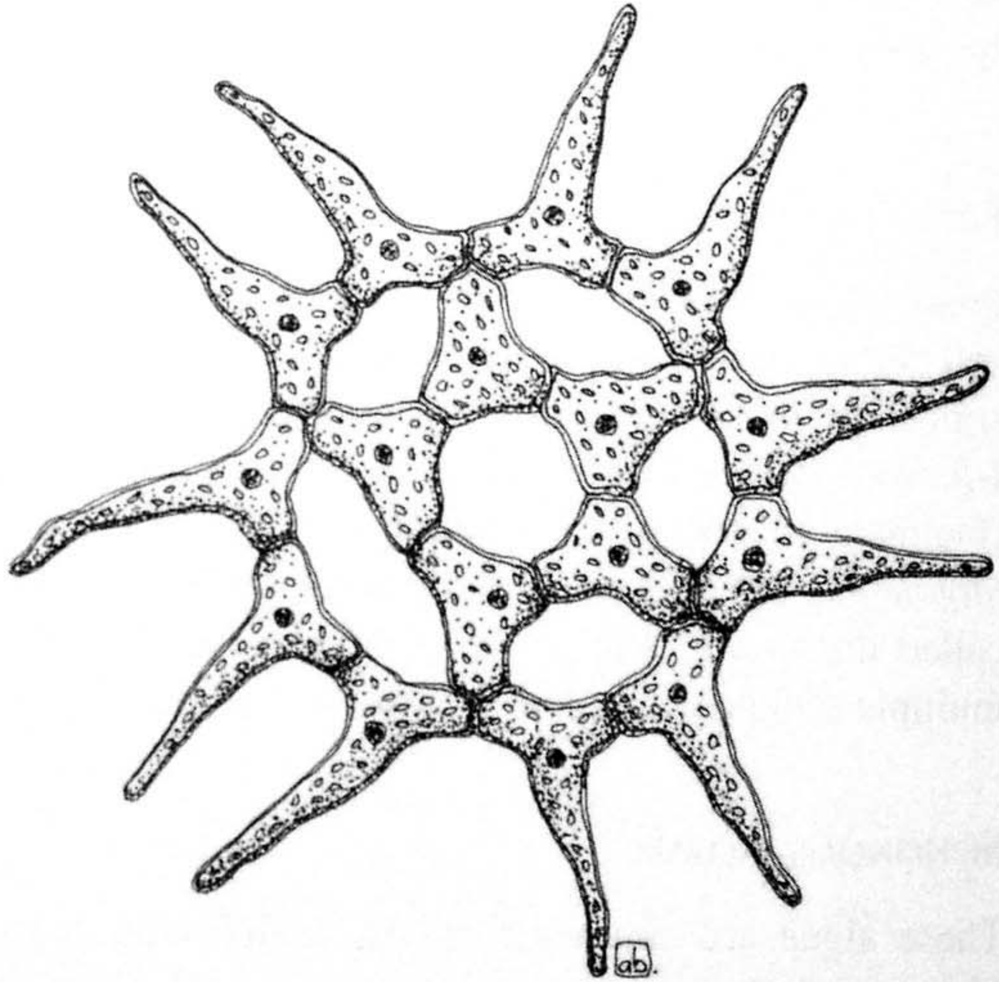


FIGURE 1.6 Non-motile coenobium of *Pediastrum simplex.*

Structure o thallus

Filamentous algae

- result from cell division in plane perpendicular to axis of filament – cell chain
  - ─**simple**
  - └**branched – true/false**
  - ─**uniseriate** – 1 layer of cells
  - └**multiseriate** – up to multiple layer

Syphonous algae

- siphonous/coenocytic construction of tubular filaments lacking transverse cell walls
- unicellular but multinucleate (coenocytic)

FIGURE 1.7 Simple filament of *Oscillatoria* sp.

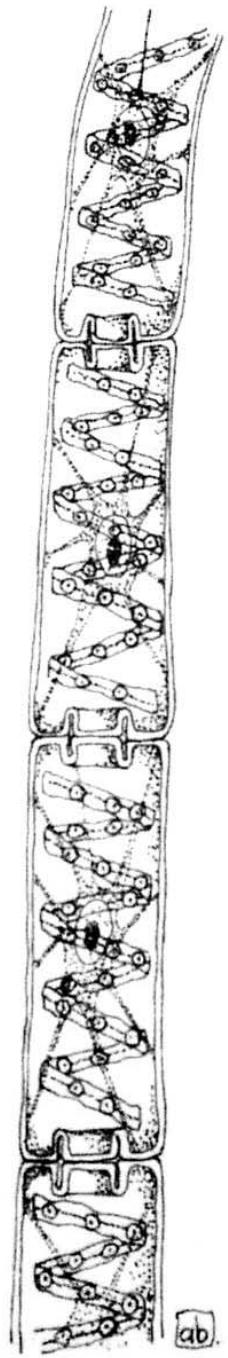


FIGURE 1.8 Simple filament of *Spirogyra* sp.

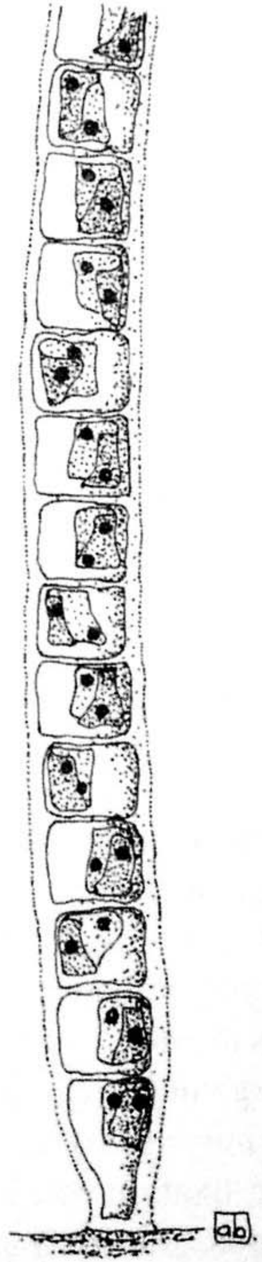


FIGURE 1.9 Simple filament of *Ulothrix variabilis.*

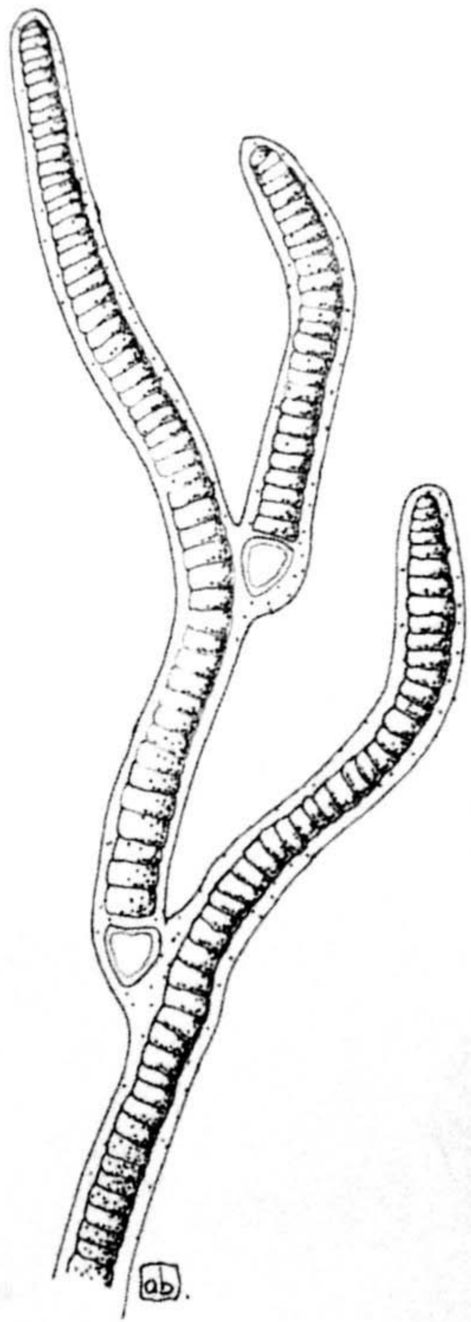


FIGURE 1.10 False branched filament of *Tolypothrix byssoidea.*

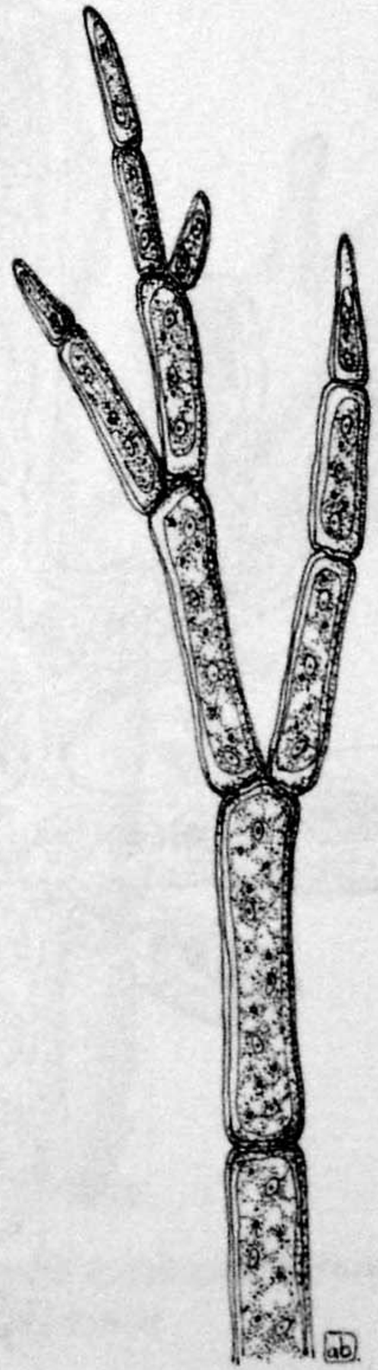


FIGURE 1.11 True branched filament of *Cladophora glomerata.*

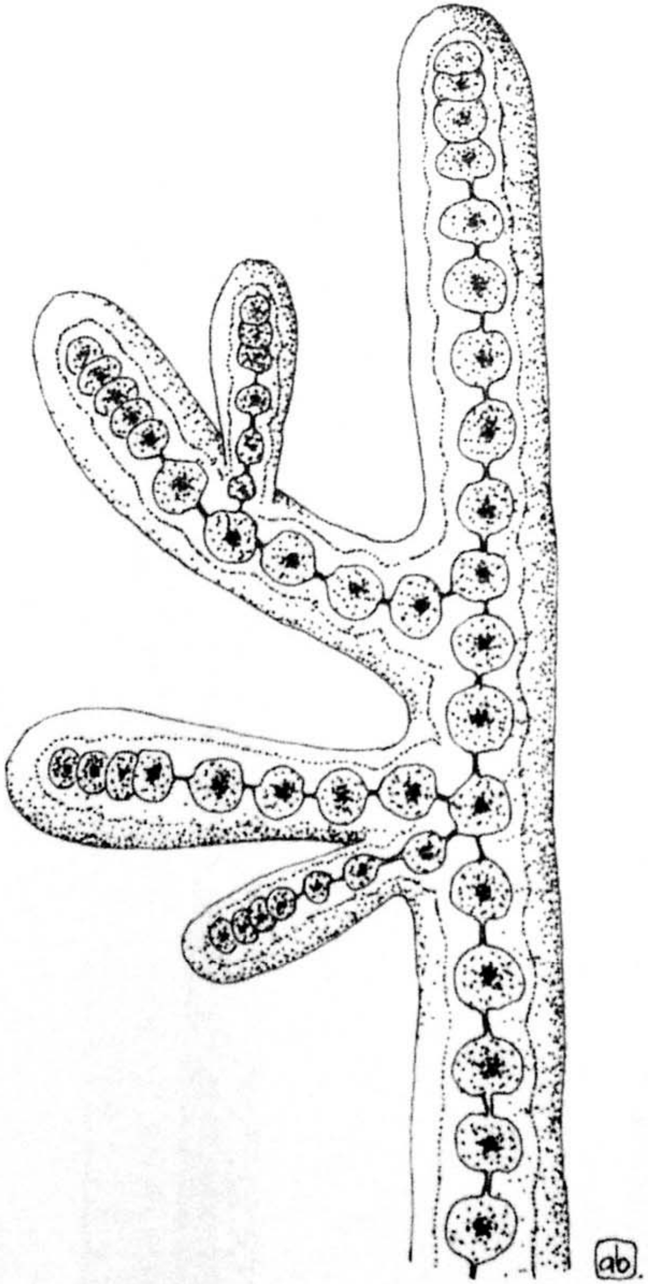


FIGURE 1.12 Uniseriate filament of *Stigonema ocellatum.*

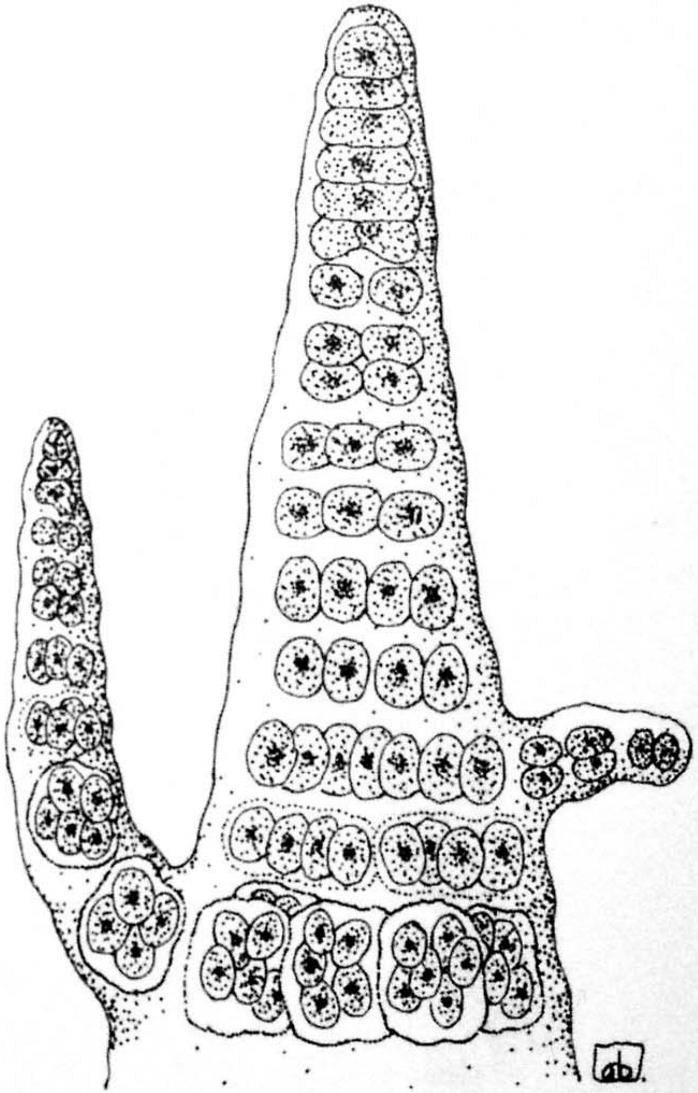


FIGURE 1.13 Multiseriate filament of *Stigonema mamillosum.*

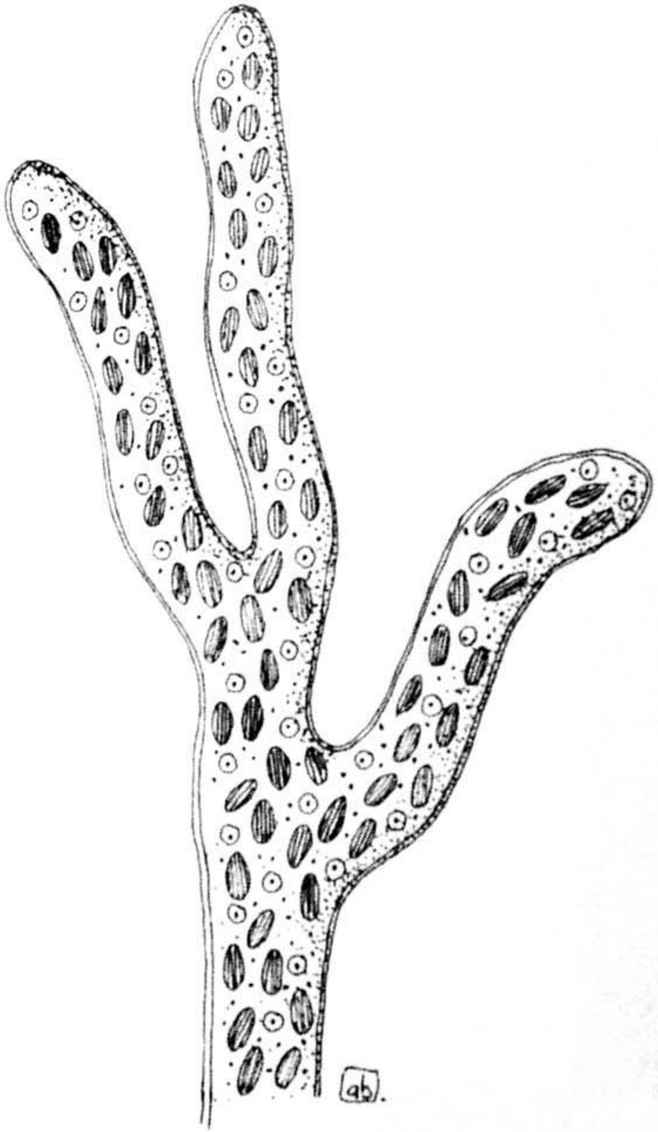


FIGURE 1.14 Siphonous thallus of *Vaucheria sessilis.*

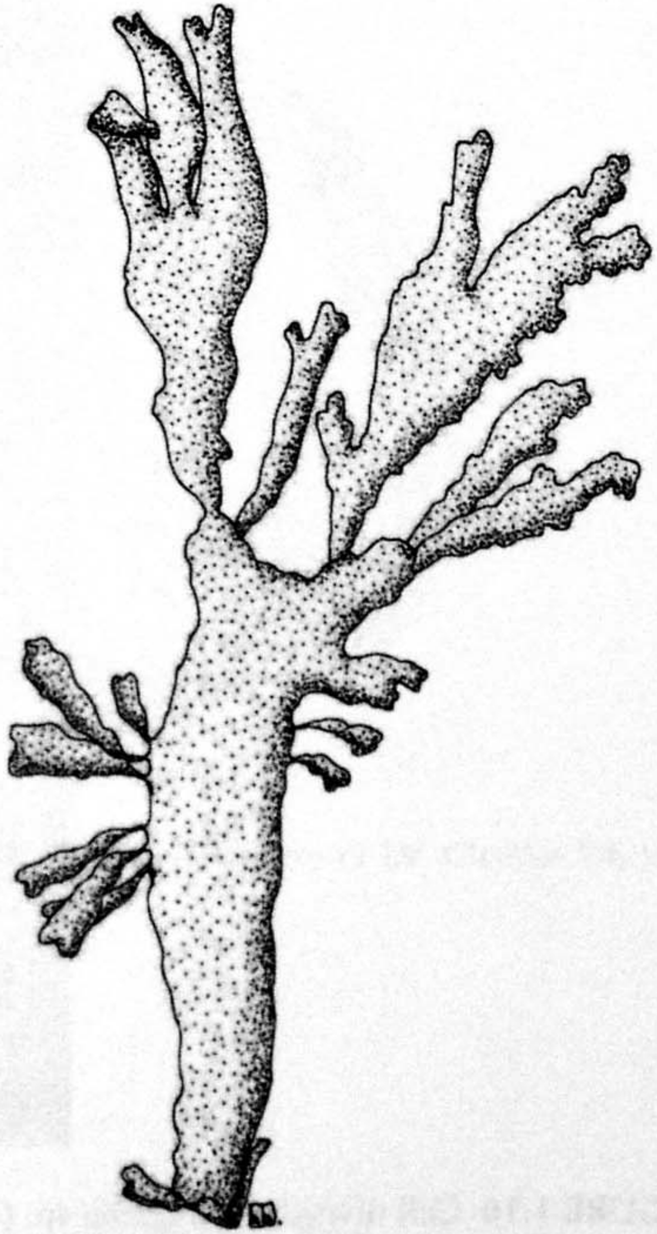


FIGURE 1.15 Pseudoparenchymatous thallus of *Palmaria palmata.*

Structure o thallus

Parenchymatous & pseudoparenchymatous algae

– mostly macroscopic

- **parenchymatous**
  - » originated from division of primary filament (all directions)
  - » lost filamentous structure
- **pseudoparenchymatous**
  - » originated from close aggregation of branched filaments, forming thallus held together with mucilages (red algae)

TABLE 1.3
Thallus Morphology in the Different Algal Divisions

Division	Unicellular and non-motile	Unicellular and motile	Colonial and non-motile	Colonial and motile	Filamentous	Siphonous	Parenche-matous
Cyanophyta	*Synechococcus*	n.d.	*Anacystis*	n.d.	*Calothrix*	n.d.	*Pleurocapsa*
Prochlorophyta	*Prochloron*	n.d.	n.d.	n.d.	*Prochlorothrix*	n.d.	n.d.
Glaucophyta	*Glaucocystis*	*Gloeochaete*	n.d.	n.d.	n.d.	n.d.	n.d.
Rhodophyta	*Porphyridium*	n.d.	*Cyanoderma*	n.d.	*Goniotricum*	n.d.	*Palmaria*
Heterokontophyta	*Navicula*	*Ochromonas*	*Chlorobotrys*	*Synura*	*Ectocarpus*	*Vaucheria*	*Fucus*
Haptophyta	n.d.	*Chrysochro-mulina*	n.d.	*Corym-bellus*	n.d.	n.d.	n.d.
Cryptophyta	n.d.	*Cryptomonas*	n.d.	n.d.	*Bjornbergiella*	n.d.	n.d.
Dynophyta	*Dinococcus*	*Gonyaulax*	*Gloeodinium*	n.d.	*Dinoclonium*	n.d.	n.d.
Euglenophyta	*Ascoglena*	*Euglena*	*Colacium*	n.d.	n.d.	n.d.	n.d.
Chlorarachniophyta	n.d.	*Chlorarachnion*	n.d.	n.d.	n.d.	n.d.	n.d.
Chlorophyta	*Chlorella*	*Dunaliella*	*Pseudo-sphaerocystis*	*Volvox*	*Ulothrix*	*Bryopsis*	*Ulva*

Note: n.d., not detected.

Nutrition

- algae = phototrophs
- most algal divisions – contain colorless heterotrophic spec.
  - **osmotrophy, phagotrophy**
  - **auxotrophy** – cannot synthesize essential components (vitamin B_{12}, fatty acids,..) and have to import them
- algae can use wide spectrum of <u>nutritional strategies</u> combining:
  - phototrophy
  - heterotrophy
  - mixotrophy (relative contribution of photo.&hetero. can vary)
    - often in extreme environment (limiting light,…)
  - after nutritional strategies:
    - **obligate heterotrophic algae** – primarily heterotrophs, but capable phototrophy in limiting prey concentration *(Gymnodium gracilentum - Dinophyta)*
    - **obligate phototrophic algae** – primarily phototrophs, but capable <u>phagotrophy/osmotrophy</u> when light is limiting *(Dinobryon divergens - Heterocontophyta)*
    - **facultative mixotrophic algae** – can equally well grow as photo-/heterotrophs *(Fragilidinium subglobosum - Dinophyta)*
    - **obligate mixotrophic algae** – primary mode is phototrophy & phago-&/osmotrophy provides essential substances *(e.g.*photoauxotrphs, *Euglena gracilis - Euglenophyta*)

Reproduction

- **vegetative** by division of single cell or fragmentation of colony
- **asexual** by production of motile spores
- **sexual** by union of gametes
  - vegetative & asexual
    - » allow stability of addapted genotypes from generation to the next
    - » fast & economical increase of number of individual
    - » lack genetic variability
  - sexual
    - » involves - **plasmogamy** (union of cells)
      - **karyogamy** (union of nuclei) - chromosome/gene association & meiosis >> genetic recombination
    - » allow variation, but is more costly

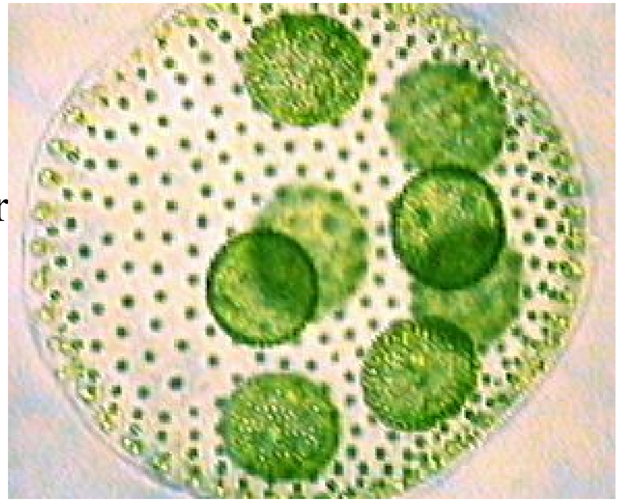
Vegetative & Asexual reproduction

- **Binary fission & Cellular bisection**
  - simplest form
  - parent org. divides into two equal parts of the same hereditary info as parent
  - unicellular a. - longitudinal
    - transverse
  - growth of population - lag > exponential > log > stationary (plateau) phase
  - in multicellular a. & colonies - leads to the growth of individual
- **Zoospore, Aplanospore & Autospore**
  - zoospores - flagelate motile spores that may be produced within parental vegetative cell *(Clamydomonas - Chlorophyta)*
  - aplanospores - aflagelate spores that begin their development within parent cell wall before being released
    - can dvelop into zoospores
  - autospores - aflagelate daughter cells released from ruptured cell wall of parental cell, - replicas of vegetative cells that produce them & lack the capacity to develop into zoospore *(Nannochloropsis - Heterocontophyta, Chlorella - Chlorophyta)*

spores - may be produced within - ordinary cells
- specialized sporangia

Vegetative & Asexual reproduction

- **Autocolony formation**
  - coenobium/colony - each cell can produce new colony similar to parent.
  - cell division produce multicellular group (not the unicellular individuals) > differs from the parent in cell size not in number *e.g. Volvox (Chlorophyta)*
    - gonidia - series of cells which produce a hollow sphaere within the hollow of parental colony (released after its ruptur



- **Fragmentation**
  - ± random process whereby non-coenobic colonies/filaments break into two/several fragments having capacity to develop into new individual

Vegetative & Asexual reproduction

- **Resting stages**
  - under unfavourable conditions (desiccation)
  - thick-walled cells
    - **hypnospores & hypnozygotes**
      - thick-walled, produced *ex novo* from cells previosly separated from parent cells
        - » **hypnospores** - *Ulothrix spp., Chlorococcum (Chlorophyceae)*
        - » **hypnozygotes** - *Spyrogyra spp. (Chlorophyceae), Dinophyta*
      - enables algae to survive temporary drying out of small water bodies & allow transport to another (*e.g.* via birds)
    - **statospores**
      - endogenous cysts formed within vegetative cells by members of *Chrysophyceae e.g. Ochromonas spp.*
        - » cyst walls consist of silica >> preserved as <u>fossils</u>
      - spherical, ellipsoidal, often ornamented with spines or other projections
      - wall - with pores sealed by unsilicified bung
      - within cysts lie nucleus, chloroplasts, reserve material
      - after dormancy - germination - form one/several flagelate cells
    - **akinetes** occurrence in blue-green algae
      - enlarged vegetative cells that develop thickened wall in response to limiting env. nutrients or light *(e.g. Anabaena cylindrica - Cyanophyta)*
      - extremely resistant to drying & freezing
      - long-term anaerobic storage of genetic material, remain viable in sediments for many years in hard conditions
      - in suitable conditions > germination into new vegetative cells

Sexual reproduction

Gametes

- morphologicaly identical/different with/from vegetative cells (a. group speciphic sign)
- haploid DNA content
- possible different gamete types

• **isogamy** - both gametes types motile & indistinguishable

• **heterogamy** - gametes differ in size

» **anisogamy** - both gametes are motile, 1. small - sperm
2. large - egg

» **oogamy** - 1. motile, small - sperm
2. non-motile, very large - egg

Algae exhibit 3 different life cycles with variation within differrent groups

• main difference - where meiosis occure & type of cells it produces & whether there is more than one free-living stages

Sexual reproduction

Haplontic or zygotic life cycle

- single predominant haploide vegetative phase, with meiosis after germination of zygote
  - » *Chlamydomonas (Chlorophyta)*

Diplontic or gametic life cycle

- single predominant diploid vegetative phase
- meiosis gives rise to haploid gametes
  - » *Fucus (Heterocontophyta), Diatoms*

Haplontic or zygotic life cycle

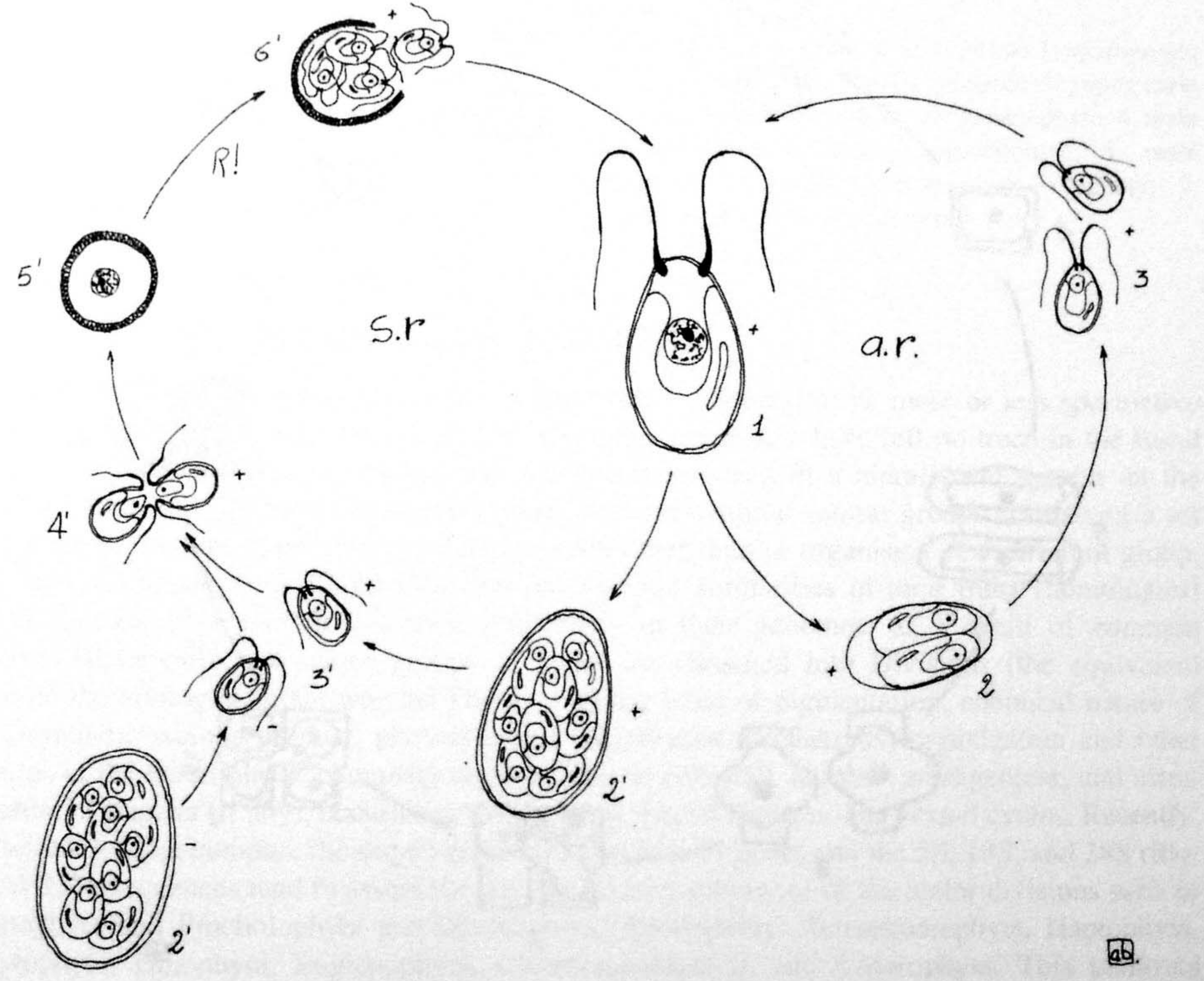


FIGURE 1.19 Life cycle of *Chlamydomonas* sp.: 1, mature cell; 2, cell producing zoospores; 2′, cell producing gametes (strain+ and strain−); 3, zoospores; 3′, gametes; 4′, fertilization; 5′, zygote; 6′, release of daughter cells. R!, meiosis; a.r., asexual reproduction; s.r., sexual reproduction.

Diplontic or gametic life cycle

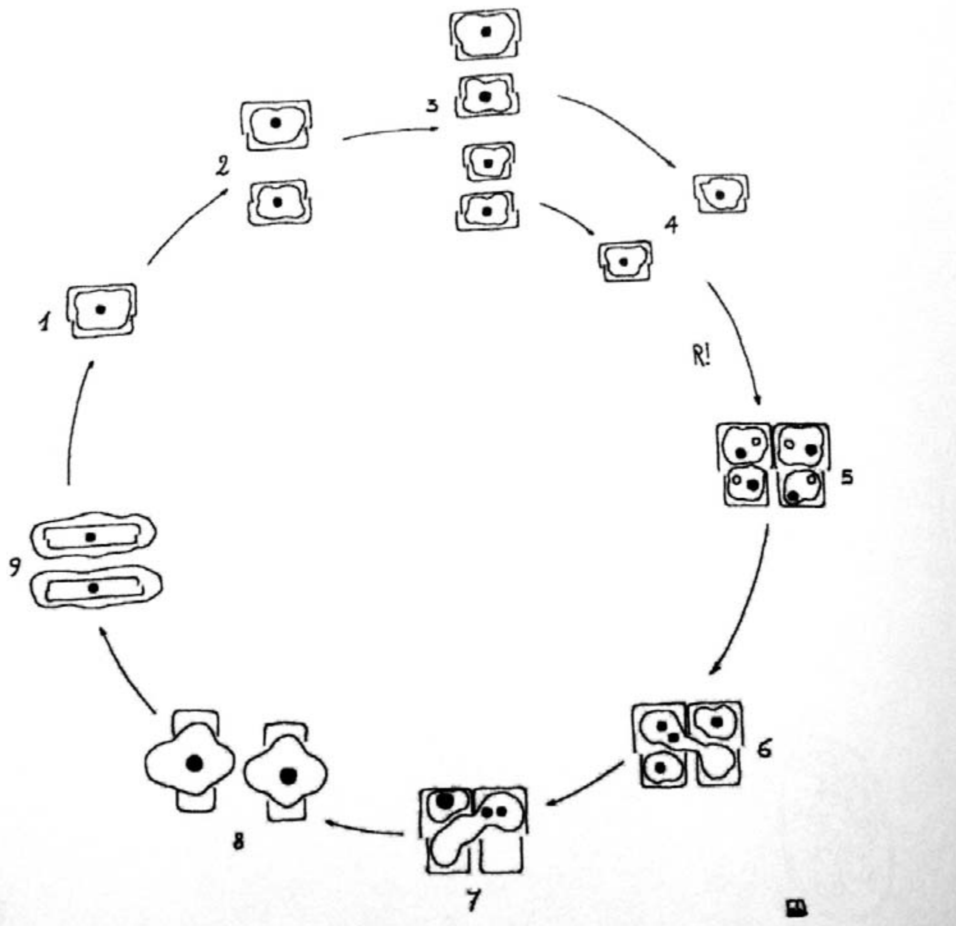


FIGURE 1.20 Life cycle of a diatom: 1, vegetative cell; 2, 3, vegetative cell division; 4, minimum cell size; 5, gametogenesis; 6, 7, fertilization; 8, auxospores; 9, initial cells. R!, meiosis.

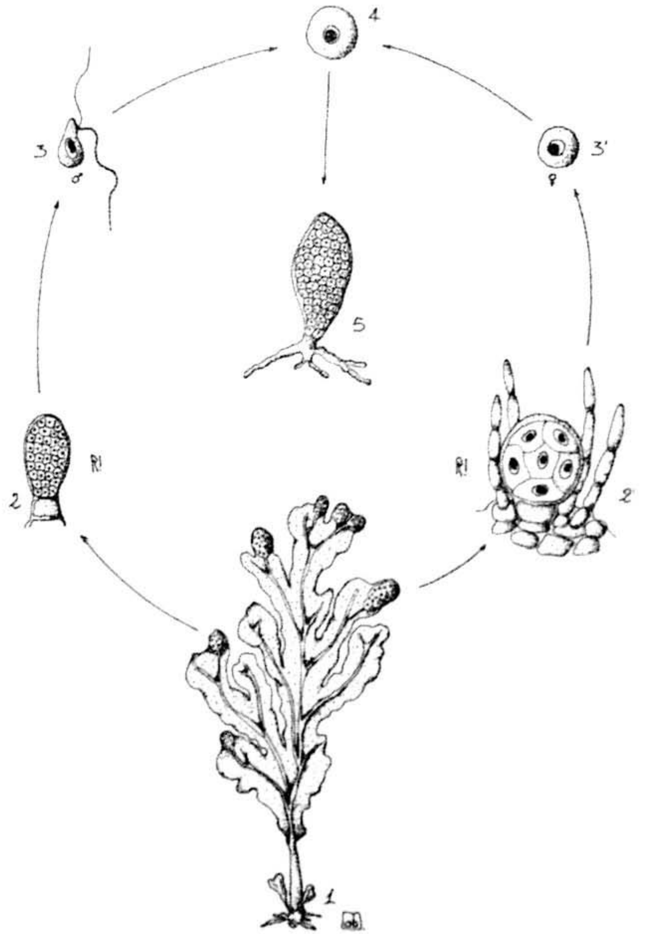


FIGURE 1.21 Life cycle of *Fucus* sp.: 1, sporophyte; 2, anteridium; 2′, oogonium; 3, sperm; 3′, egg; 4, zygote; 5, young sporophyte. R!, meiosis.

Sexual reproduction

Diplohaplontic or sporic life cycle

- present alternations of generation between two different phases consisting of haploide gametophyte & diploid sporophyte
  - **gametophyte** - produce gamete by mitosis
  - **sporophyte** - produce spore by meiosis
- alternation of generations can be
  - **isomorphic** - both phases morphologicaly identical
    - » *Ulva (Chlorophyta)*
  - **heteromorphic** - with predominance of
    - sporophyte - *Laminaria (Heterocontophyta)*
    - gametophyte - *Porhyra (Rodophyta)*

Diplohaplontic or sporic life cycle

-isomorphic alternation of generations

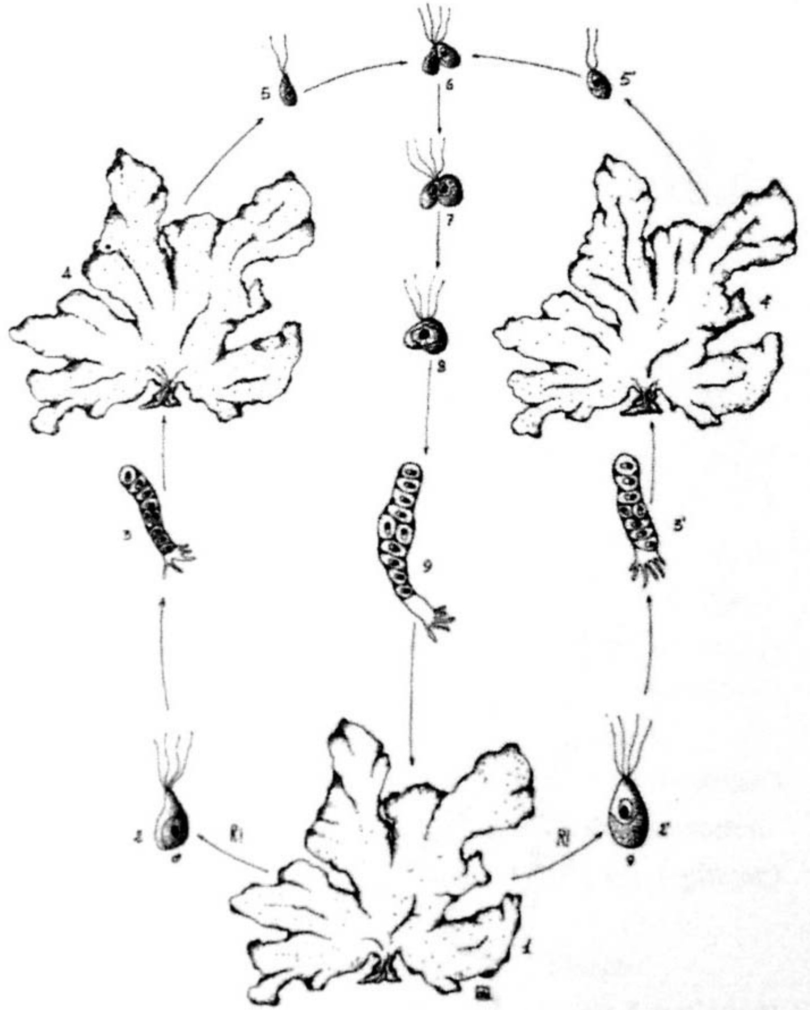


FIGURE 1.22 Life cycle of *Ulva* sp.: 1, sporophyte; 2, male zoospore; 2′, female zoospore; 3, young male gametophyte; 3′, young female gametophyte; 4, male gametophyte; 4′, female gametophyte; 5, male gamete; 5′, female gamete; 6–8, syngamy; 9, young sporophyte. R!, meiosis.

Diplohaplontic or sporic life cycle

-heteromorphic alternation of generations

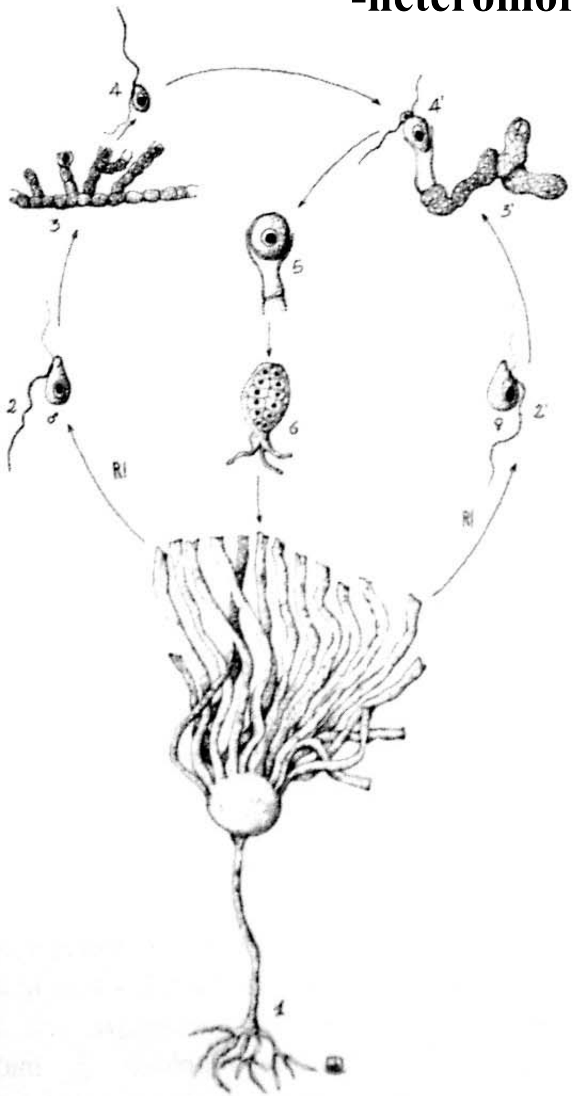


FIGURE 1.23 Life cycle of *Laminaria* sp.: 1, sporophyte; 2, male zoospore; 2′, female zoospore; 3, male gametophyte; 3′, female gametophyte; 4, sperm; 4′, egg and fertilization; 5, zygote; 6, young sporophyte. R!, meiosis.

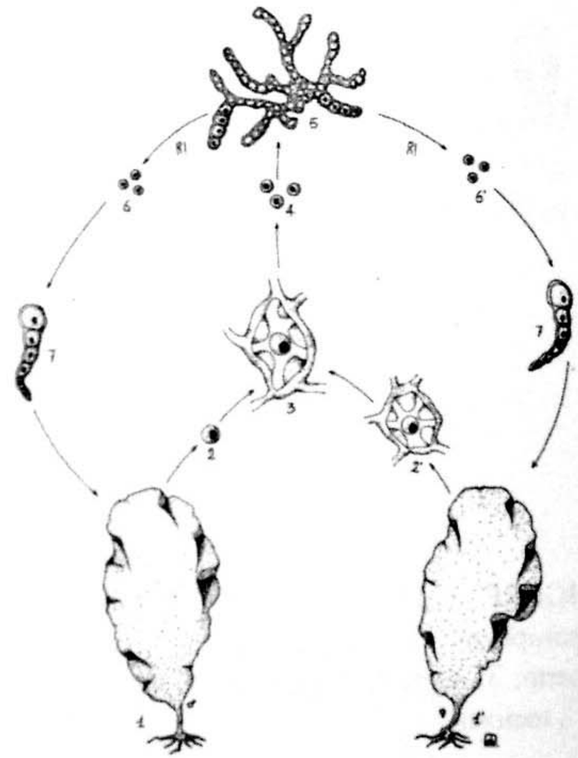


FIGURE 1.24 Life cycle of *Porphyra* sp.: 1, male gametophyte; 1′, female gametophyte; 2, sperm; 2′, egg; 3, fertilization and zygote; 4, spores; 5, sporophyte; 6, male spore; 6′, female spores; 7, young male gametophyte; young female gametophyte. R!, meiosis.

Physiology and Cultivation of Algae and Cyanobacteria

2.

Summaries of the ten algal divisions

- Historically
  - classified on basis of: pigmentation, chem. structure of storage products, thylakoid membrane organization, chemistry, structure of cell wall, number, arrangement, and ultrastructure of flagella, occurrence of special features, sex.cycles
- Recently
  - comparison of sequence of 5S; 18S; 28S rRNA, internal genetic coherence

TABLE 1.4
The Main Pigments, Storage Products, and Cell Coverings of the Algal Divisions

Division	Pigments				Storage Products
	Chlorophylls	Phycobilins	Carotenoids	Xanthophylls	
Cyanophyta	*a*	*c*-Phycoerythrin *c*-Phycocyanin Allophycocyanin Phycoerythrocyanin	β-Carotene	Myxoxanthin Zeaxanthin	Cyanophycin (argine and asparagine polymer) Cyanophycean starch (α-1,4-glucan)
Prochlorophyta	*a, b*	Absent	β-Carotene	Zeaxanthin	Cyanophycean starch (α-1, 4-glucan)
Glaucophyta	*a*	*c*-Phycocyanin Allophycocyanin	β-Carotene	Zeaxanthin	Starch (α-1,4-glucan)
Rhodophyta	*a*	*r,b*-Phycoerythrin *r*-Phycocyanin Allophycocyanin	α- and β-Carotene	Lutein	Floridean starch (α-1,4-glucan)
Cryptophyta	*a, c*	Phycoerythrin-545 *r*-Phycocyanin	α-, β-, and ε-Carotene	Alloxanthin	Starch (α-1,4-glucan)
Heterokontophyta	*a, c*	Absent	α-, β-, and ε-Carotene	Fucoxanthin, Violaxanthin	Chrysolaminaran (β-1,3-glucan)
Haptophyta	*a, c*	Absent	α- and β-Carotene	Fucoxanthin	Chrysolaminaran (β-1,3-glucan)
Dinophyta	*a, b, c*	Absent	β-Carotene	Peridinin, Fucoxanthin, Diadinoxanthin Dinoxanthin Gyroxanthin	Starch (α-1,4-glucan)
Euglenophyta	*a, b*	Absent	β- and γ-Carotene	Diadinoxanthin	Paramylon (β-1,3-glucan)
Chlorarachniophyta	*a, b*	Absent	Absent	Lutein, Neoxanthin, Violaxanthin	Paramylon (β-1,3-glucan)
Chlorophyta	*a, b*	Absent	α-, β-, and γ-Carotene	Lutein Prasinoxanthin	Starch (α-1,4-glucan)

	Cyanophyta	Prochlorophyta	Glaucophyta	Rhodophyta	Heterokontophyta Chrysophyceae	Heterokontophyta Xanthophyceae	Heterokontophyta Eustigmatophyceae	Heterokontophyta Bacillariophyceae	Heterokontophyta Raphidophyceae	Heterokontophyta Dictyochophyceae	Heterokontophyta Phaeophyceae	Haptophyta	Cryptophyta	Dinophyta I	Dinophyta II	Euglenophyta	Chlorarachniophyta	Chlorophyta
chlorofyly																		
chlorofyl a	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
chlorofyl b		*														*	*	*
chlorofyl c_1					*	+		*	*		*	*			*			
chlorofyl c_2					*	+		*	*	}*[4]	*	*	*	*	*			} ±[8]
chlorofyl c_3					±			*			*							
fykobilíny																		
fykocyanín	*		*	*									*					
alofykocyanín	*		*	*														
fykoerytrín	*			*									*					
fykobilizómy	*		*	*														
karotény																		
α-karotén				*	+							+	*		+			±
β-karotén	*	*	*	*	*	*	*	*	*	*	*	*	±	*	*	*		*
γ-karotén																±		±
ε-karotén					+			+			+		±					
xantofyly																		
zeaxantín	*	*	*	*	+		±		+[3]		±		+			±		+
echinenón	*	+						+				+		+		±		±
kantaxantín	*						+	+				+		+				
myxoxantofyl	*																	
oscillaxantín	*																	
α-kryptoxantín				±	+		±					+						
β-kryptoxantín	+	+	+	±		±	±									±		±
izokryptoxantín	+	+																
mutatochróm	+	+																
luteín				+						+							+	*
anteraxantín				±	+		+				+							+
violaxantín				±	+		*		+[3]		*						+	*
fukoxantín					*			*	*[3]	*	*	*			*			
neofukoxantín					+			+							+			
fukoxantín- der.[1]												*[5]			*[5]			
fukoxantín- der.[2]												*[5]						
diatoxantín					+	*		*		+	+	*			*	±		
diadinoxantín					+	*		*	*	+	+	*		*	*	+		
vaucheriaxantín						*	*		+									
heteroxantín						*			+									
aloxantín													*					
dinoxantín									+			+		+				
peridinín														*				
neoxantín					+	+	+	+	+		+					*	+	*
sifoneín																		+[6]
sifonoxantín																		+[6,7]
krokoxantín													+					
monadoxantín													+					
pyrroxantín														+				

[1,2] deriváty fukoxantínu, **[3]** v morských druhoch, **[4]** bližšie nešpecifikovaný chlorofyl c, **[5]** v niektorých druhoch, **[6]** v Bryopsidophyceae, **[7]** roztrúsene v niekoľkých triedach, **[8]** pigment podobný chlorofylu c, v asi piatich zelených riasach triedy Prasinophyceae

Tab. 2.: Najdôležitejšie zásobné látky rias (upravené podľa van den Hoeka)

	Cyanophyta	Prochlorophyta	Glaucophyta	Rhodophyta	Heterokontophyta Chrysophyceae	Heterokontophyta Xanthophyceae	Heterokontophyta Eustigmatophyceae	Heterokontophyta Bacillariophyceae	Heterokontophyta Raphidophyceae	Heterokontophyta Dictyochophyceae	Heterokontophyta Phaeophyceae	Haptophyta	Cryptophyta	Dinophyta	Euglenophyta	Chlorarachniophyta	Chlorophyta
cyanofycínové zrná *(bohaté na arginín a asparagín)*	*																
α-1,4 glukány																	
sinicový škrob	*	*															
florideový škrob				*													
škrob			*										*	*			*
β-1,3 glukány																	
chryzolaminarín					*	*	*	*	?	?	*	*					
paramylón												*[1]			*	?	

[1] v rode Pavlova.

Cyanophyta

- together with prochlorophyta – non-motile G- eubacteria

- 1-cell. to filament (un-/branched), & colonial aggr.
- widely distrib.; planctonic, blooms, picoplancton, benthic, soil, mud, hot springs; symbiotic
- Pigm.: chl *a*, blue & red phycobilins, carotenes
- phycobilisomes in rows on outher surface of thylakoids
- Thylacoids – free in cytoplasm, non-stacked, singled&equidistant
- Res.polysach.: cyanophycean starch (granules betw. thylakoids), cyanophycin
- some marine contain gas vesicles
- some filamentous form heterocysts & akinetes
- some produce hepato-, neurotoxins

Prochlorophyta

- 1-cell. / filamentous (un-/branched)
- free-living component of pelagic nanoplancton, obligate symbionts within didemnid ascidians & holoturians
- mainly limited to tropical&subtropical env.
- Pigm.: chl *a*, chl *b*, β-carotene, xanthophyls lack phycobilins,
- Thylakoids – free in cytoplasm, stacked
- Res.polysach.: cyanophycean starch (starch-like)

Cyan.&Prochlor.

- able to fix nitrogen
- contain polyhedral bodies (carboxysomes) with RuBisCO
- in cell wall peptidoglycane layer
- obligate photoautotrophs
- asexual reproduction (cell division / fragmentation of colonies)

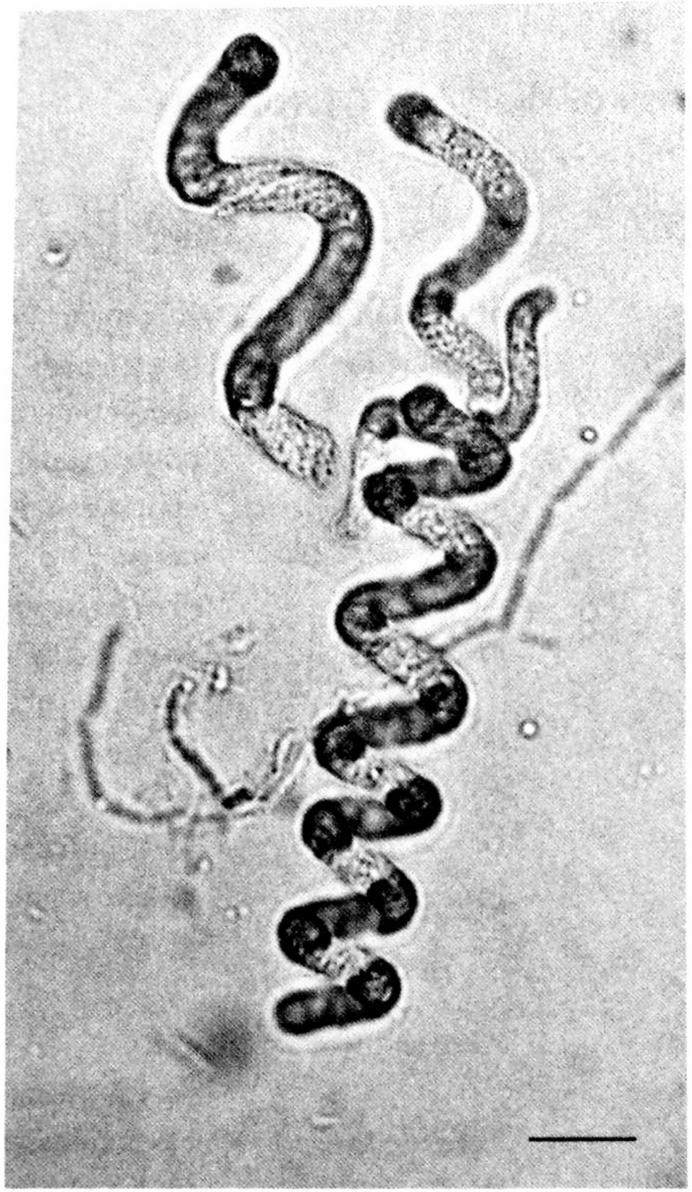


FIGURE 1.25 Trichome of *Arthrospira* sp. (Bar: 20 µm.)

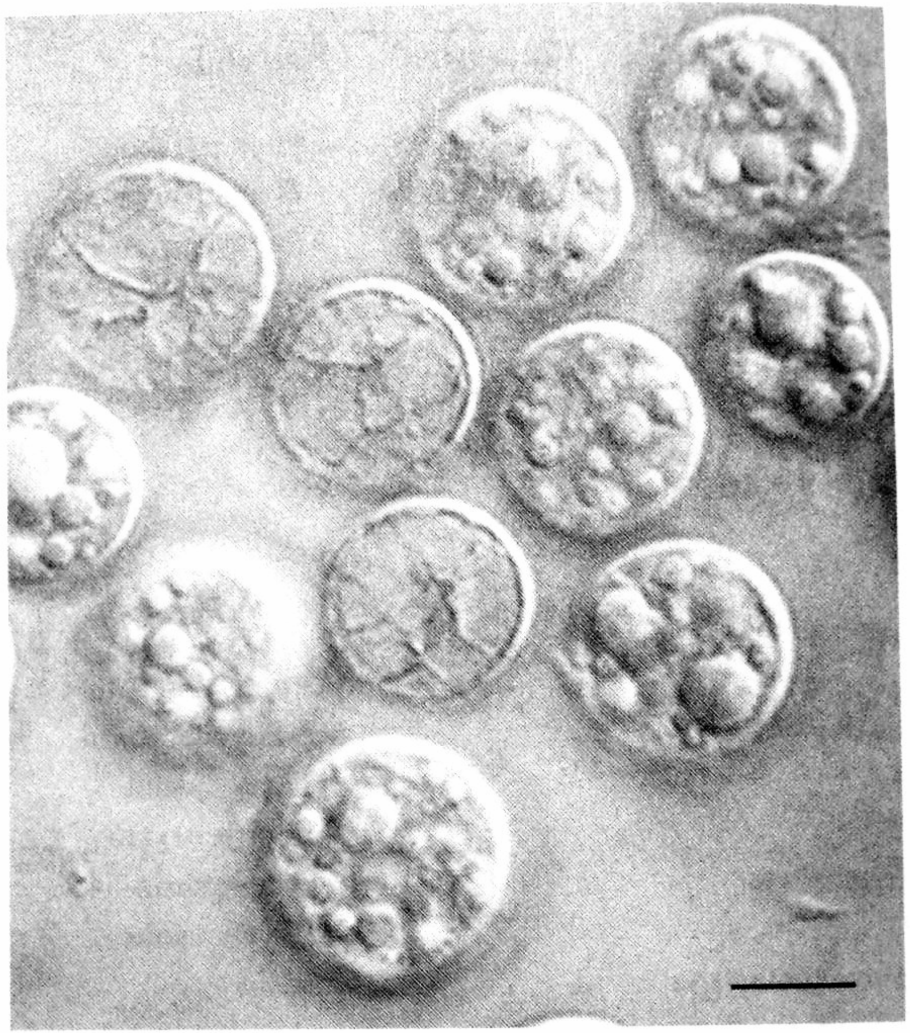


FIGURE 1.26 Cells of *Prochloron* sp. (Bar: 10 µm.)

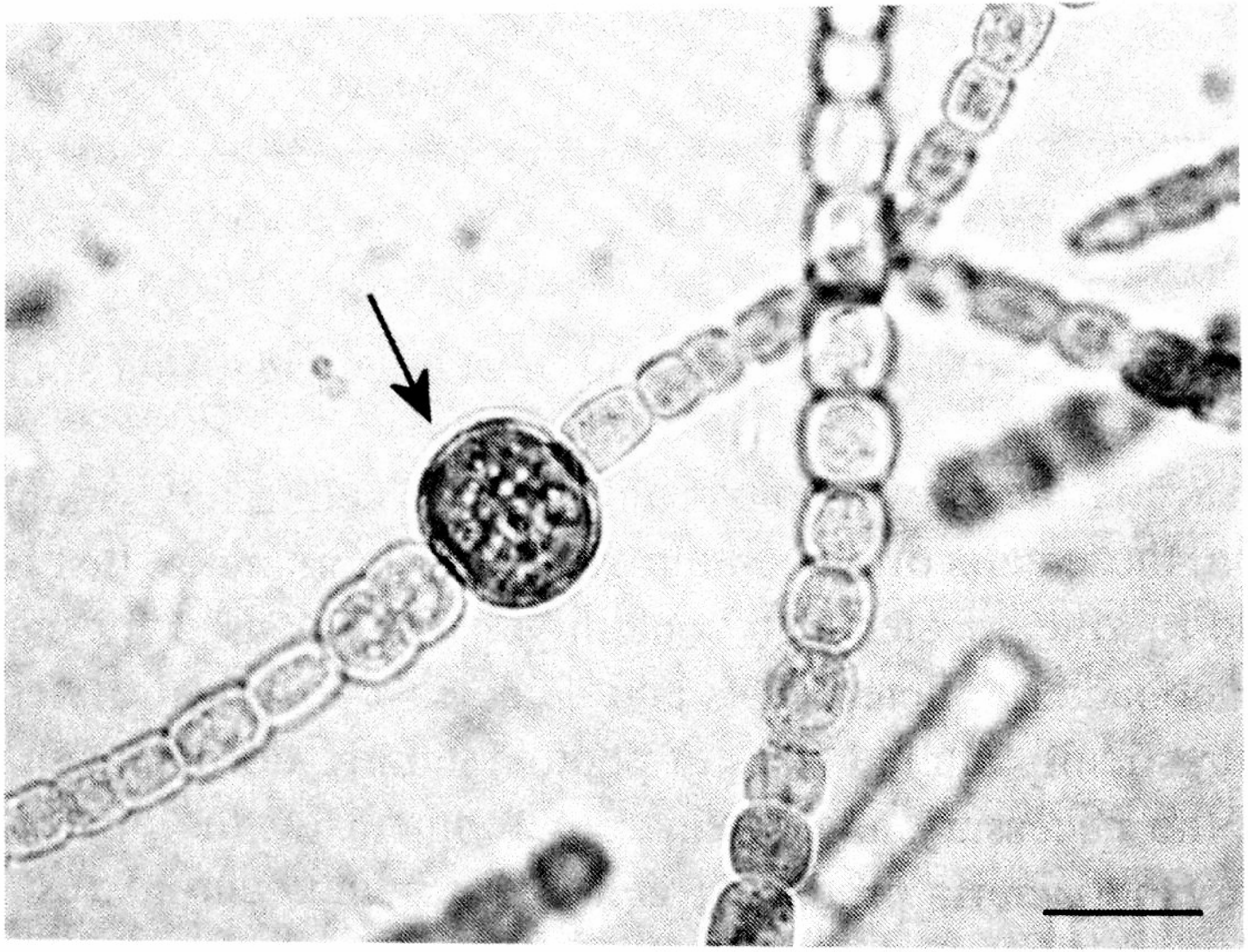


FIGURE 1.27 Heterocyst (arrow) of *Anabaena azollae*. (Bar: 10 μm.)

Glaucophyta

- 1-cell., flagellate, dorsiventral construction, 2 unequal flagella inserted in shallow depression below apex
- marine, rare freshw., solme soil
- Pigm.: chl *a*, acces.pigm.: phycoerythrocyanin, phycocyanin, allophycocyanin in phycobilisomes, carotenoids
- Chlpl. lie ni spec. vacuole, present thin peptidoglycan wall betw. 2 outher plastid membranes
- Thylacoids non-stacked
- ctDNA in center of chlpl. near carboxysomes (RuBisCO)
- Res.polysach.: starch (granules in cytoplasm outside of chlpl.)
- photoautotrophs with blue-green plastids – **cyanelles**
  - presumed to be phylogenetically derived from endosymbiotic cyanobacterium
- unknown sex. reproduction

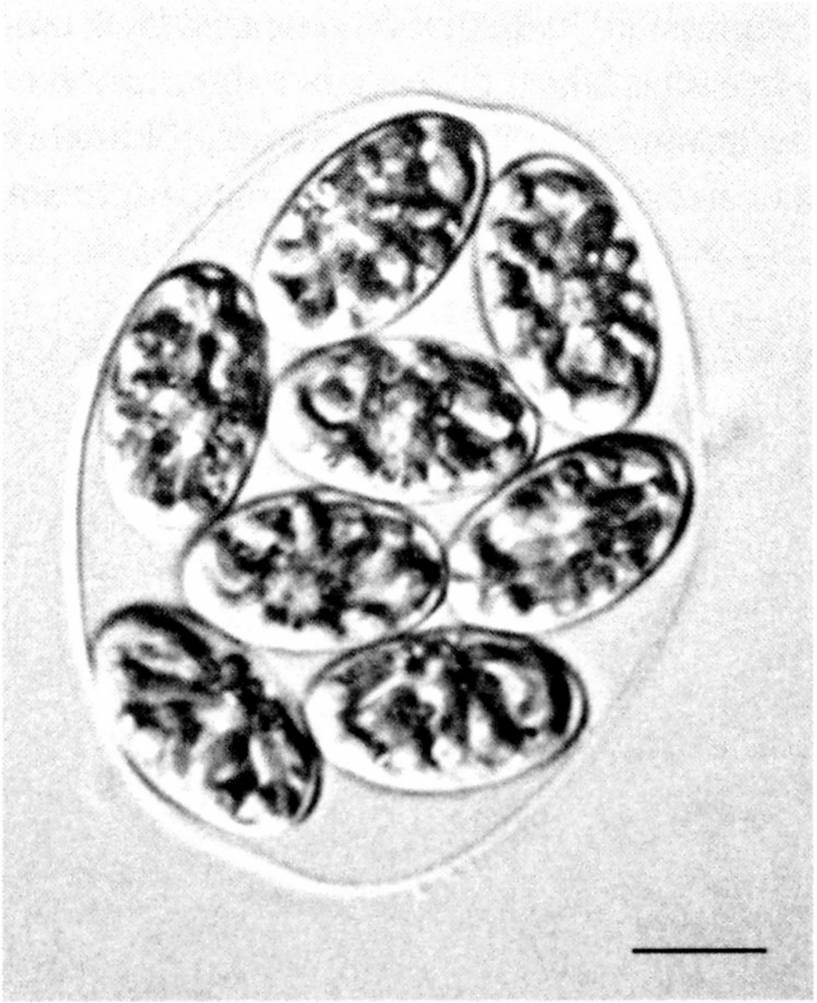


FIGURE 1.28 A group of eight autospore of *Glaucocystis nostochinearum* still retained within parent cell wall. (Bar: 10 μm.)

Rhodophyta

- red algae, mostly seaweeds, can free-living 1-cell.
- mostly marine
- lack flagellate stages
- Pigm.: chl *a*, phycobiliproteins in phycobilisomes
- Chlpl. 2-membrane enclosure
- thylakoids – non-stacked, single&equidistant within chlpl.
- ctDNA scattered throughout chlpl.
- Res.polysach.: floridean starch & α-1.4-glucan polysach. – grains in cytoplasm
- mostly photoautotrophs
- Repr.
  - in majority – cytokinesis incomplete >> pit connection >> proteinacous plug >> plug
  - sexual.: isomorphic / heteromorphic diplohaplontic life cycle

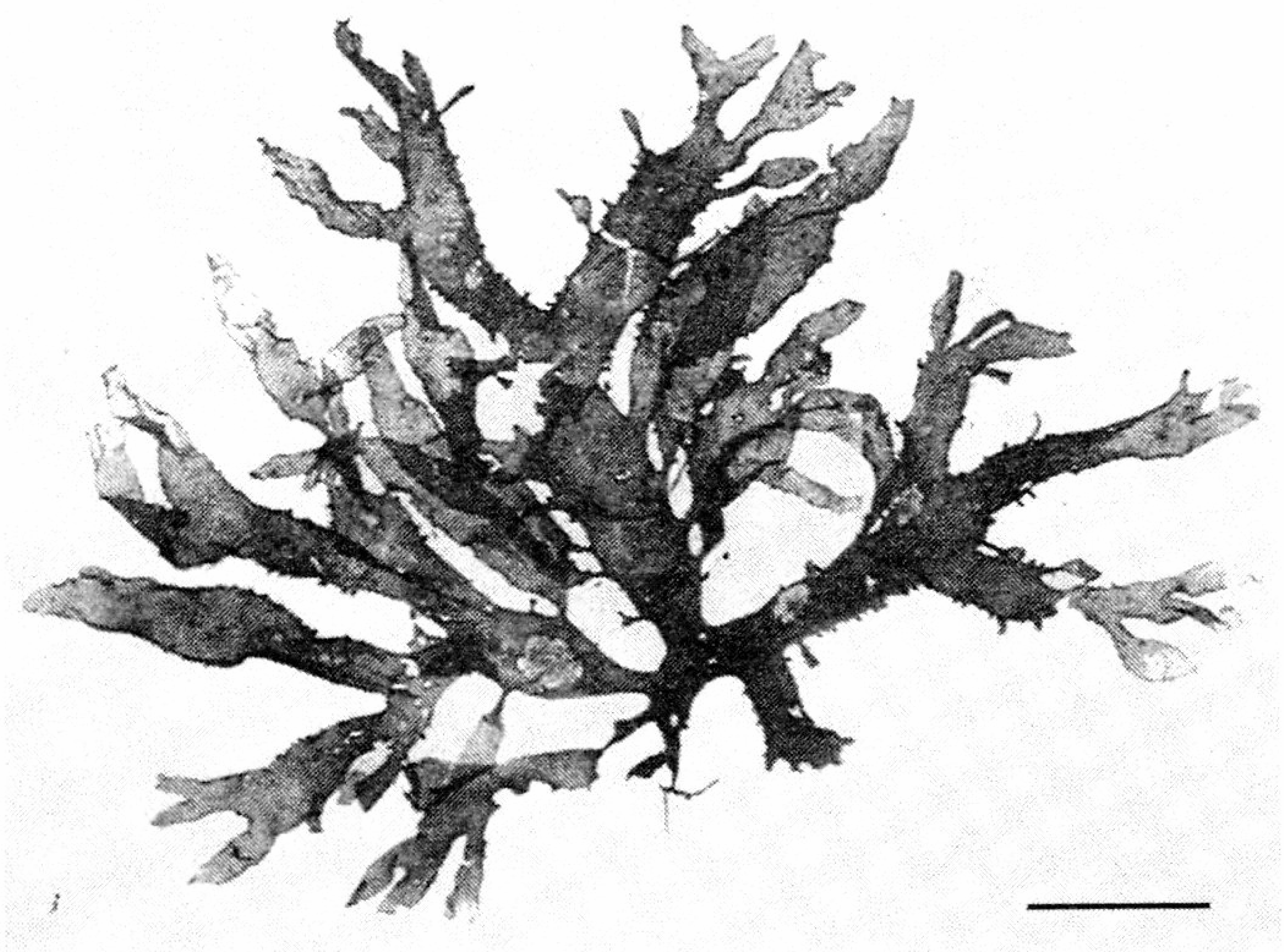


FIGURE 1.29 Frond of *Rhodophyllis acanthocarpa*. (Bar: 5 cm.)

Heterokontophyta

- when 2 flagella they are different
- flagelate cells – **heterokont** – long mastigonemate flagellum directed forward during swimming & short smooth fl., points backwards along the cell
- 1-cell-to-colonial, filamentous, siphonous, multicellular complex kelp; various type of flagellum
- mostly marine (can freshw,&terestrial)
- Pigm.: prepoderance of carotenoids over chlorophylls
  - chl *a*, c_1, c_2, c_3, access.: b-carotene, fucoxanthin, vaucheriaxanthin
- thylakoids stacked in three – **lamellae**
    - one lamellae usually runs along whole chlpl. = **girdle lamellae**
- chlpl. 2-membrane & fold of ER
- ctDNA – ring-shaped
- Res.polysach.: chrysolaminarin in cytoplasm in spec. vacuole
- eyespot – layer of globules, enclosed within chlpl. together with photoreceptor loc. in smooth flagellum, forms photoreceptive apparatus
- photoautotrophy can be combine with heterotrophy
- sex. reproduc.: life cycle – haplontic (Chrysophyceae), diplontic (Bacillariophyceae), diplohaplontic (Phaeophyceae)

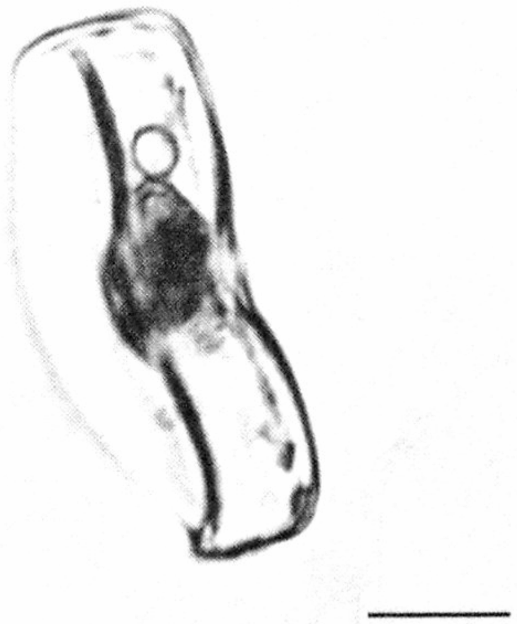


FIGURE 1.30 Marine diatom. (Bar: 10 μm.)

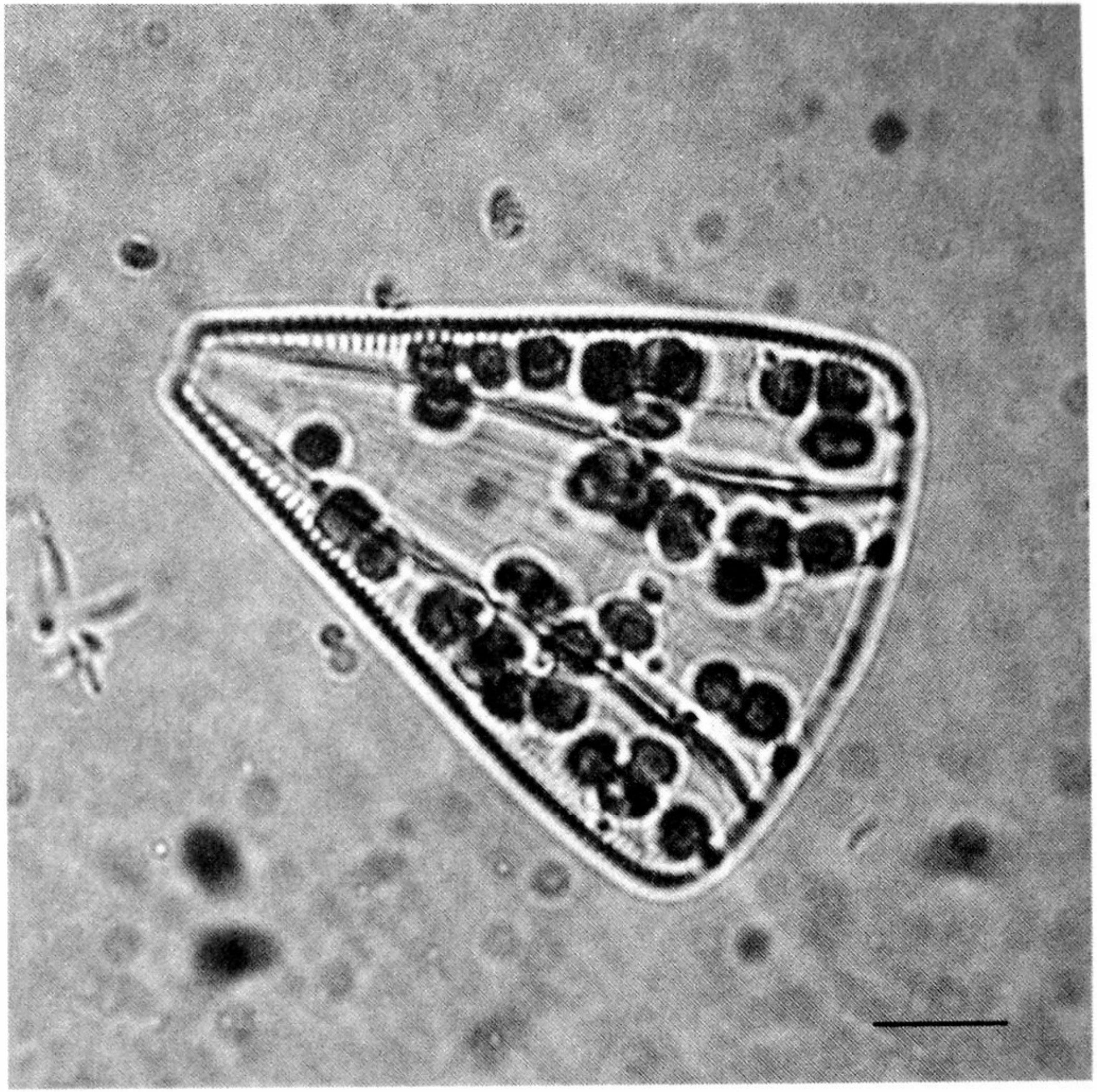


FIGURE 1.31 Freshwater diatom. (Bar: 20 μm.)

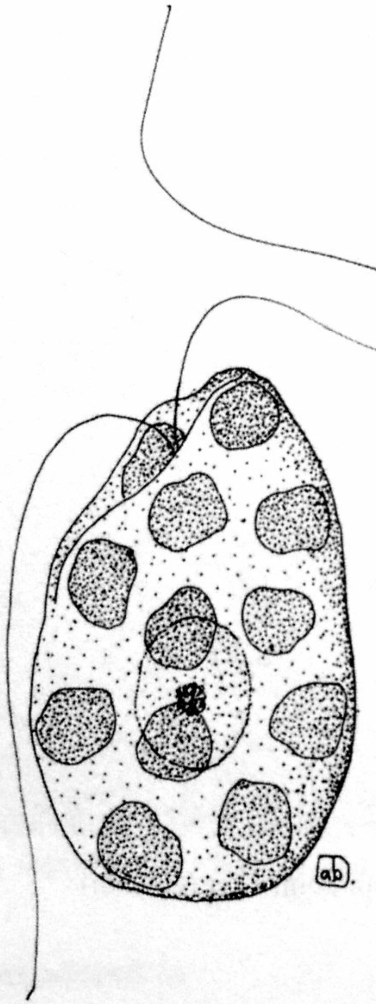


FIGURE 1.32 Unicell of *Heterosigma akashiwo.*

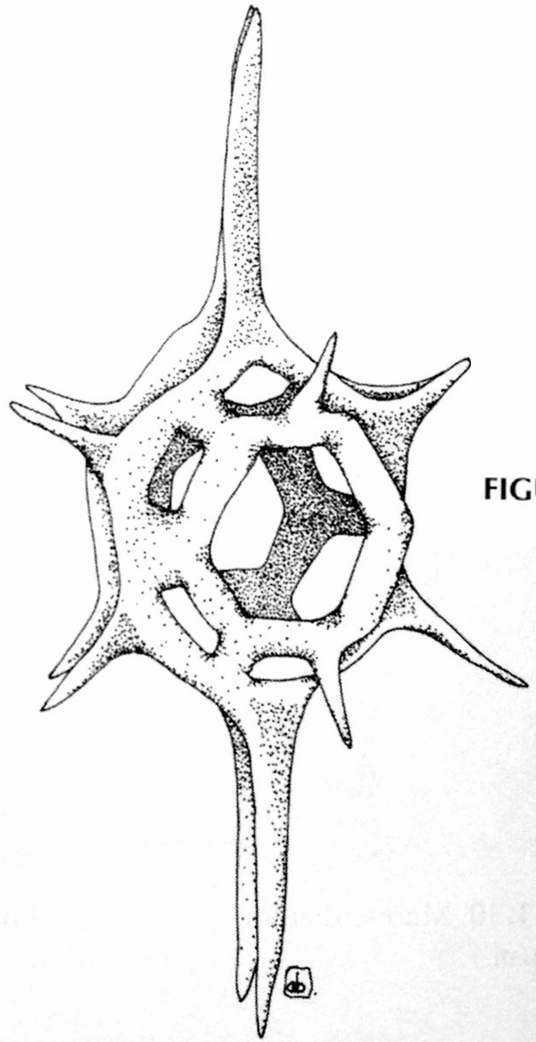


FIGURE 1.33 The silicoflagellate *Distephanus speculum.*

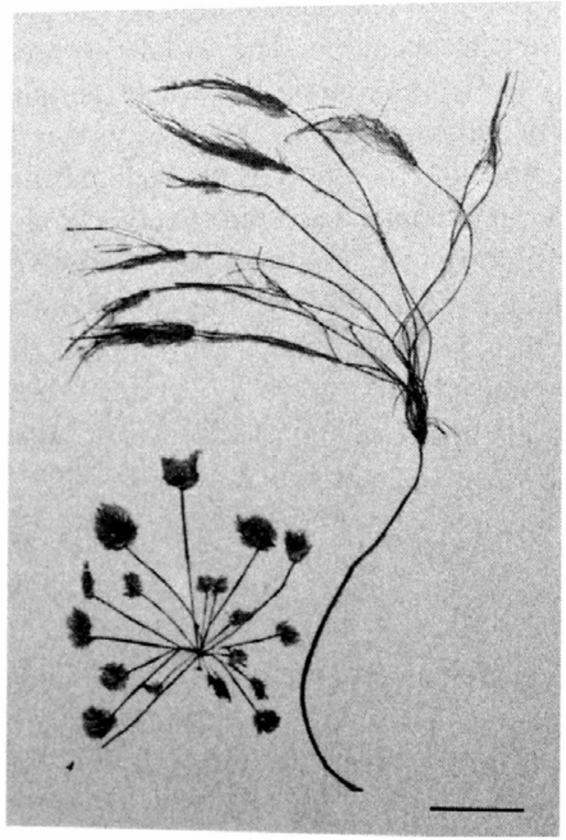


FIGURE 1.34 Frond of *Bellotia eriophorum.* (Bar: 5 cm.)

Haptophyta

- mostly 1-cell., motile, palmelloid / coccoid (rare colonial/filament.)
- generaly marine
- flagellate cells – 2 naked flagella inserted laterally or apically (may have diff. lenght)
- **haptonema** – typically long organelle flagellar structure (diff ultrastruct.)
- Pigm.: chl *a*, c_1, c_2, access.: fucoxanthin, β-carotene, xanthins
- Chlpl. enclosed within fold of ER
- Thylakoids – stacked in three, no girdle lamellae
- ctDNA – nucleoid scattered in chlpl.
- can be eyespot – row of globules inside chlpl., no associated flagellar struct.
- Res.polysach.: chrysolaminarin
- Cell surface – tiny celulosic scales or calcified scales bearing spoke-like fibrils
- Phototrophs / heterotrophs, phagotrophy in forms that lack cell covering
- Sex. reprod.: heteromorphic diplohaplontic life cycle

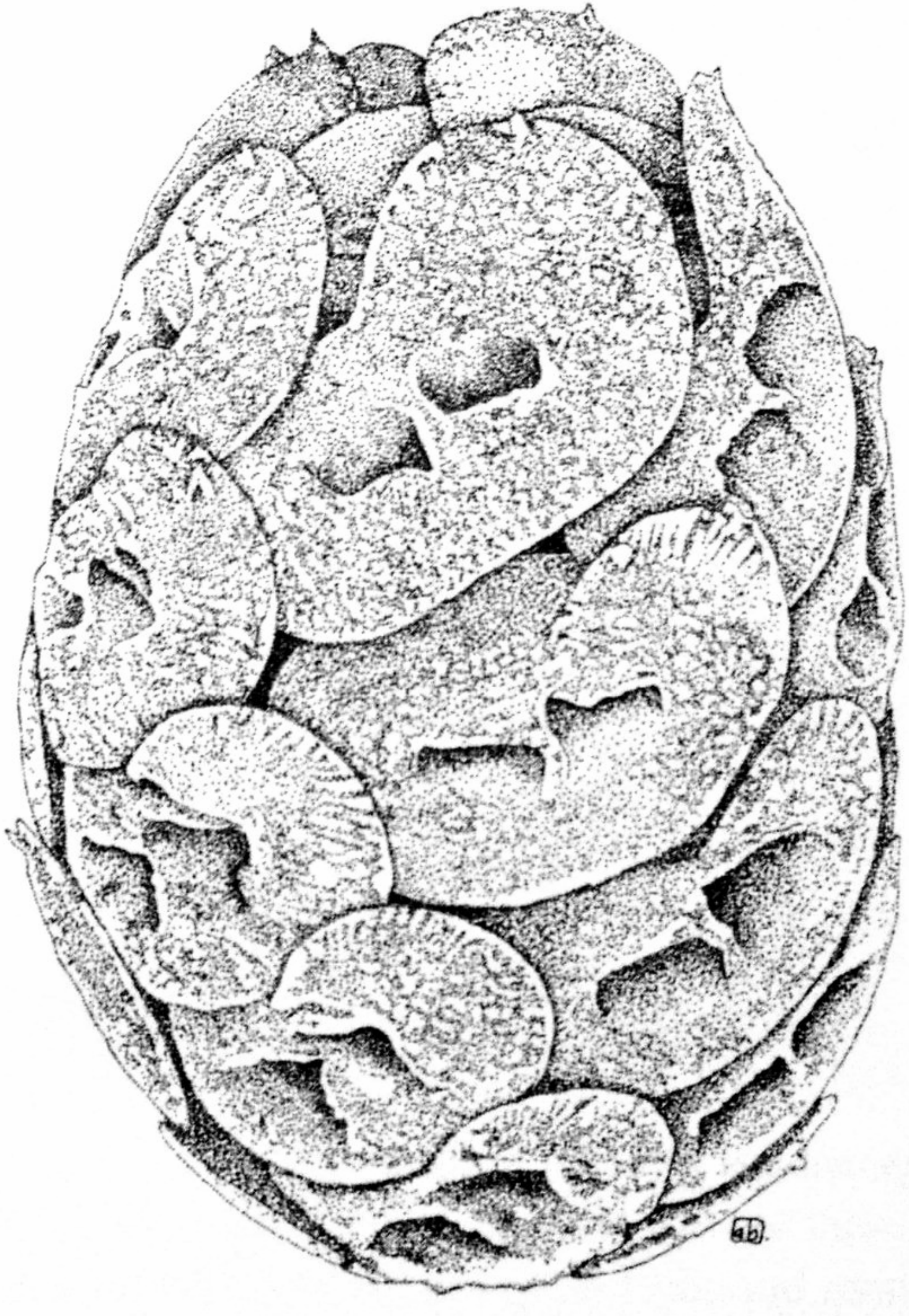


FIGURE 1.35 Unicell of *Helicosphaera carteri*.

Cryptophyta

- 1-cell. flagellate
- assymetric cells, dorsiventrally constructed
- 2 unequal hary flagella, subapically inserted, emerging from above gullet loc. on ventral side of cell – lined by ejectosomes
- free-swimming in freshw. & marine
- Pigm.: chl *a, c2,* phycobilins in thylakoid lumen > in phycobilisomes
- Chlpl. – 1 - 2 per cell, surrounded by fold of ER - in these intermembrane space – peculiar organemme – **nucleomorph -** ? nucleus of red algal symbiont
- Thylakoids in pairs, no girdle lamellae
- Pyrenoid projects out from the inner side of chlpl.
- ctDNA condensed in small nucleoid inside chlpl.
- Res.polysach.: starch ganules in periplastidial space
- eyespot sometimes inside plastid, no association with flagella
- Cell enclosed in stiff, proteinaceous periplast – polygonal plates
- mostly photosynthetic nutrition, can be heterotrophs
- Repr.: primary longitudinal cell division, can be sexual

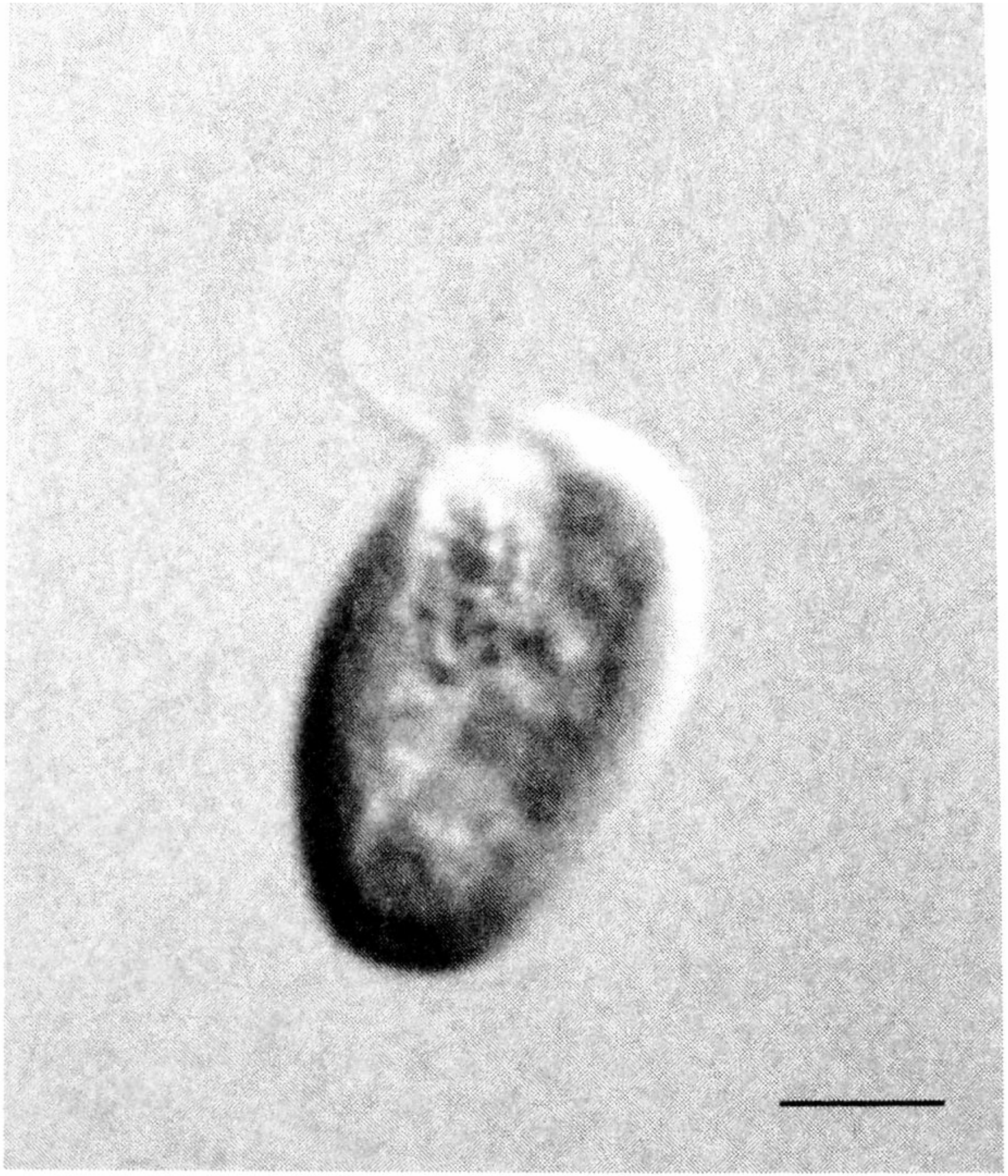


FIGURE 1.36 Unicell of *Cryptomonas* sp. (Bar: 6 μm.)

Dinophyta

- 1-cell., flagelates (can be ameboid, coccoid, palmelloid or filament.)
- 2 flagella with independent beating pattern (1^{st} - training, 2^{nd} - girdling) >> rotatory whirling motion
- characteristic cell covering components beneath cell membrane (layer of flat, polygon. vesicles – empty or celulose filled)
- dinokont type – **theca** – bi-partite armor (upper, anterior [epiconus]; lower, posterior [hypoconus]) separated by groove (cingulum) loc. transversal flagellum; smaller groove (sulcus) – extended posteriorly – host longitudinal flagellum
- important freshw. & marine microplancton
    - consumed by filterfeeders; parasites; endosymbionts of tropical corals
- Pigm.: chl *a, b, c1, c2,* fucoxanthin, carotenoids, xanthophylls
- Chlpl. (if present) surrounded by 3 membranes
- Thylakoids stacked in 3; ctDNA in small nodules scattered in whole chlpl.
- complex photoreceptive system “compound eye” (Warnowiaceae) – lens & retinoid
- <u>Dinocaryon</u> – eukaryotic nucleus, chromosomes condensed during mitosis, karyotheca unbroken – endomitosis
- Res.polysach.: starch - grains in cytoplasm; oil droplets in some genera
- At cell suraface – trichocysts – discharge explosively when stimulated
- Photoautotrophy & hi. diversity of nutrit. types
- can form blooms, possib. bioluminiscence
- Sex. reproduct. – haplontic life cycle; can form hypnospores

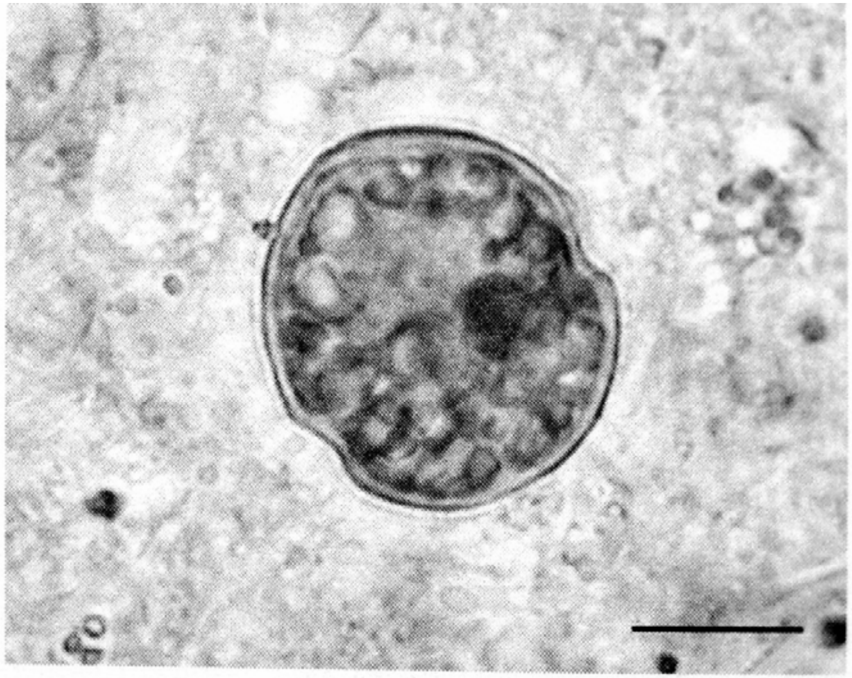


FIGURE 1.37 A marine dinoflagellate. (Bar: 30 μm.)

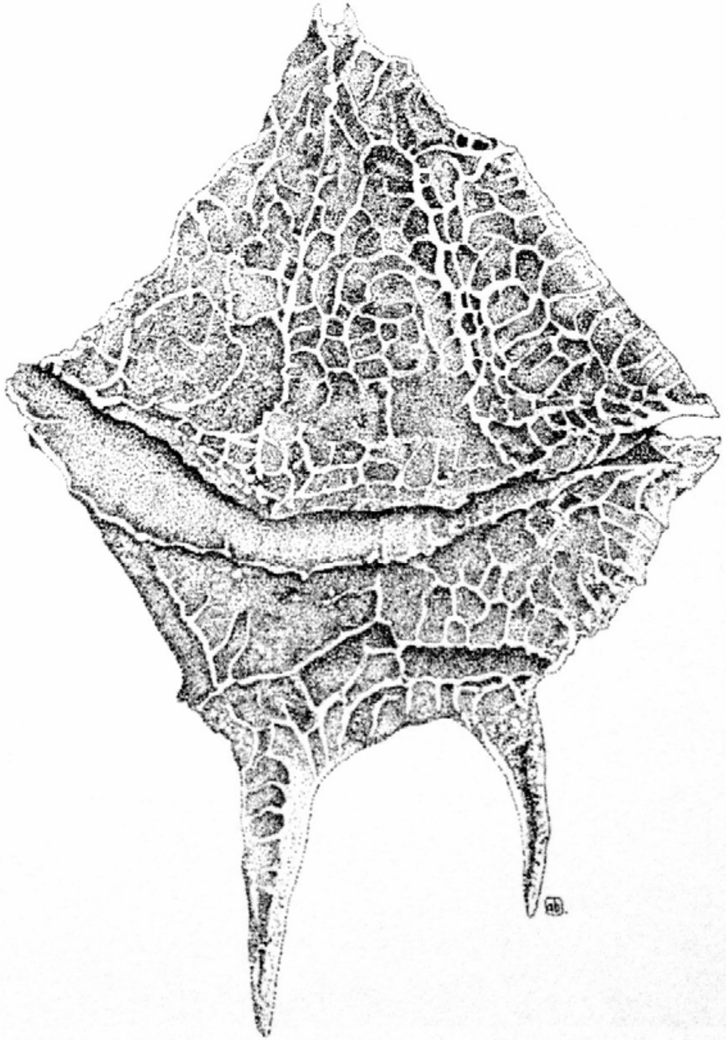


FIGURE 1.38 Dorsal view of *Gonyaulax* sp., a brackish water dinoflagellate.

Euglenophyta

- mostly unicell.flagellates, can be colonial
- widely distributed, freshwater, brakish & marine, abundant in hi. heterotrophic env.
- flagella arise from bottom of cavity – reservoir (loc. in anterior end of cell); can live in mud; presence of pellicle – proteinaceous wall inside cytoplasm – spiral construction
- Pigm.: chl *a* & *b*, β & γ-carotenes, xanthins, some spec. can absent plastids
- Chlpl.- 3 membrane envelope
- Thylakoids – group of three without girdle lamella
- photoreceptive system – orange eyespot loc. free in cytoplasm; true photoreceptor loc. at base of flagellum
- Res.polysach.: paramylon (β-1,3-glucan); granules inside cytoplasm(not in chlpl.)
- obligate mixotrophs, require vitamins of B group; colorless can be phagotrophs, osmotrophs
- Reproduction – only asexual

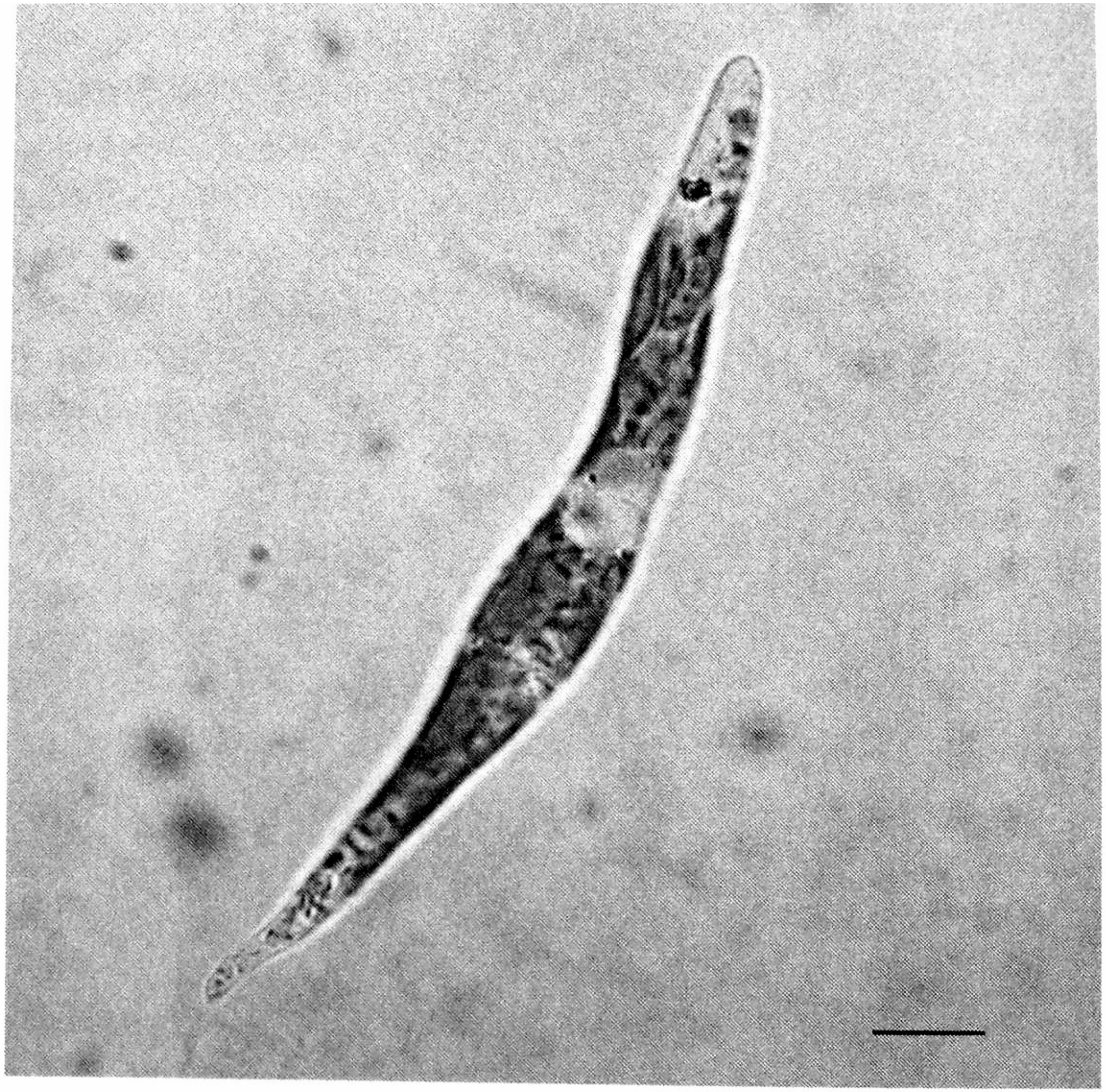


FIGURE 1.39 Unicell of *Euglena mutabilis.* (Bar: 10 μm.)

Chlorarachniophyta

- naked, unicell., form net-like plasmodium via filopodia
- life cycle forms – amoeboid, coccoid, flagellate stages
- ovoid zoospores bear single flagellum
- marine
- Pgm.: chl *a* & *b*
- Chlpl. – 4-membrane envelope, each has prominent projecting pyrenoid
- Thylakoids – stacked in one to three
- nucleomorph present btw. 2^{nd} and 3th membrane originated from green algal endosymbiont
- Res.polysach.: paramylon (β-1,3-glucan)
- phototrophs & phagotrophs engulfs bacteria, flagelates & eukaryotic algae
- Repr. – mostly asexual – mitosis or zoospore formation
  - heterogamy rarely

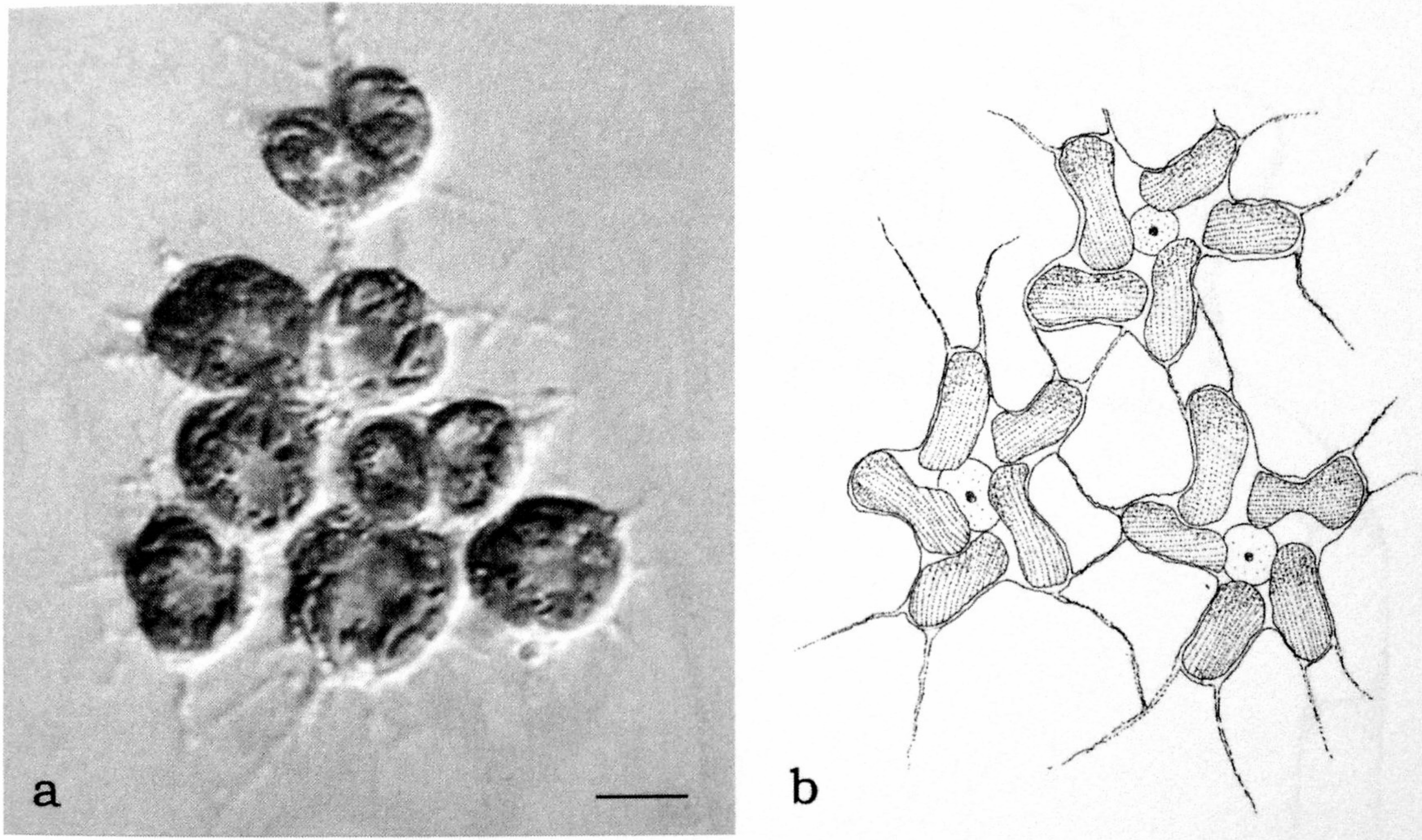


FIGURE 1.40 Plasmodial reticulum of *Chlorarachnion*, bright field microscope image (a) and schematic drawing (b). (Bar: 4 μm.)

Chlorophyta

- great range of somatic differentiation (flagellates to differentiated multicell. thalli)
- thallus organization <--> basis of classification
- flagella -wide diversity in number & arrangement (1-8 in apical / subapical region)
- zoids are isokont (similar struct. but can differ in lenght)
- freshwater, marine & terestrial
- Pigm.: chl. *a* & *b*, β & γ-carotenes, xanthophylls
- Chlpl. – 2-membrane envelope
- Thylakoids – stacked, form grana
- pyrenoid (if present) in chlpl.
- ctDNA – circular
- Res.polysach.: starch – most important – grain inside chlpl.
- glucan & β-1,4-mannan can present in cell wall
- eyespot (if present) loc. inside chlpl.
- photoautotrophs, can be heterotrophs
- Repr. – sex. – variety of life cycle – group specific
  - similarity to higher plants - *Trentepohliaceae* – cell division using phrogmoplast disc where the cells will divide
- probably land plants derived directly from these freshwater algae

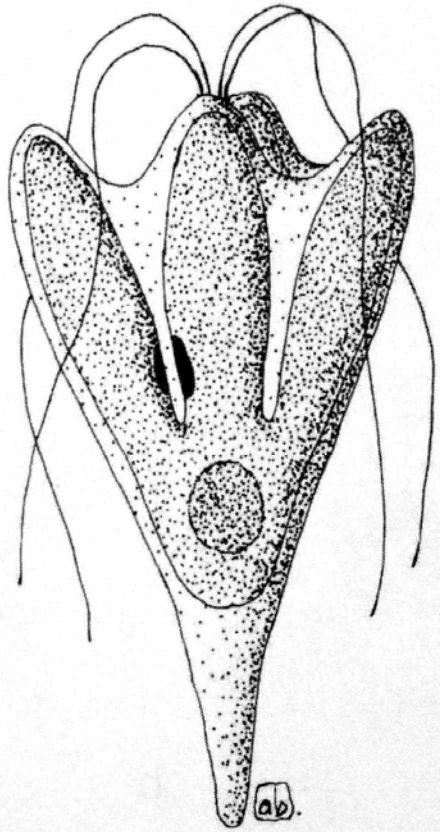


FIGURE 1.41 Unicell of *Pyramimonas longicauda.*

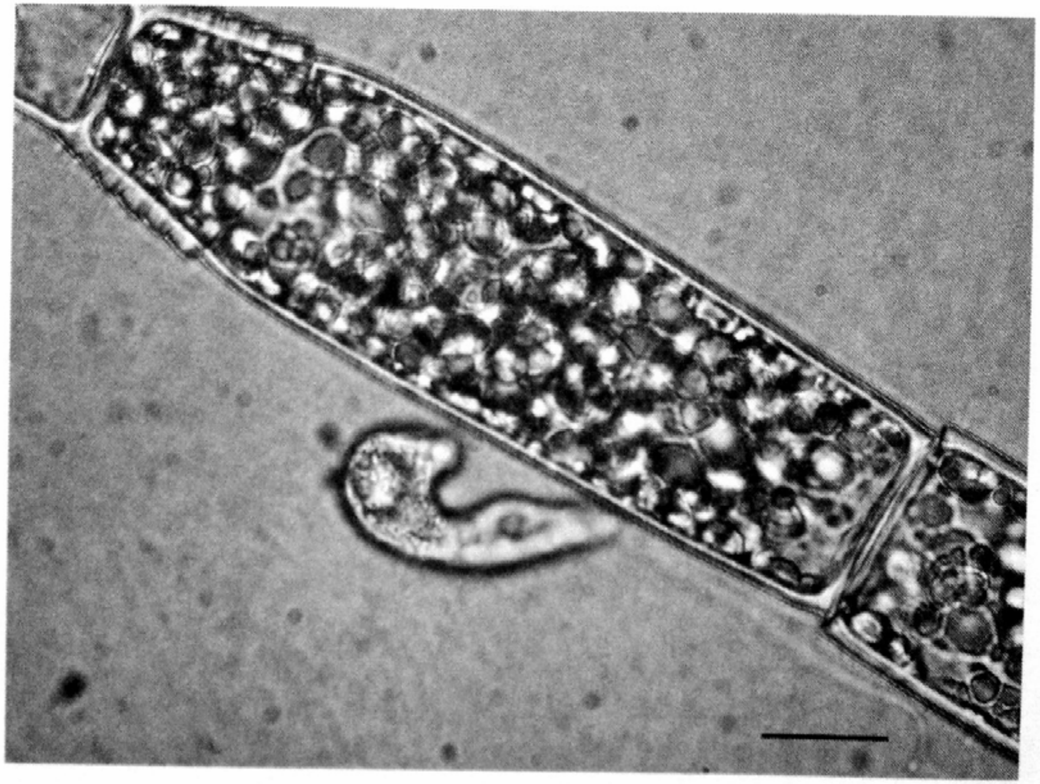


FIGURE 1.42 Filament of *Oedogonium* sp., with a *Peranema* sp. cell. (Bar: 20 μm.)

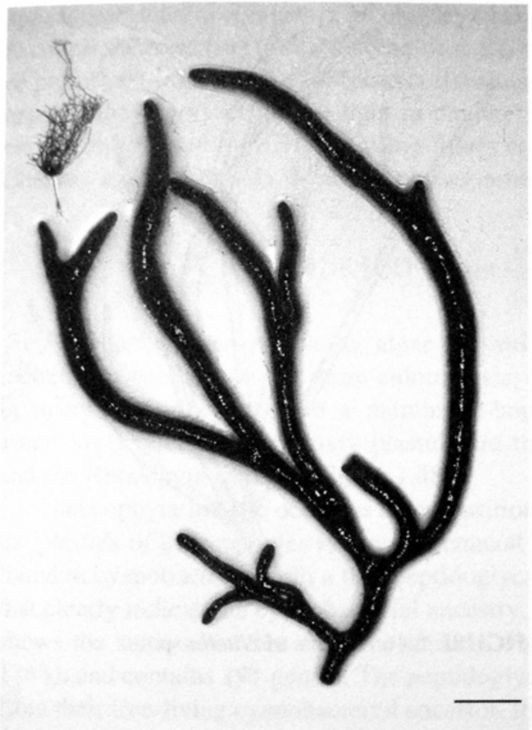


FIGURE 1.43 Thallus of *Codium* sp. (Bar: 2 cm.)

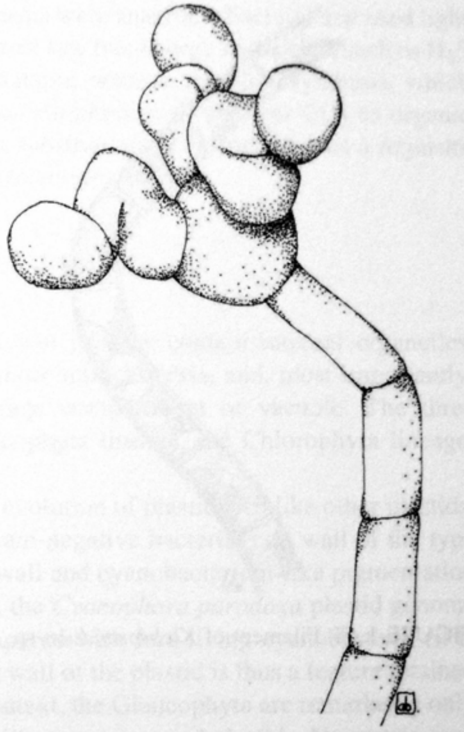


FIGURE 1.44 Thallus of *Trentepohlia arborum.*

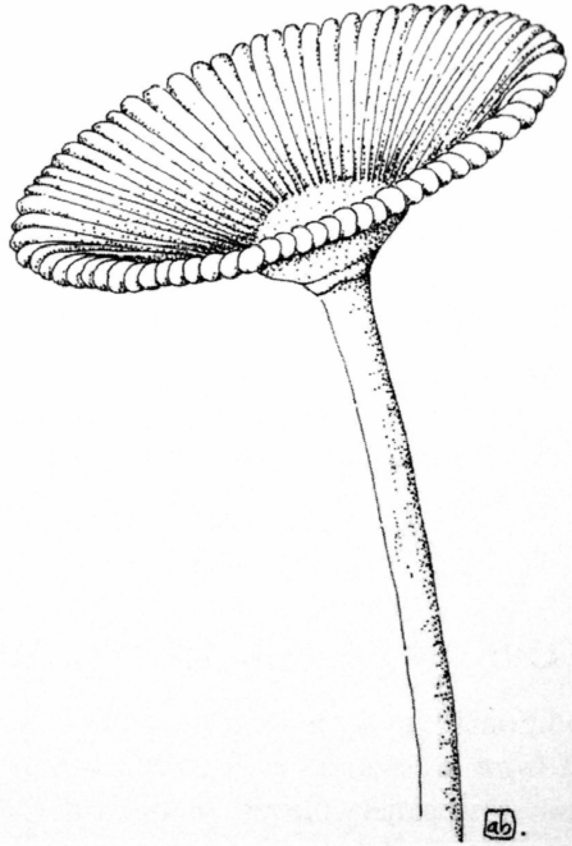


FIGURE 1.47 Portion of the thallus of *Acetabularia* sp.

FIGURE 1.45 Filament of *Klebsormidium* sp.

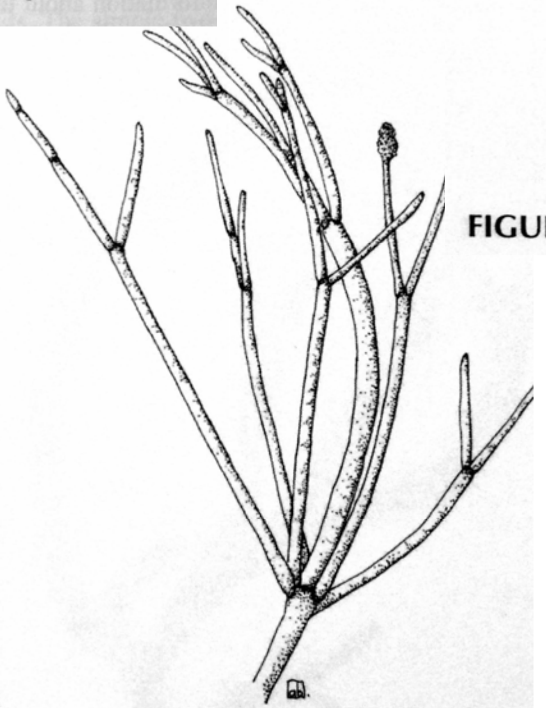


FIGURE 1.46 Thallus of *Nitella* sp.

Endosymbiosis & origin of eukaryotic algae

- procaryotic ancestor → endosymbiotic events → primary plastid
- secondary / tertiary endosymbiosis → plastid (sec. eukaryotic cell)
- arrangement of cellular compartments inside the the other – info about evolut. history
- Cyanobacteria
  - evolved >2.8 bill. years ago
  - fundamental roles in ocean carbon, oxygen, nitrogen fluxes
  - turning point in biogeochemistry of Earth
  - photosynthesis – energy of VIS, oxidation of H2O, reduction of CO2
  - $CO_2 + H_2O + \text{light} \xrightarrow{\text{chl } a} (CH_2O)n + O_2$

Three major algal lineages of primary plastids

- <u>Glaucophyta lineage</u>
  - plastids – thin peptidoglycan cell wall & cyanobacter.-like pigments
    - » cyanobacterial ancestry
  - neither green nor red plastid present
  - any secondary plastid derived from Glacophyta is known

Endosymbiosis & origin of eukaryotic algae

- Chlorophyta lineage "green algae"
  - green algal plastid
  - 2-membrane system surround.
    - chl *b* – sec. pigment, phycobiliproteins lost
  - other – prochlorophyte based hypothesis
  - major role in oceanic food webs & carbon cycle from -2.2 bill. years until Permian extinction (-250mil y.)
  - origin of land plants

  - >Secondary endosymbiosis → Euglenophyta (3-membrane plastid) & Chlorarachniophyta (4-membrane plastid
- Rodophyta leneage " red algae"
  - red algal plastid
  - 2-membrane system
    - chl *a* & phycobiliproteins organized into phycobilisomes attached on thylakoid membrane
    - Thylakoids with phycobilisomes don't form stacks – similar to cyanobacteria

  - >Secondary endosysmbiosis → Cryptomonades (Cryptophyta)
    - (4-membrane plastid) + chl *c* (also Haptophyta, Heterocontophyta & Dinophyta)

  - stacked thylakoids found in lineages lacking phycobilisomes

  - >a few groups of Dinoflagelates Tertiary endosymbiosis – uptake of secondary plastid containing endosymbiont

  - All these groups are relatively modern org.
  - dinoflagelates & coccolithophorids rise paralel with dinosaurs
  - diatoms rise with mammals

members of red lineages in shallow seas in Jurassic period provide petroleum

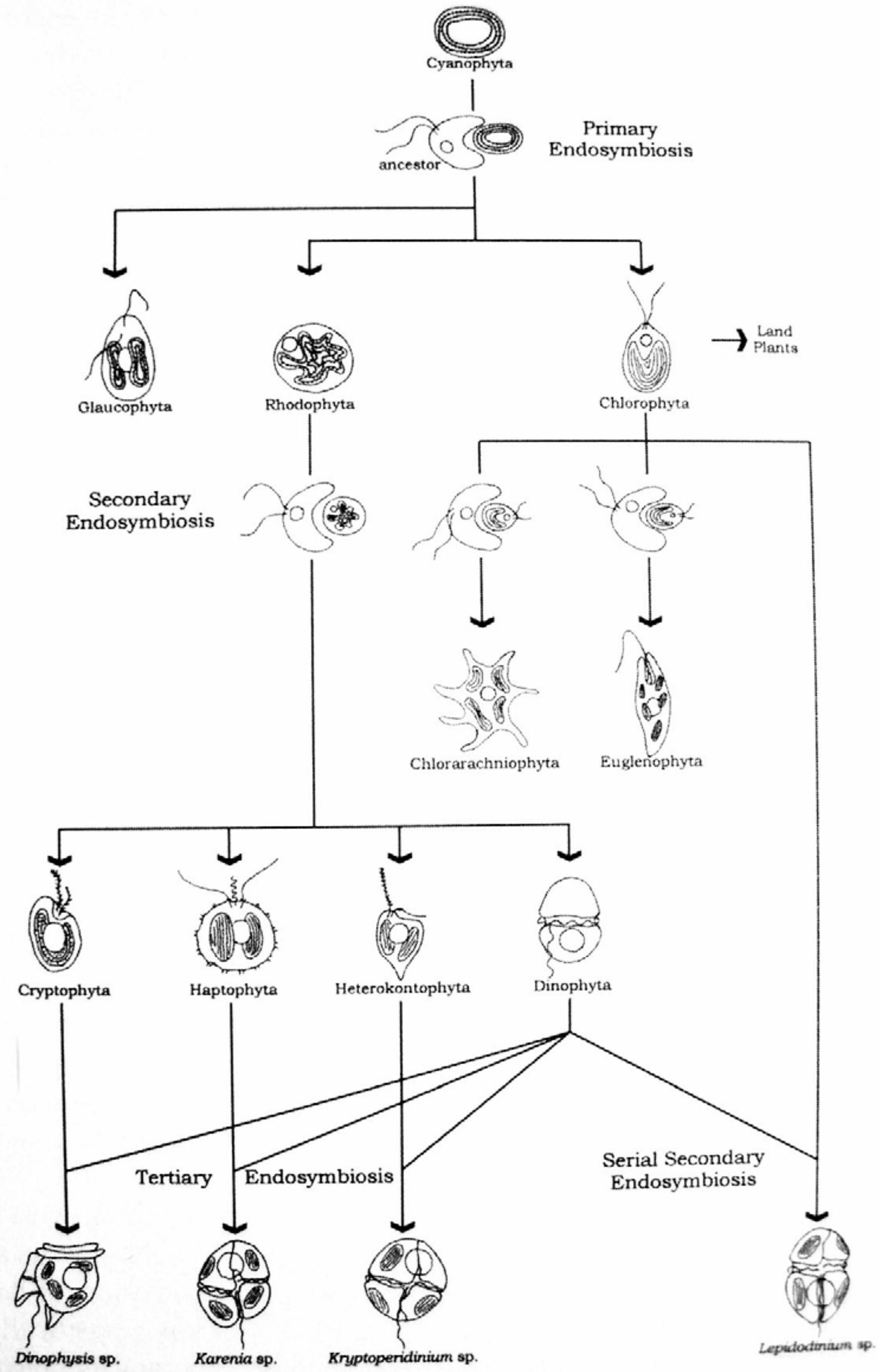


FIGURE 1.48 Algal evolution and endosymbiotic events.

Physiology and Cultivation of Algae and Cyanobacteria

3.

Photosynthesis

- light
- structure & function
  - membrane structure
  - pigments
  - photosystems
- photosynthesis – reactions
  - light dependent
  - light independent
- energy transfers

Light

- sunlight *vs.* PAR
- units; W m^{-2} s^{-1}; μmol m^{-2} s^{-1}
- spectrum
- absorption, transmittation, reflection, scattering, interference
- environmental accessibility (spectrum, int..)

TABLE 3.1
Sun Light Reflected by Sea Surface

Angle between Sun rays and zenith	0°	10°	20°	30°	40°	50°	60°	70°	80°	90°
Percentage of reflected light	2	2	2.1	2.1	2.5	3.4	6	13.4	34.8	100

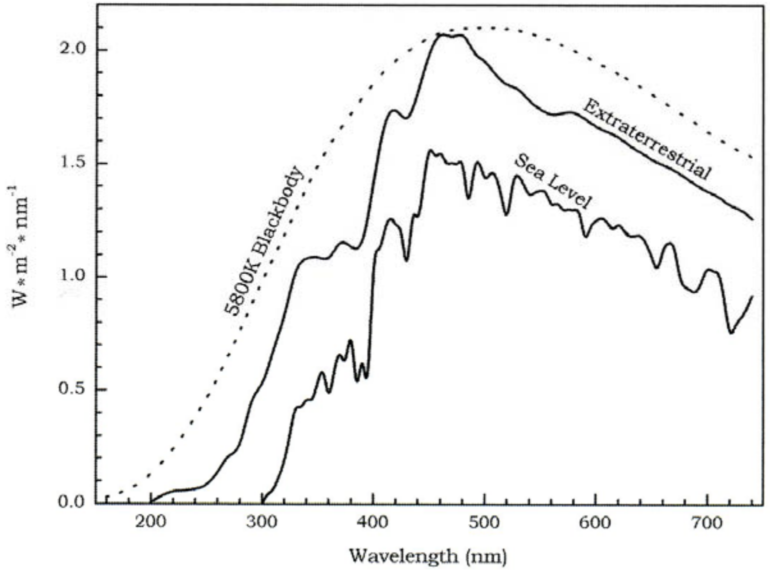


FIGURE 5.10 Spectral irradiance of the incoming sun radiation outside the atmosphere and at sea level compared with that of a perfect blackbody at 5800 K

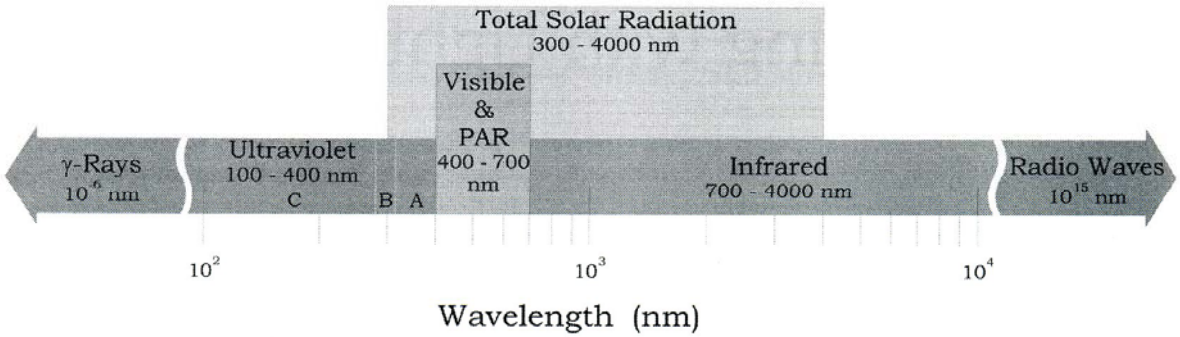


FIGURE 5.1 The electromagnetic spectrum from γ-rays (10^{-6}) to radio waves (10^{15}).

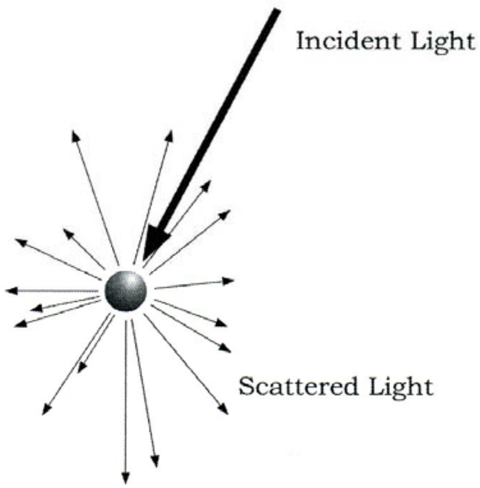


FIGURE 5.2 Light interaction with matter: the scattering process.

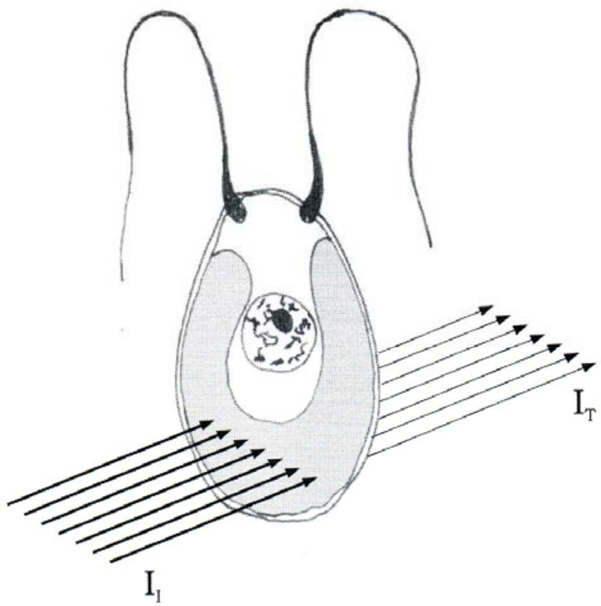


FIGURE 5.3 Light absorption by a unicellular alga: I_I, light incident on the cell and I_T, light transmitted by the cell.

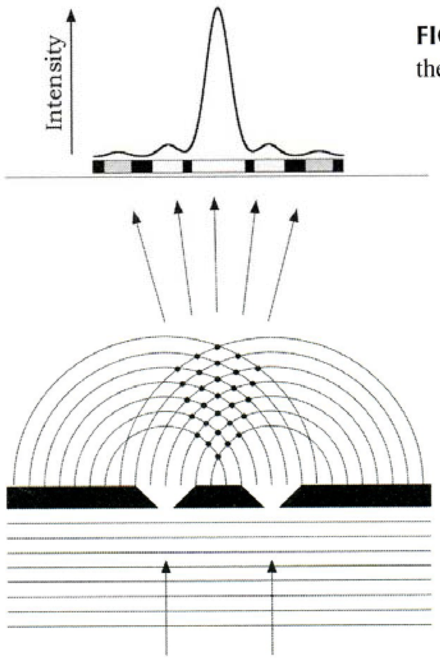


FIGURE 5.4 Interference of light passing through two narrow slits, each acting as a source of waves. The superimposition of waves produces a pattern of alternating bright and dark bands. When crest meets crest or trough meets trough, constructive interference occurs, which makes bright bands; when crest meets trough destructive interference occurs, which makes dark bands. The dots indicate the points of constructive interference. The light intensity distribution shows a maximum that corresponds to the highest number of dots.

Structure & function

- thylakoid membrane structure
- pigments
- photosystems

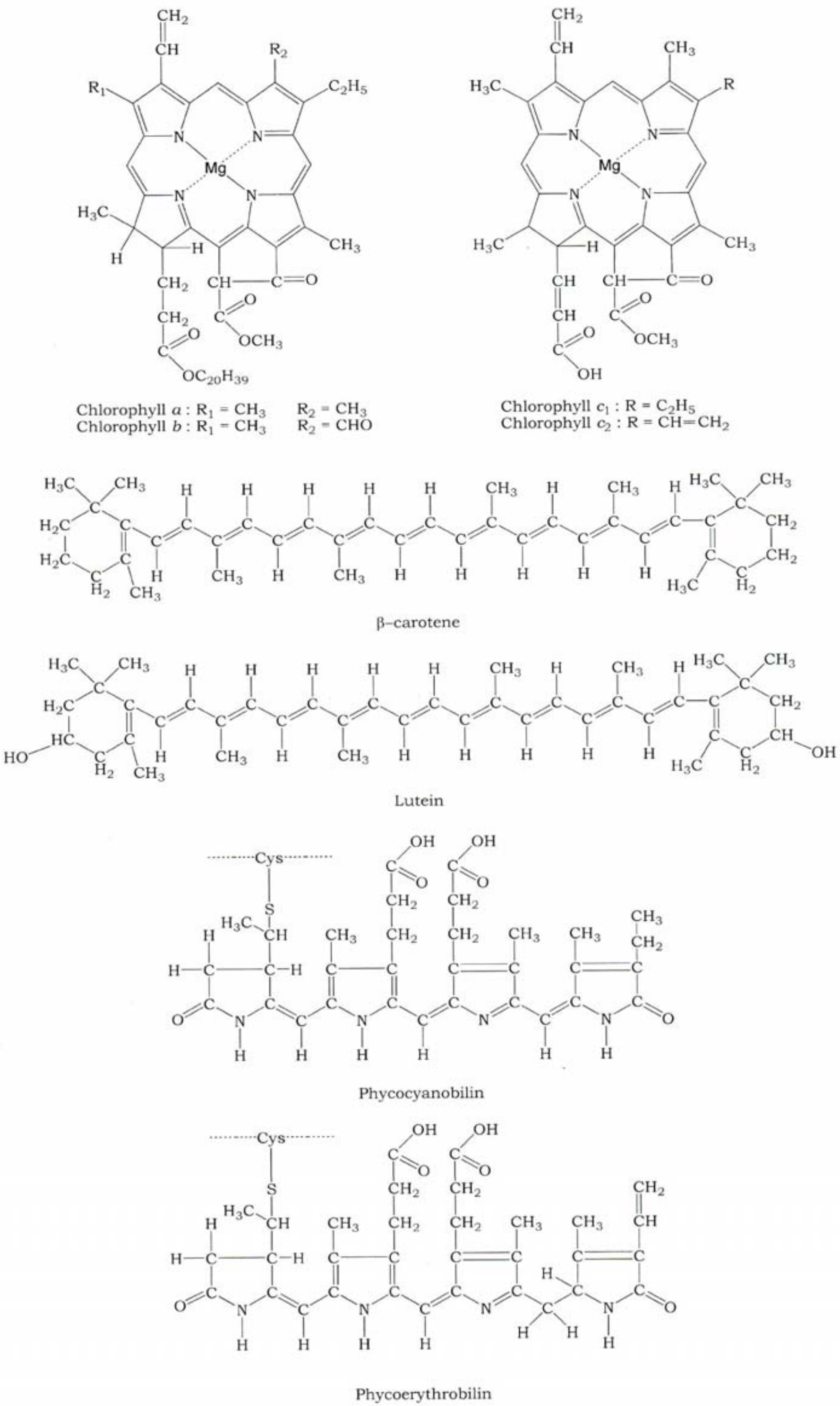


FIGURE 3.2 Structure of the main pigments of the thylakoid membrane.

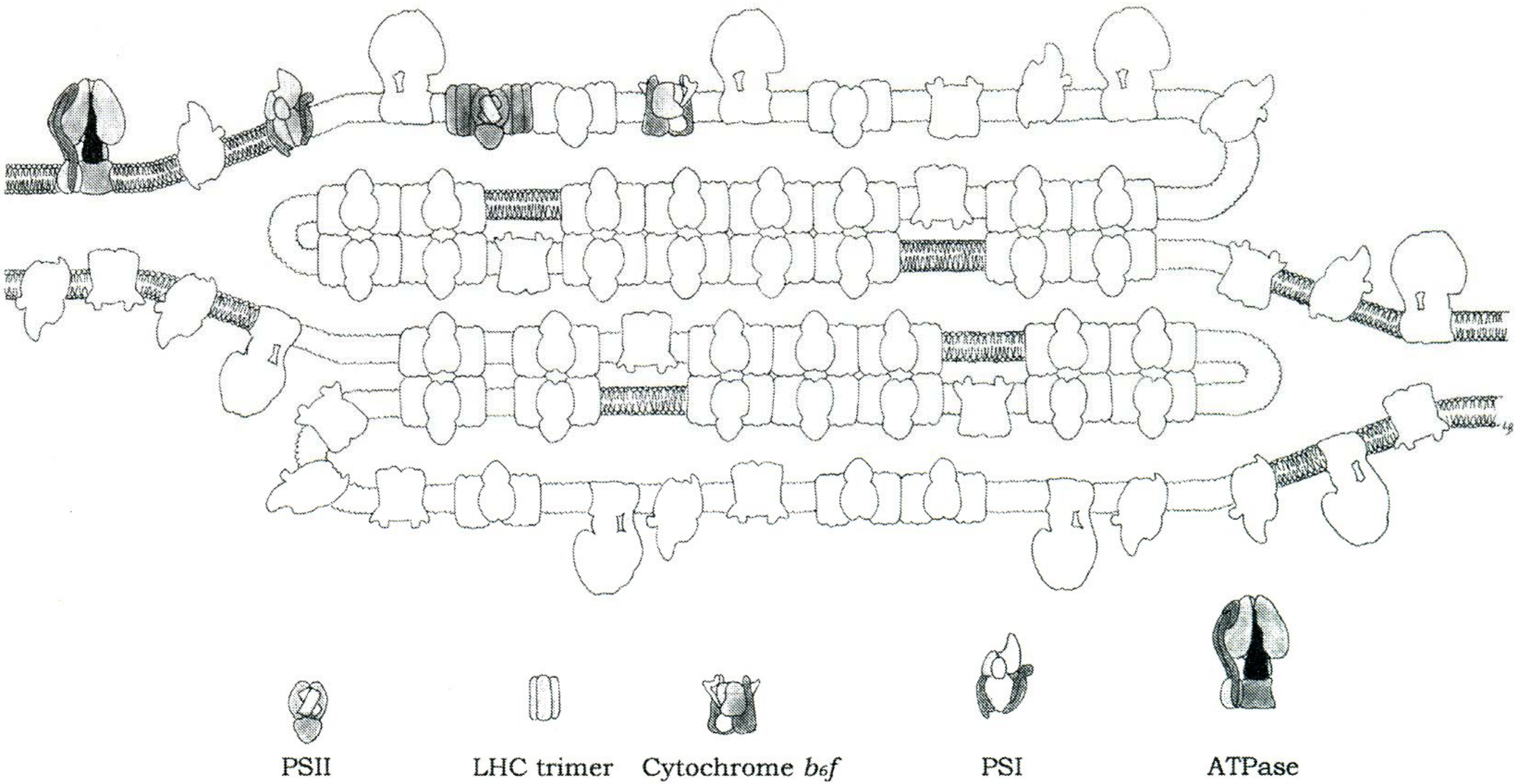


FIGURE 3.3 Model for the topology of chloroplast thylakoid membrane, and for the disposition within the chloroplast of the major intrinsic protein complexes, PSI, PSII, LHCII trimer, Cytochrome b_6f dimer and ATPase. (Redrawn after Allen and Forsberg, 2001.)

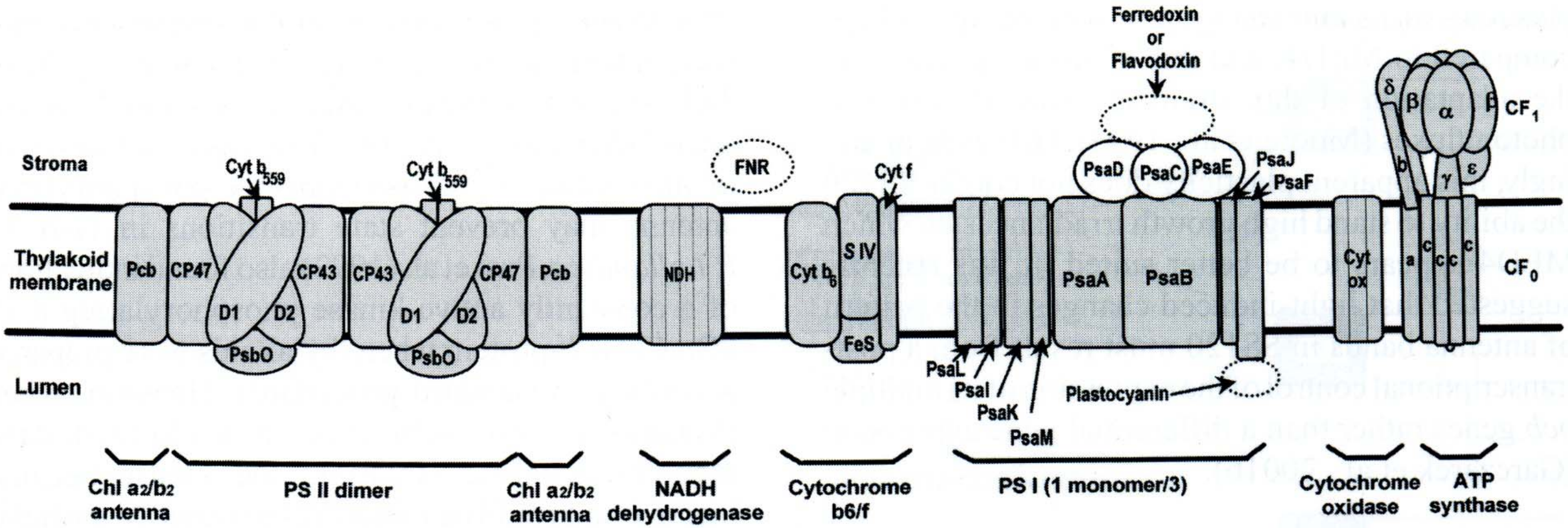


Fig. 5. Diagram showing the photosynthetic apparatus of *Prochlorococcus* sp. MED4. Genes encoding the proteins shown here have either been individually sequenced (see text) or are present in the MED4 genome and have been identified by homology with those of model organisms, such as *Synechocystis* PCC6803. Note that the MED4 strain possesses a single Pcb protein type which is thought to be associated with PS II (T. S. Bibby, F. Partensky, J. Barber (unpublished). A number of putative minor PS II proteins identified in *Synechocystis* (PsbH-M, PsbP, PsbY, PsbZ, Psb27 and Psb28; Kashino et al., 2002) have homologs in the MED4 genome, but are not shown for readability. PsaM, PsbU and PsbV are lacking from the MED4 genome. PS I is organized as trimers (see text), but only one monomer is shown here. The precise organization of the different subunits of NADH dehydrogenase (NDH; 11 subunits) and cytochrome *c* oxidase (Cyt Ox; 3 subunits) are not shown. Dotted forms indicate nonmembrane polypeptides which in Cyanobacteria are known to move and/or exchange with another protein type, depending on physiological conditions (for details, see Bryant, 1994). The localization of the ferredoxin-NADP+ oxidoreductase (FNR), which in Cyanobacteria exists under several isoforms, including one associated with the peripheral rods of PBS, is not yet known in green oxyphotobacteria.

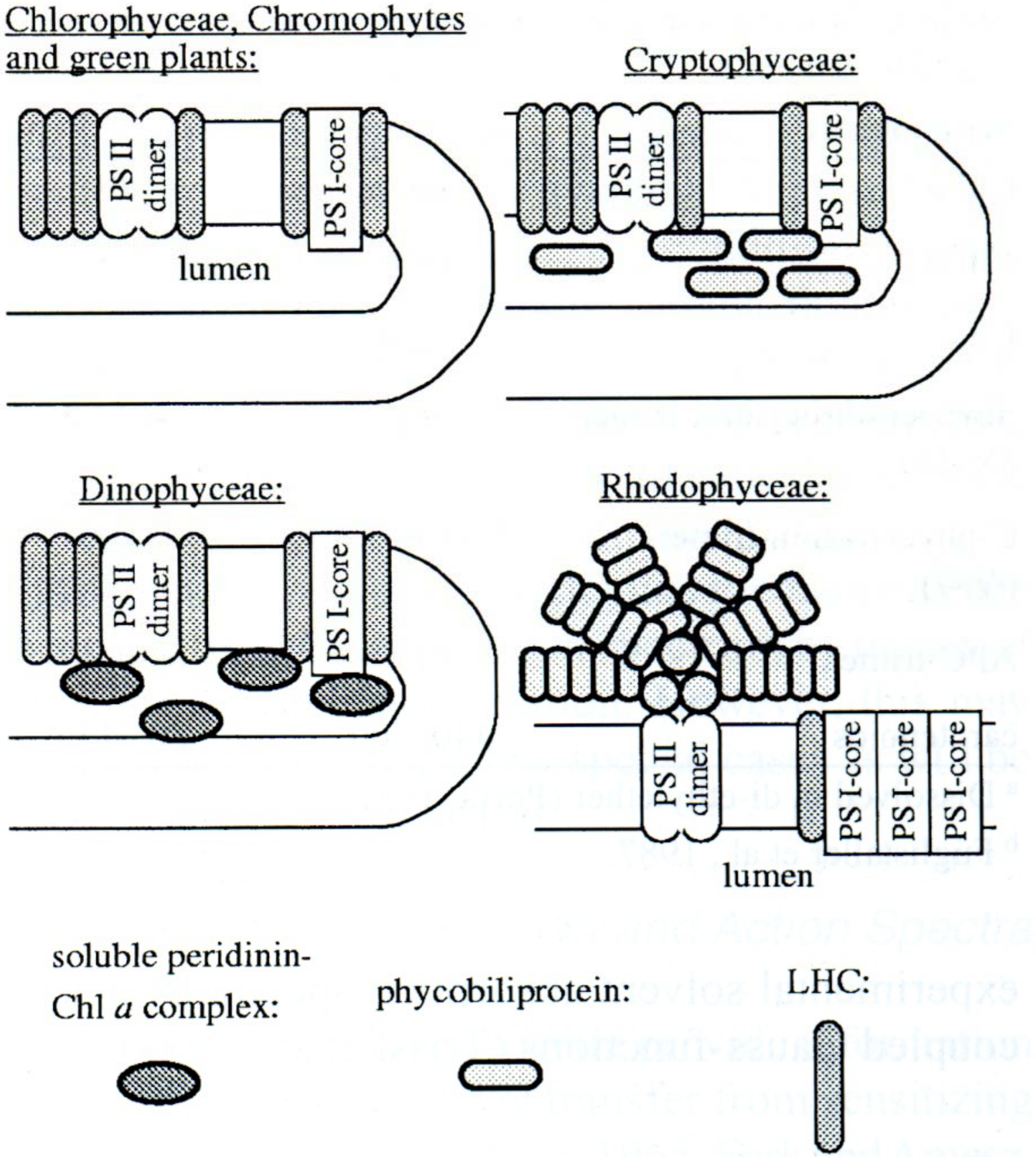


Fig. 3. Association of the different peripheral LHCs with the RCs of PS I and PS II in the thylakoid membranes of different groups of algae.

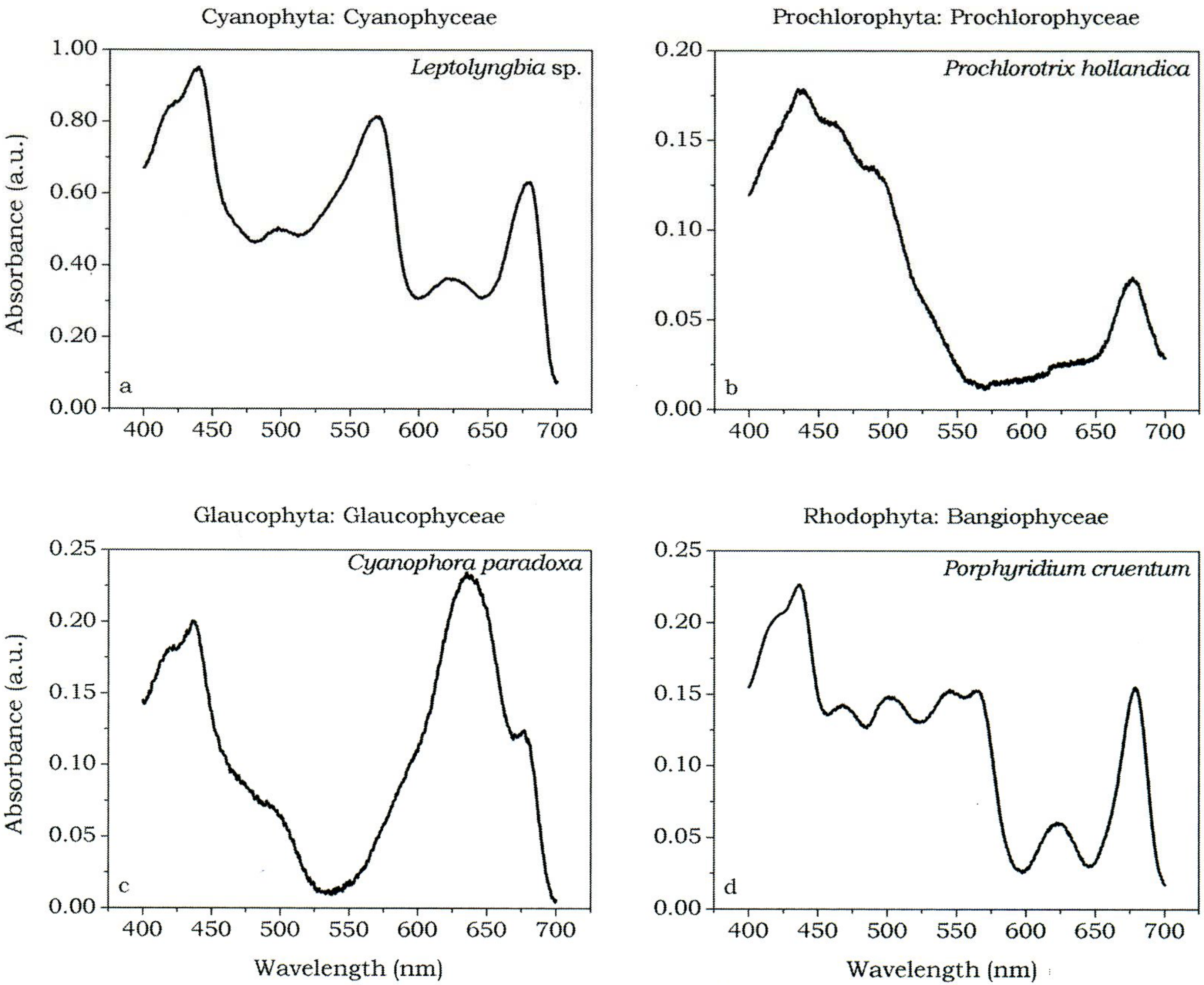


FIGURE 3.5 *In vivo* absorption spectra of photosynthetic compartments of Cyanophyta (a), Prochlorophyta (b), Glaucophyta (c), and Rhodophyta (d).

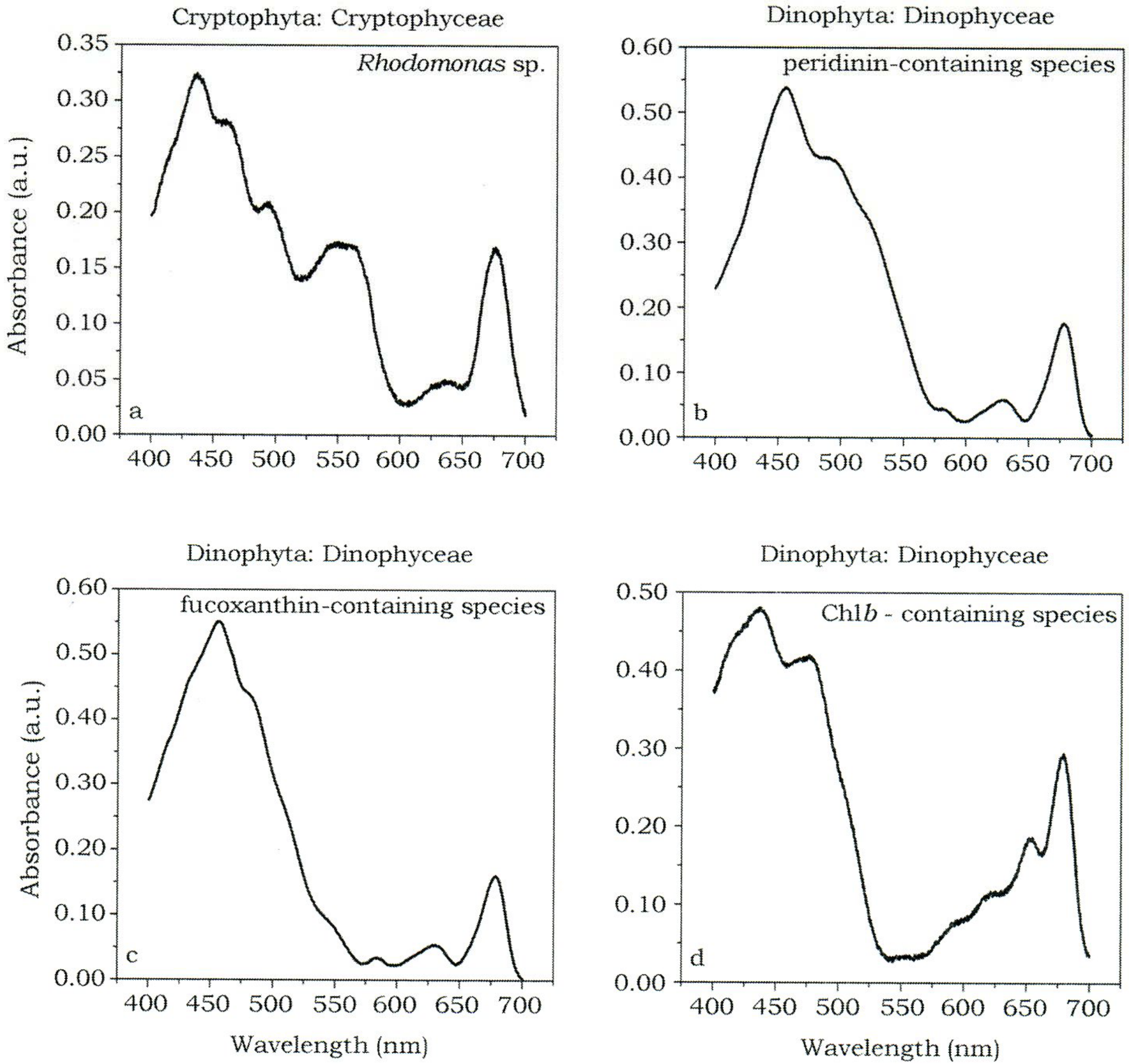


FIGURE 3.7 *In vivo* absorption spectra of photosynthetic compartments of Cryptophyta (a) and Dinophyta (b, c, and d).

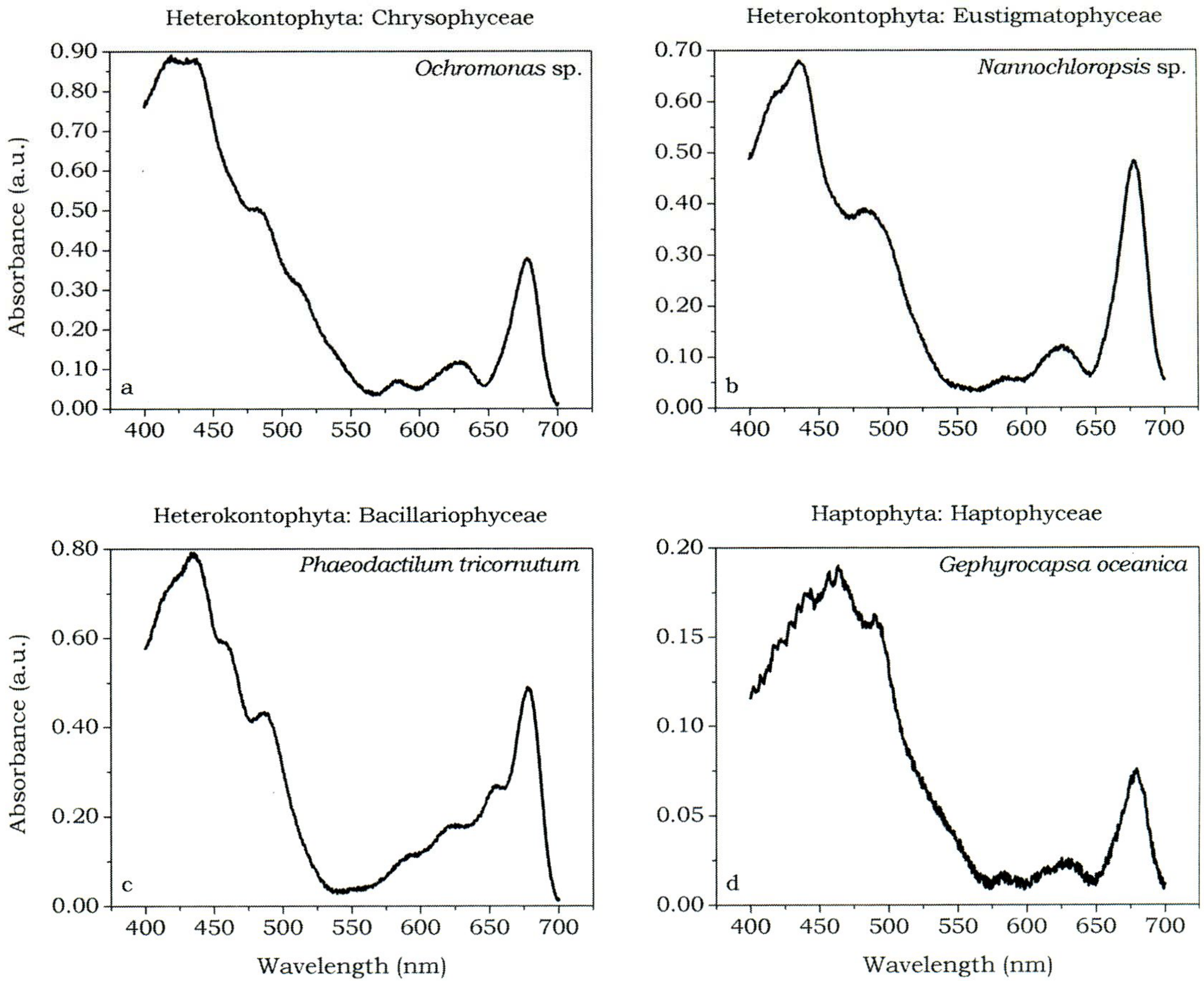


FIGURE 3.6 *In vivo* absorption spectra of photosynthetic compartments of Heterokontophyta (a, b, and c) and Haptophyta (d).

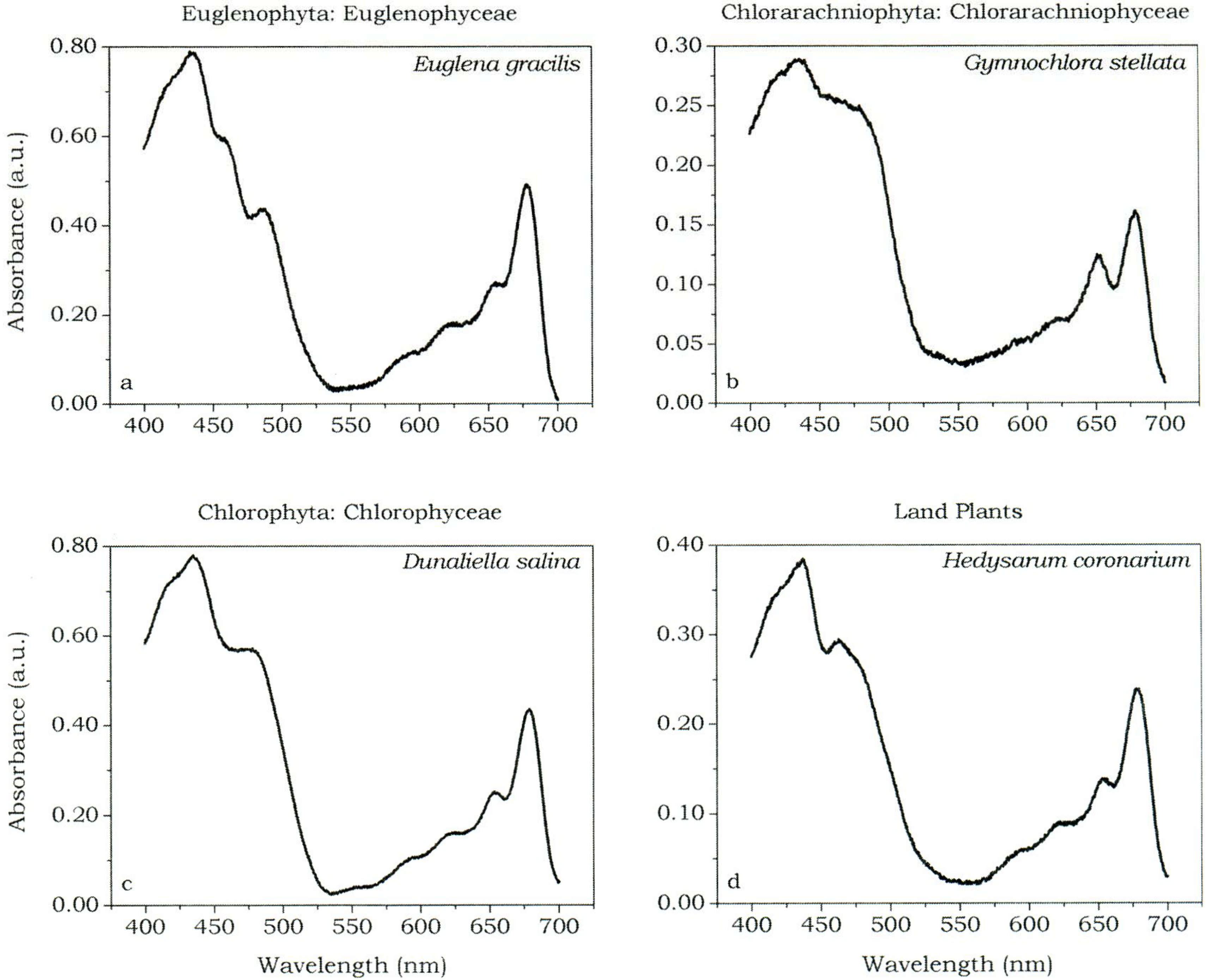


FIGURE 3.8 *In vivo* absorption spectra of photosynthetic compartments of Euglenophyta (a), Chlorarachnophyta (b), Chlorophyta (c), and Land Plants (d).

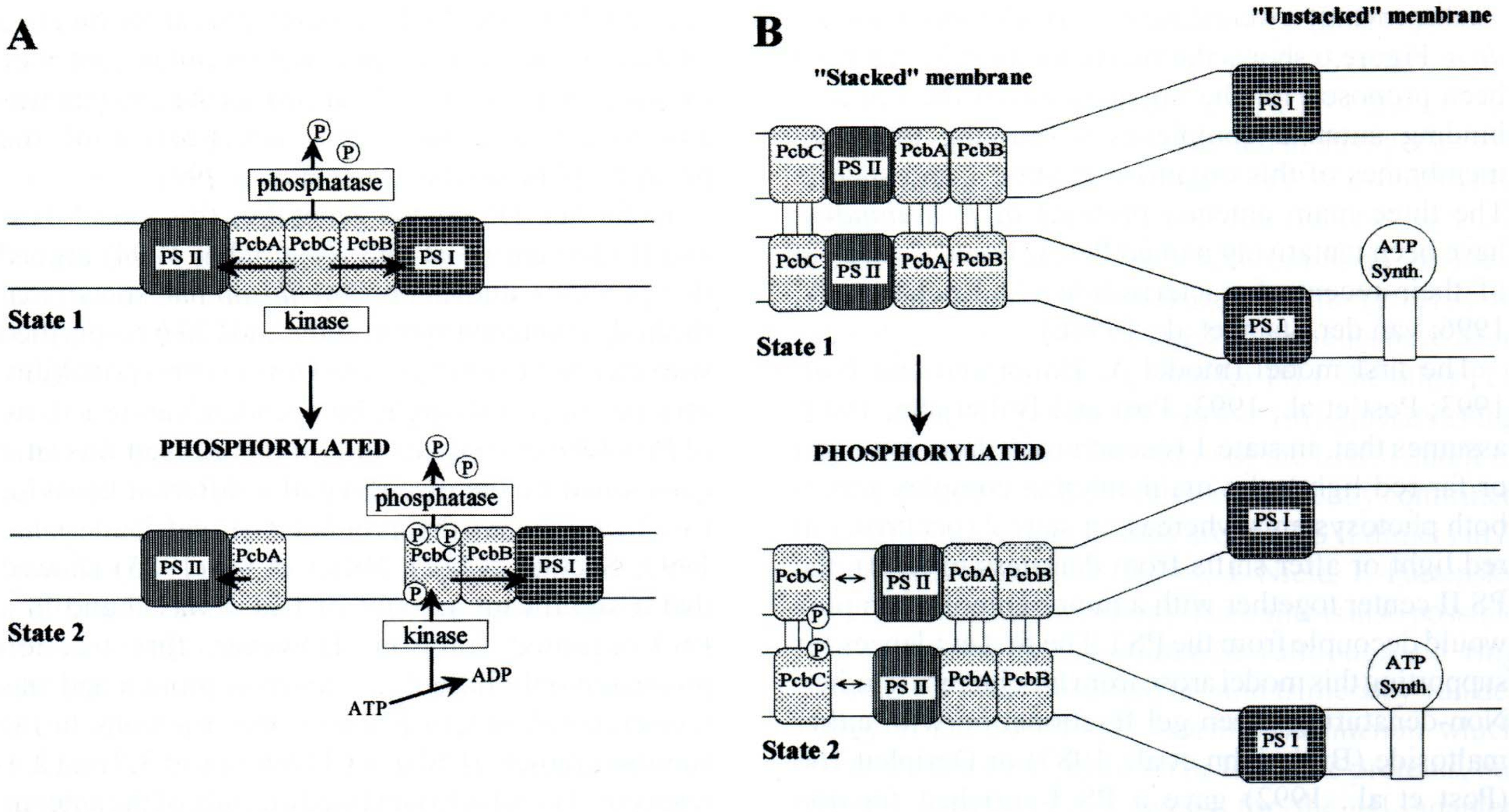


Fig. 6. Current models for the localization of the antenna proteins with regard to photosystems in thylakoid membranes of *Prochlorothrix hollandica* and effect of state 1-state 2 transitions. The original schemes have been redrawn in light of the recent identification of the three Pcb proteins and for a better homogenization. A: Model from Post et al. (1993). The Chl *a/b* antenna consists of a bulk antenna which is located on apoproteins of '30-kDa' (PcbA) and '35-kDa' (PcbC) and associated preferably with PS I. A minor Chl *a/b* antenna is carried by a '33-kDa' (PcbB) apoprotein and is found to co-purify with PS II. The '35-kDa' antenna protein forms the major target protein of light/redox controlled kinase activity. Upon phosphorylation, the bulk antenna excludes PS II centers and enters a tighter association with PS I. Under such conditions, the energy transfer to PS I is enhanced. This process reverses to a state of balanced energy transfer by the bulk antenna following dephosphorylation of the antenna in either darkness or far red illumination. B: Model from van der Staay and Staehelin (1994). As in chloroplasts, PS II and the Chl *a/b* antenna are located in 'stacked' parts of the thylakoid membrane, whereas PS I and the ATP synthase are restricted to the 'unstacked' membranes. The Chl *a/b* antenna associated with PS II comprises four polypeptides with apparent molecular masses of '32-kDa' (PcbA), '33.5-kDa', '35-kDa' (one or both of these probably correspond to PcbB) and '38-kDa' (PcbC), all of which are assembled into one antenna complex. The '38-kDa' antenna gets phosphorylated at high light. In accordance with the fluorescence data, an uncoupling of the phosphorylated antenna from PS II is assumed to occur, but it would not migrate into the 'unstacked' membranes.

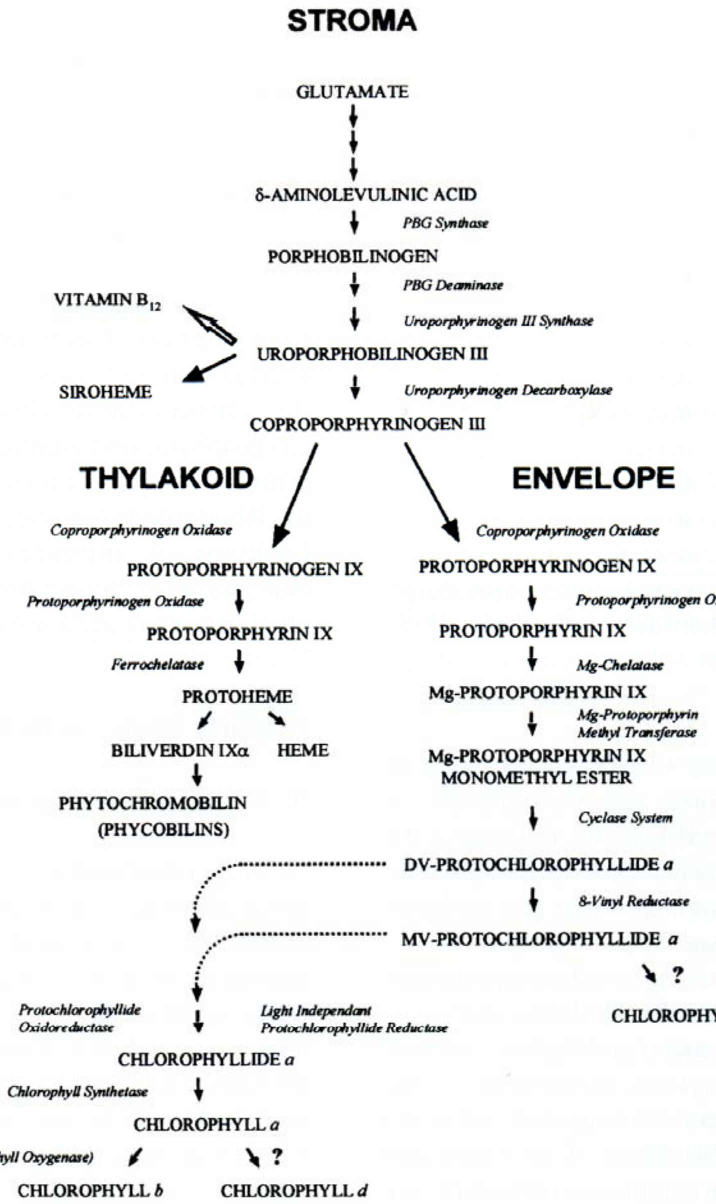


Fig 1. General outline of the tetrapyrrole biosynthesis pathway in photosynthetic organisms. Shown are the major intermediates and enzymes involved in tetrapyrrole formation in plants, algae, and photosynthetic bacteria and the proposed location of the enzymatic activities in plastids. The steps leading to phycobilin and vitamin B_{12} formation appear to be restricted to prokaryotic organisms. The dashed lines indicate the possible transport of these compounds from the plastid envelope to the thylakoids.

Photosynthetic reactions

- general equation:
  - » $nCO_2 + nH_2O + light \xrightarrow{\text{Chl } a} (CH_2O)_n + nO_2$
- light dependent reactions
  - light interception, l. energy transfer
  - excitation, charge separation
  - ETR
    - » linear
    - » cyclic
  - O_2 evolution
- light independent reactions
  - CBB – Calvin Benson Bassham Cycle
  - RuBisCO
  - CA

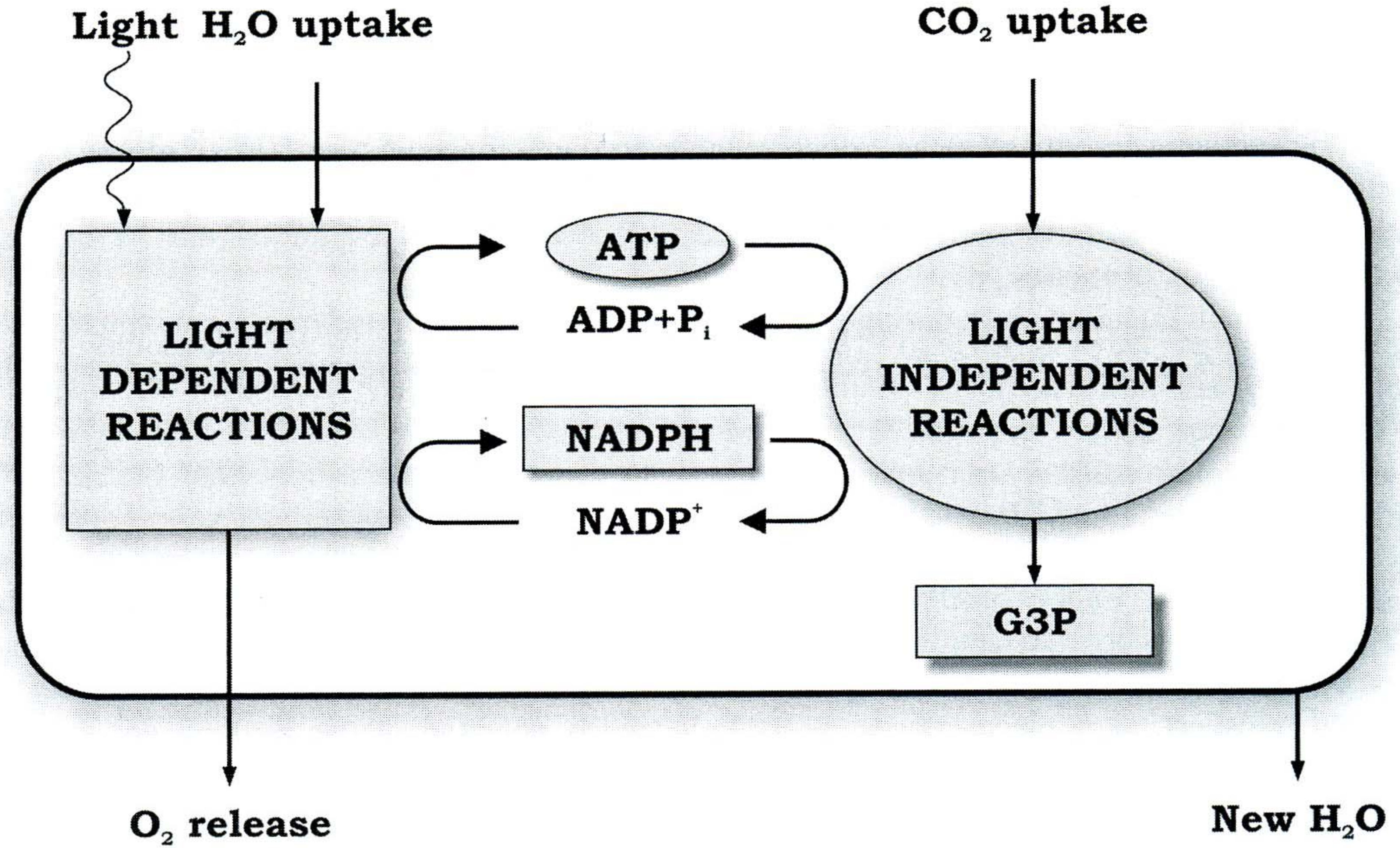
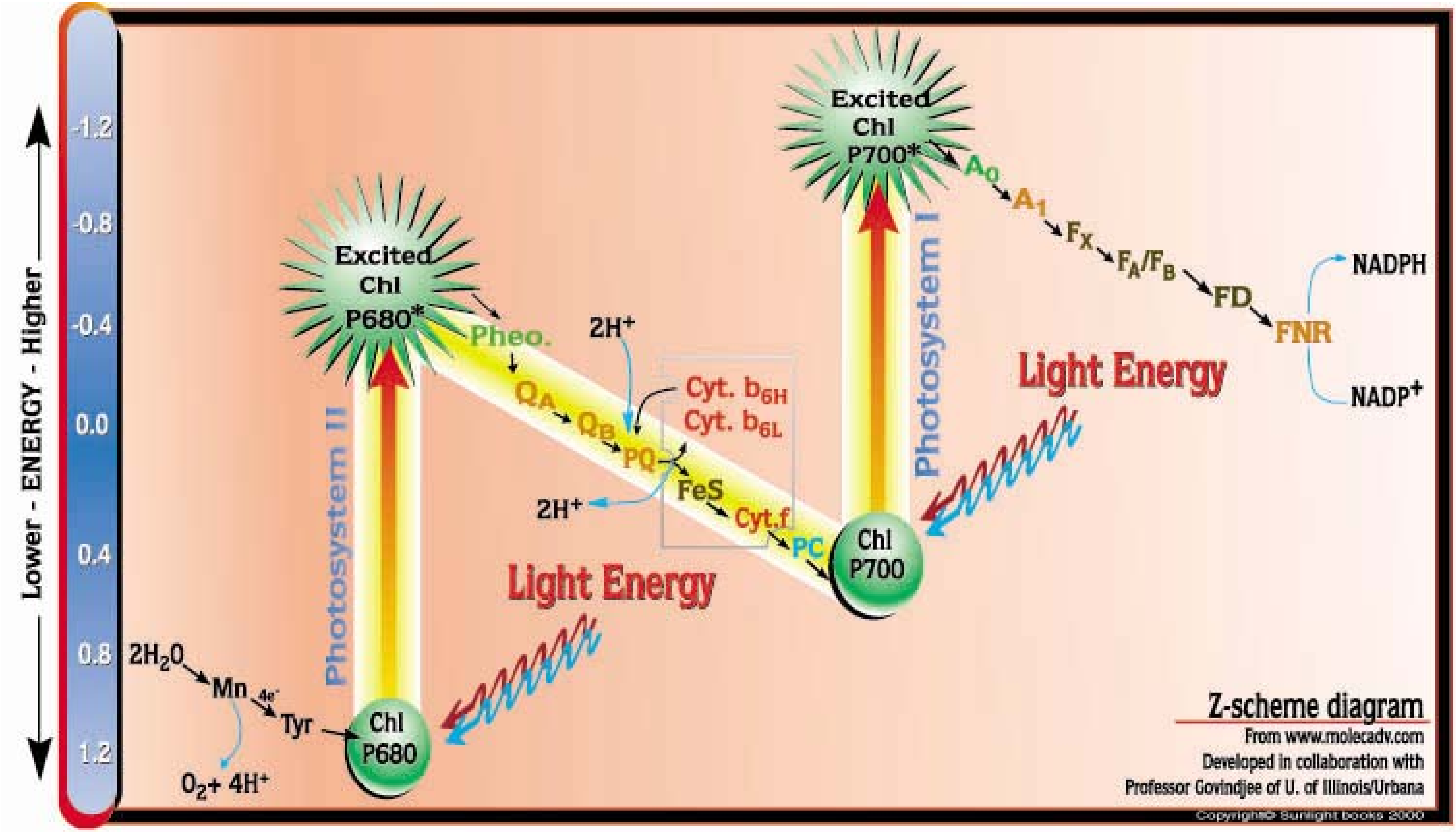


FIGURE 3.1 Schematic drawing of the photosynthetic machinery.



Lower - ENERGY - Higher
-1.2
-0.8
-0.4
0.0
0.4
0.8
1.2
Excited Chl P680*
Pheo.
Q_A
Q_B
PQ
$2H^+$
$2H^+$
Cyt. b_{6H}
Cyt. b_{6L}
FeS
Cyt.f
PC
Photosystem II
Chl P680
Light Energy
$2H_2O$
Mn
$4e^-$
Tyr
$O_2 + 4H^+$
Excited Chl P700*
A_0
A_1
F_X
F_A/F_B
FD
FNR
NADPH
$NADP^+$
Photosystem I
Chl P700
Light Energy
Z-scheme diagram
From www.molecadv.com
Developed in collaboration with
Professor Govindjee of U. of Illinois/Urbana
Copyright© Sunlight books 2000

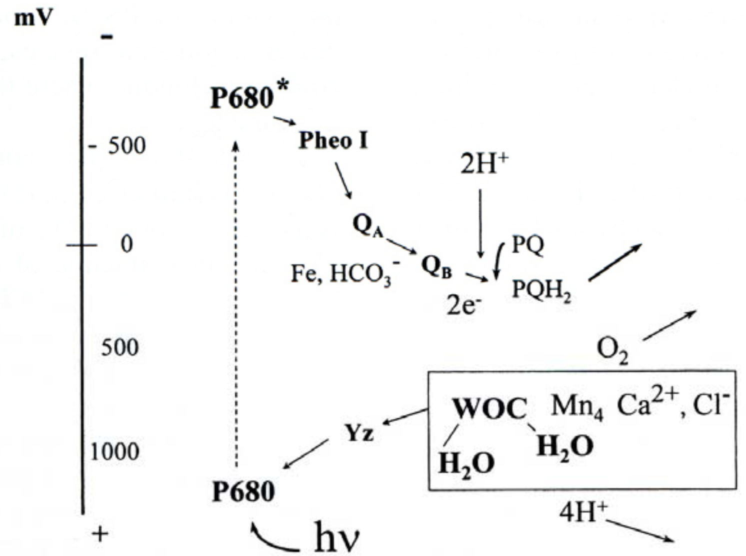


Fig. 1. Diagram showing the main cofactors of PS II, the pathway of electrons through the reaction center and the probable redox potential relationship between the cofactors. Electron flow occurs from water to Q_B. Absorption of light energy (hν) or excitons by P680, producing P680*, leads to a photochemical charge separation between P680 and Pheo I. Water donates electrons to $P680^+$ via Y_Z (D1 Tyr161). The water oxidizing complex (WOC) involves Mn, plus possibly Ca^{++} and Cl^- cofactors, oxygen evolution requiring four turnovers of the reaction center. A non-heme iron (Fe) is located between Q_A and Q_B and bicarbonate (HCO_3^-) also binds in this region. Q_B picks up two electrons and two protons, transferring these to the membrane plastoquinone pool (PQ). Key: P680, the primary chlorophyll electron donor; P680*, excited state of P680; Pheo, pheophytin electron acceptor I; Q_A and Q_B, primary and secondary plastoquinones.

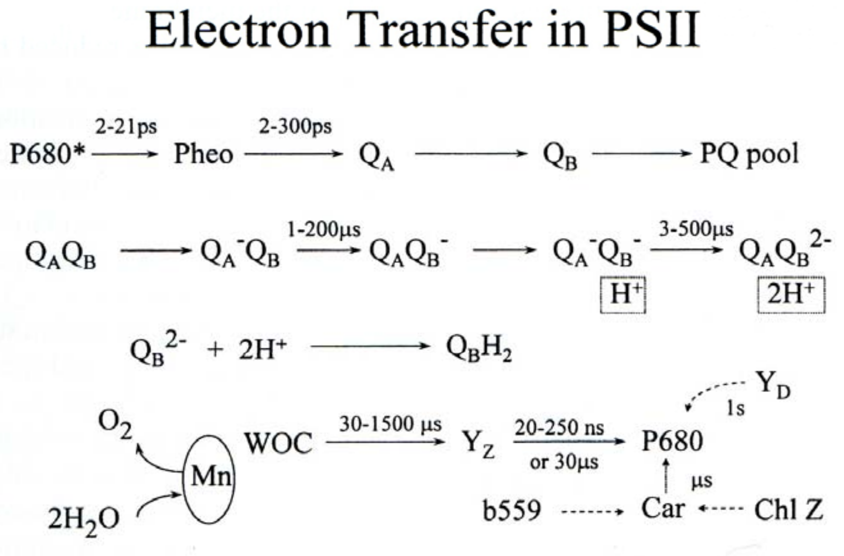


Fig. 2. Diagram showing the main kinetics of the main pathway of electrons through the PS II reaction center. Y_D (D2 Tyr161), Chlorophyll Z (Chl Z) and Cytochrome b_{559} (b_{559}) are alternate electron donors under certain conditions. See also Figure 1.

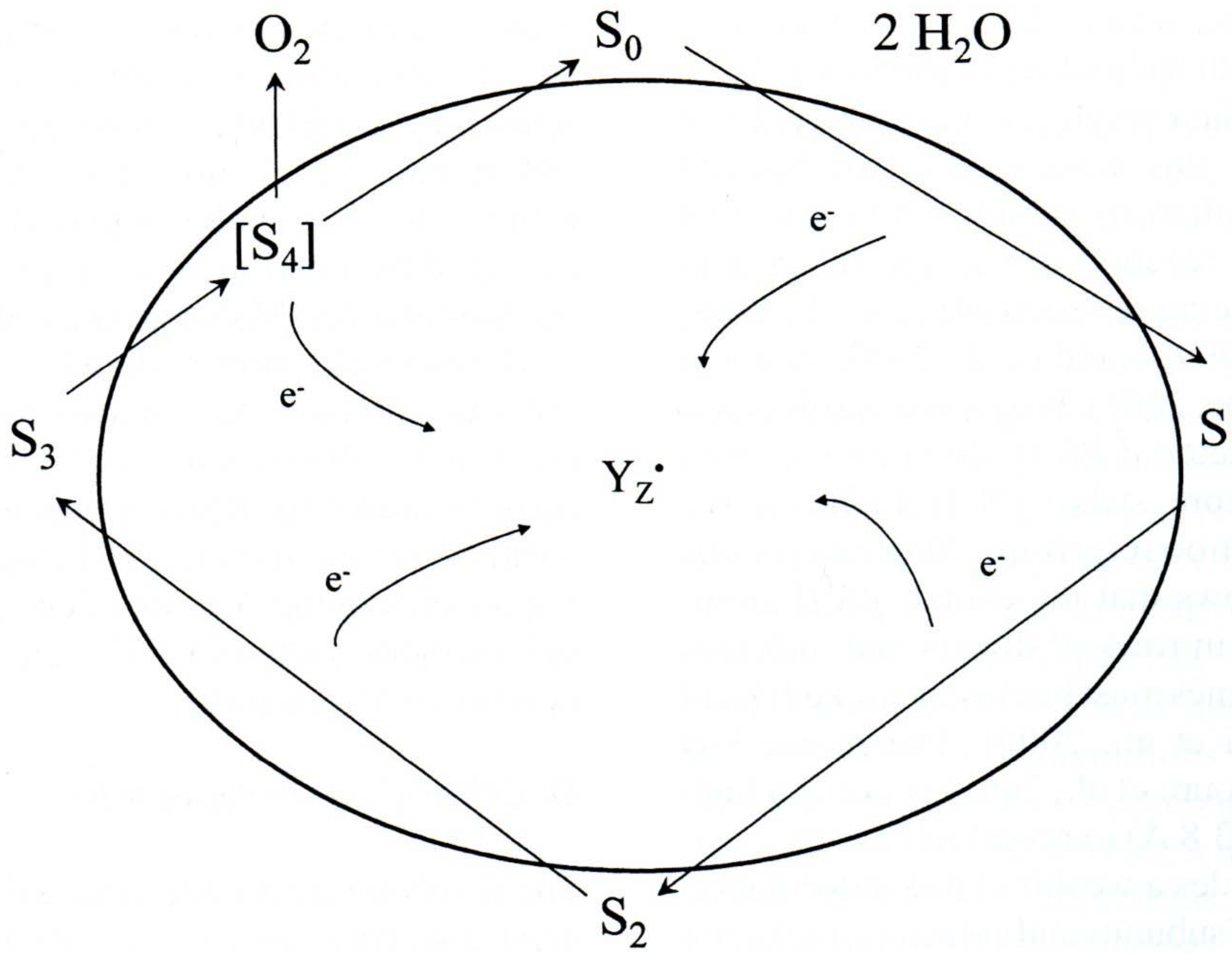


Fig. 3. The S-states of the water oxidizing complex (WOC). Electrons are removed sequentially by $P680^+$ via Y_Z. The S-state number indicates the number of oxidizing equivalents stored. On reaching S_4, oxygen is released and the cycle reset. The steps at which water may be bound, oxidized and protons released are discussed in the text.

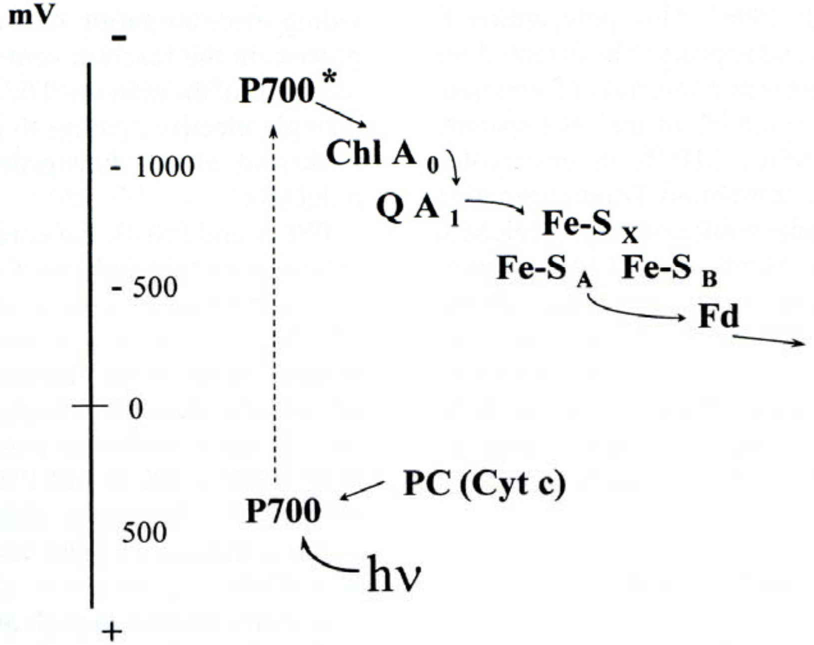


Fig. 4. Diagram showing the main cofactors of PS I, the pathway of electrons through the reaction center and the probable redox potential relationship between the cofactors. Absorption of light energy (h*ν*) or excitons by P700 leads to a photochemical charge separation. Electron flow occurs from Plastocyanin (PC) to Ferredoxin (Fd). Electron transfer from P700 occurs across the membrane to reduce the iron-sulfur centers. PC donates an electron to $P700^+$. Key: P700, primary electron donor; P700*, excited state of P700; A_0, Chl electron acceptor; A_1, phylloquinone (Q); Fe-S_X, Fe-S_A and Fe-S_B, are iron-sulfur centers; Cyt *c*, cytochrome *c* is an alternative electron donor.

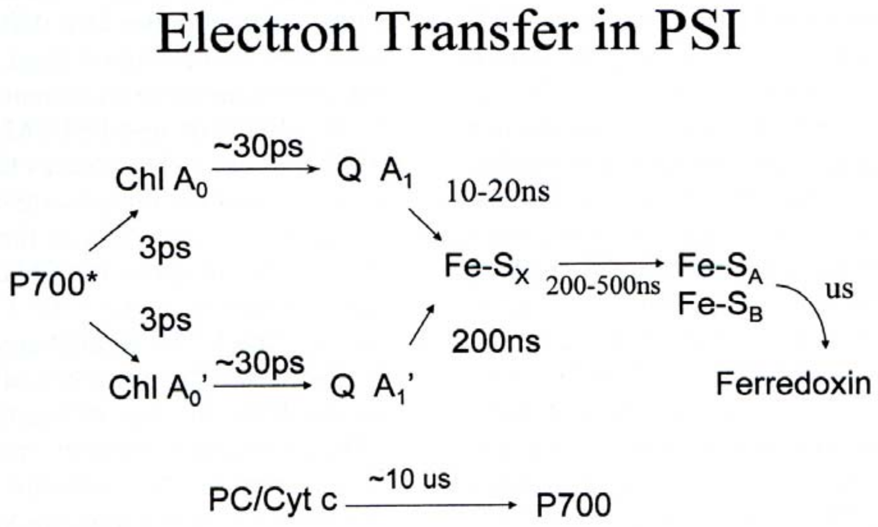
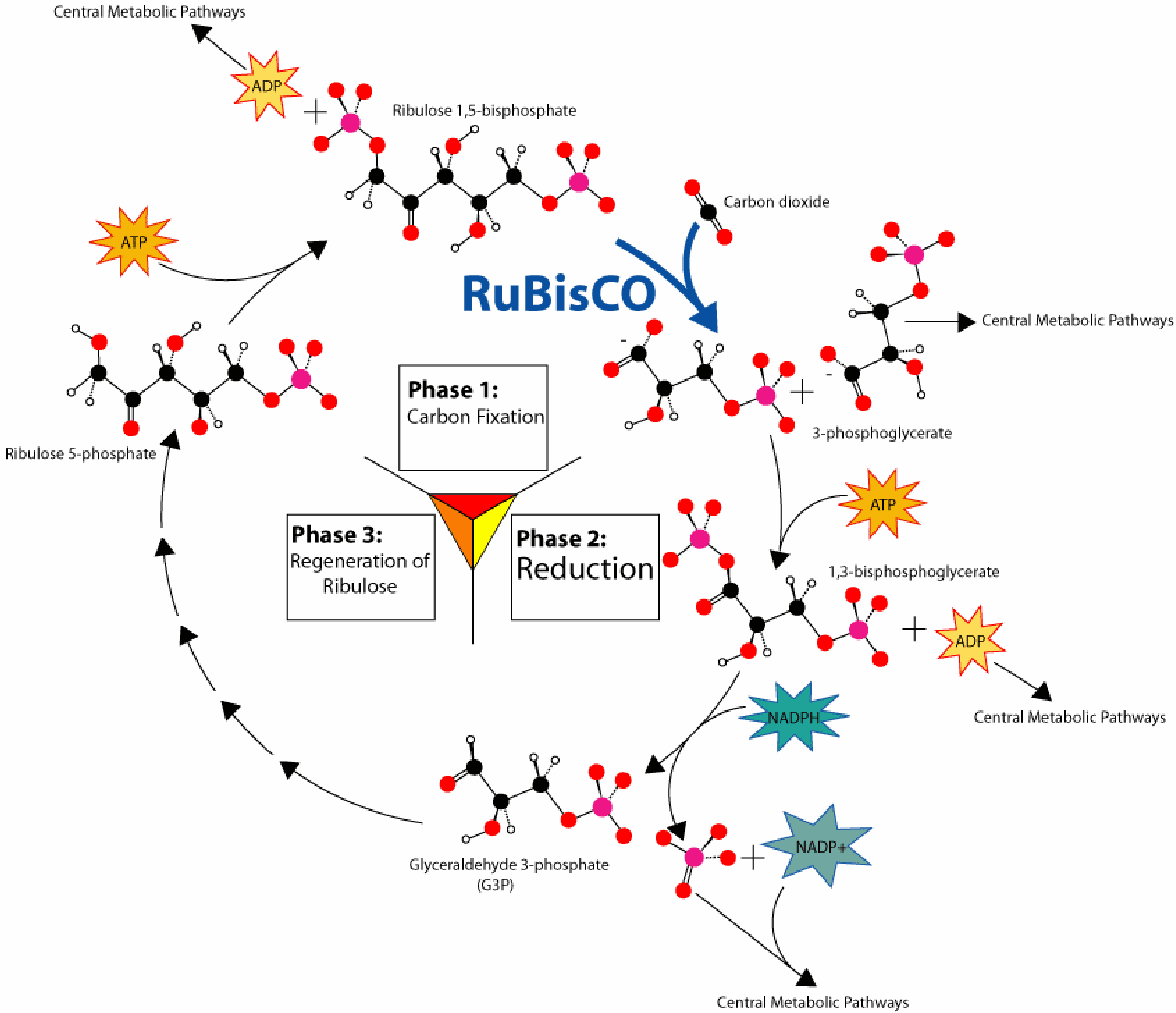


Fig. 5. Diagram showing the main kinetics of the main pathway of electrons through the PS I reaction center.



Central Metabolic Pathways
ADP
Ribulose 1,5-bisphosphate
ATP
Carbon dioxide
RuBisCO
Central Metabolic Pathways
3-phosphoglycerate
Ribulose 5-phosphate
Phase 1:
Carbon Fixation
ATP
Phase 3:
Regeneration of
Ribulose
Phase 2:
Reduction
1,3-bisphosphoglycerate
ADP
NADPH
Central Metabolic Pathways
Glyceraldehyde 3-phosphate
(G3P)
NADP+
Central Metabolic Pathways

Role of Carbon Concentrating Mechanism

- Carbonic Anhydrase
  - periplasmic space, carboxysomes (b-g algae), pyrenoid (algae)
  - CA *vs.* RuBisCO
- mechanisms of HCO_3^- uptake
  - active transp. via CMP (enough ATP)
  - symport Na^+ HCO_3^- - via icpB complex
  - Na^+/H^+ antiport help
  - difusion
- CO_2 uptake - difusion

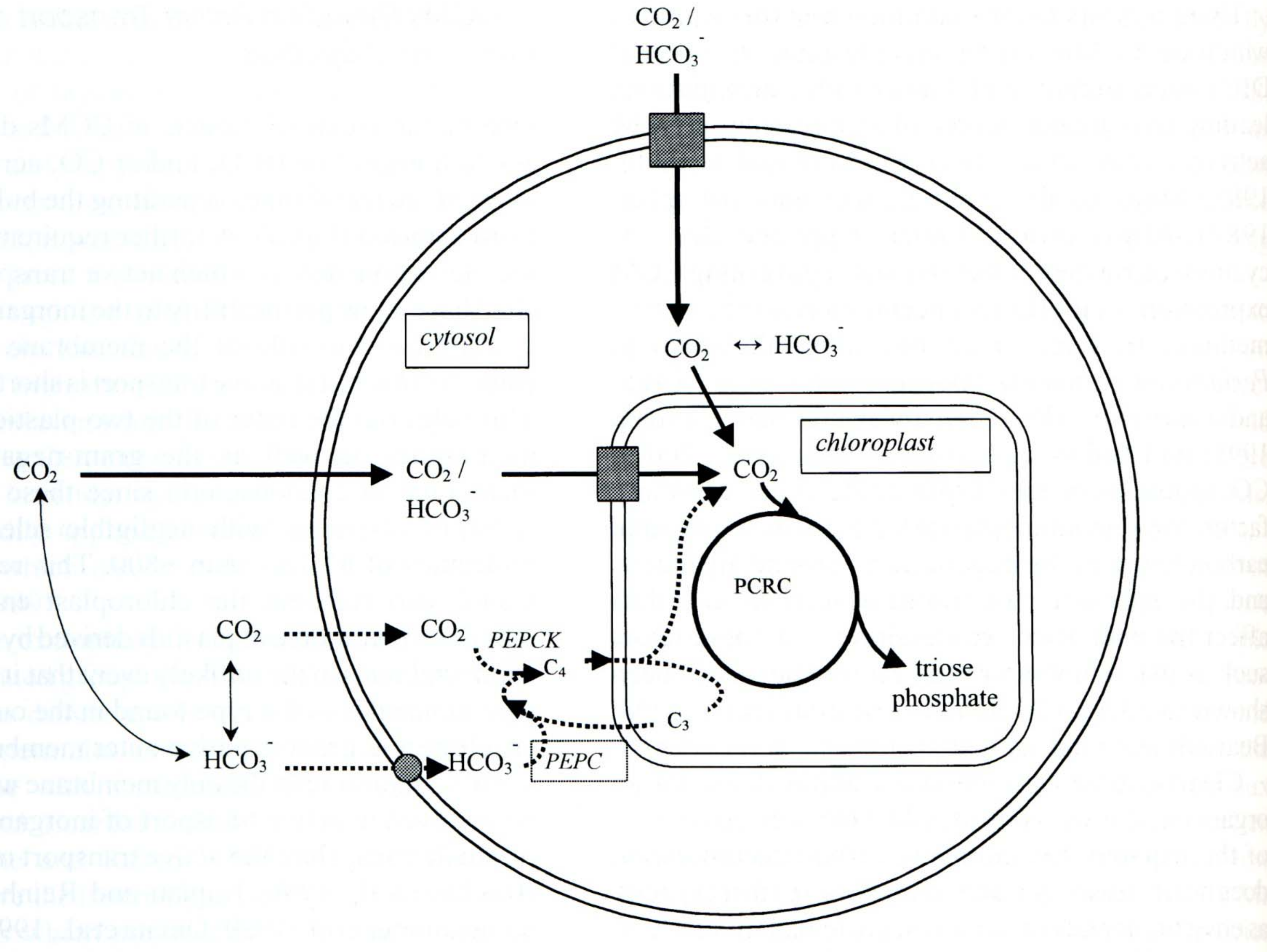


Fig. 1. A simplified scheme for transport of inorganic carbon into eukaryotic algal cells via active transport of CO_2 and/or bicarbonate. As explained in the text, CO_2 will cross membranes by diffusion, whereas active transport (shown by the shaded boxes) can be of CO_2 or HCO_3^-. Active transport can occur at the plasmalemma or at the chloroplast envelope or at both membranes. Carbonic anhydrases in the periplasmic space, cytosol and chloroplast maintain equilibrium between CO_2 and HCO_3^-. Also shown (dotted line) is a putative role for C_4-like metabolism in CO_2 concentration (see text for details). PCRC – photosynthetic carbon reduction cycle; PEPC – phosphoenolpyruvate cartboxylase; PEPCK – phosphoenolpyruvate carboxykinase. Redrawn after Sültemeyer (1998).

Energy transfers & regulations

- excitation energy (excess) dissipation pathways
  - photosynthesis
  - state stransitions
  - heat production (Xanthophyll cycle)
  - fluorescence
- photoaclimation
  - light dependent motions; state transitions; non-photochemical processes
- photoinhibition
  - photomodification
  - photodamage

- photorespiration & chlororespiration
    - RuBisCo – generation of glycolate
    - enigma; PQ pool reducion

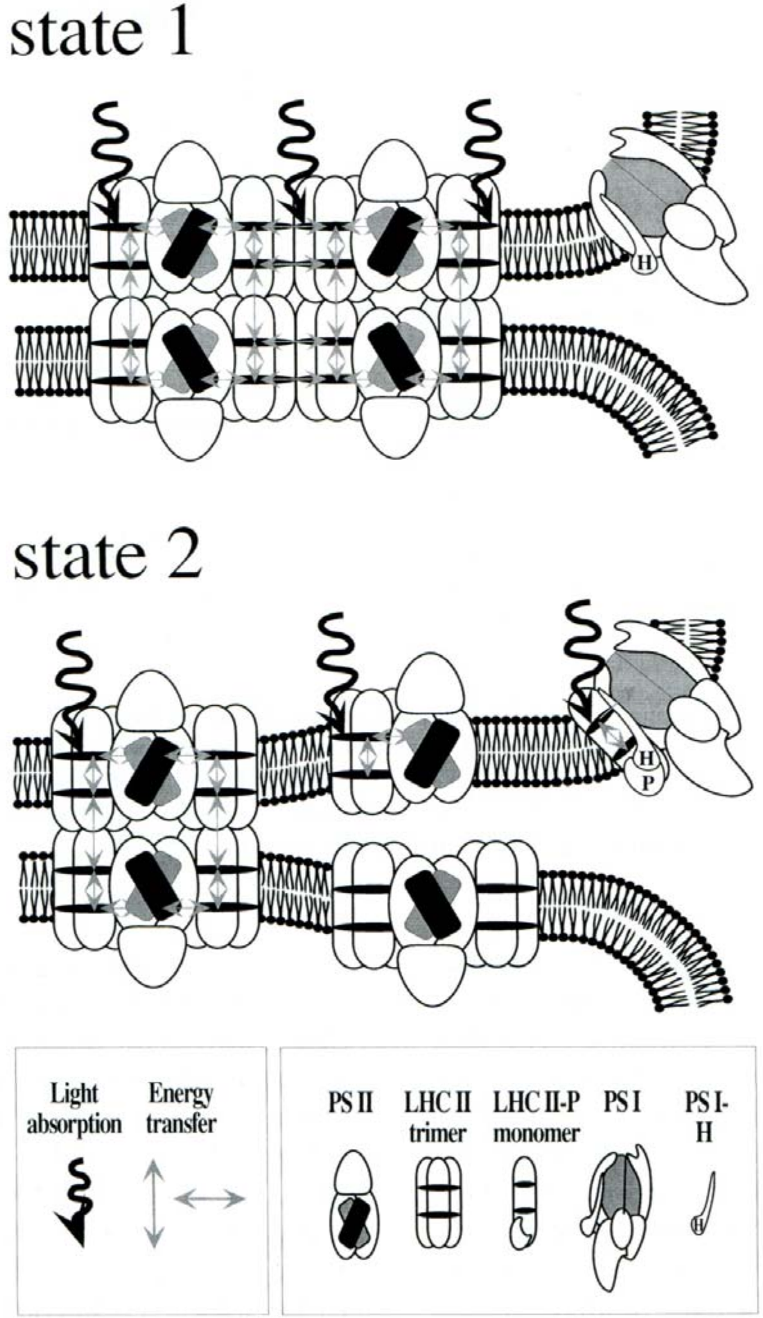
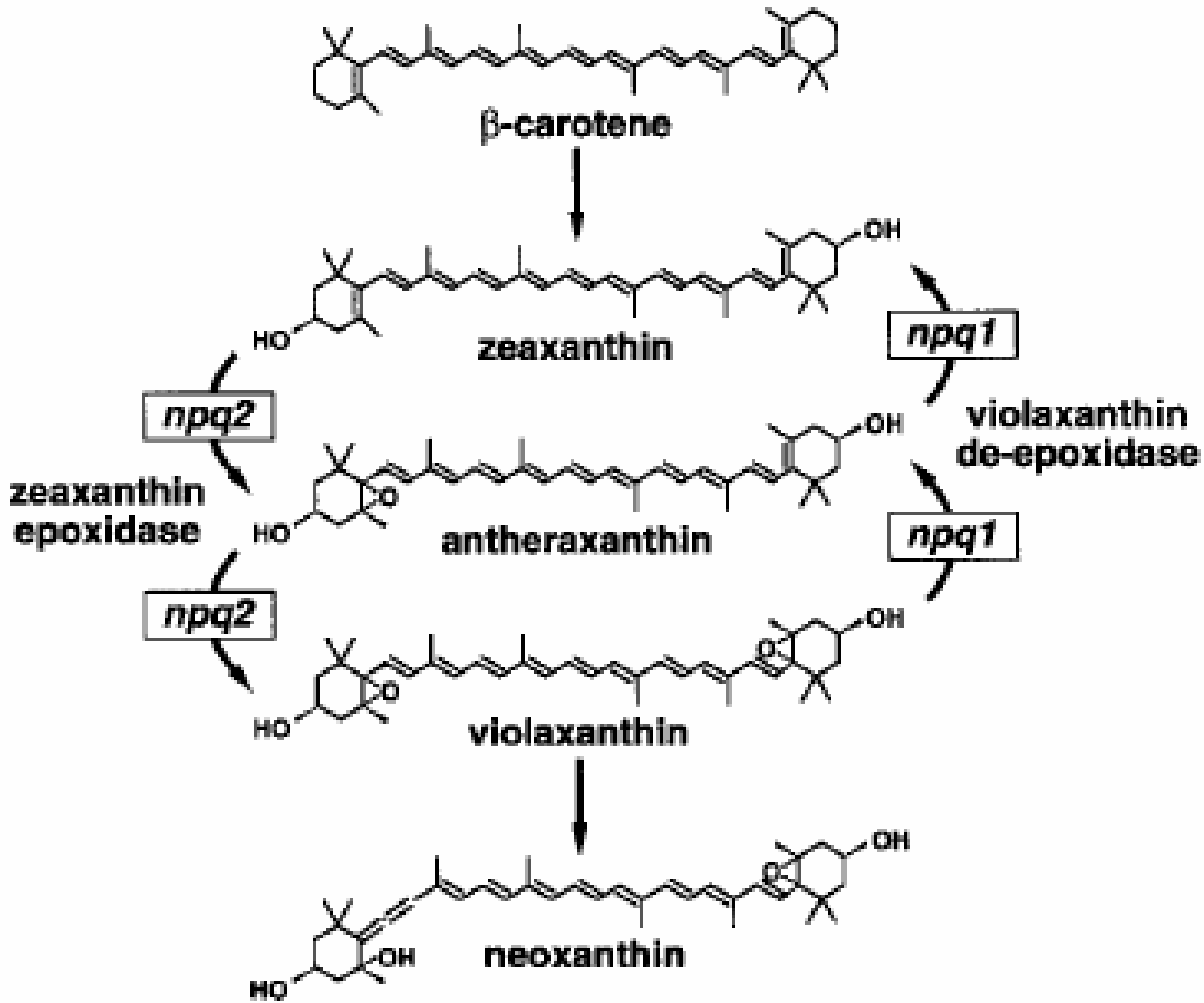


Fig 6. Artist's impression of the difference between State 1 and State 2 organization in appressed and non-appressed thylakoids in green plants. Redrawn with permission from Forsberg and Allen (2001a).



β-carotene
zeaxanthin
antheraxanthin
violaxanthin
neoxanthin
npq2
npq2
zeaxanthin epoxidase
npq1
npq1
violaxanthin de-epoxidase
HO
OH
O

Photosynthesis – Irradiance response curve (P *vs.* E curve)

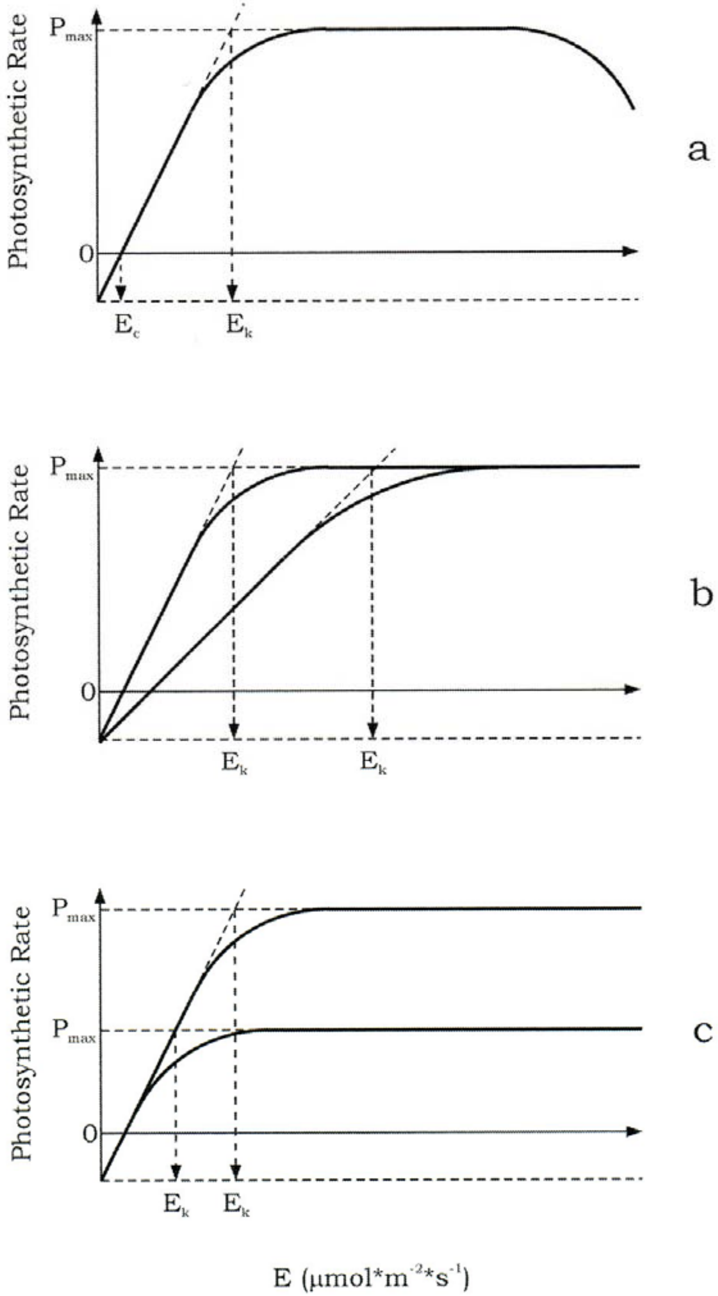


FIGURE 5.15 Photosynthesis–irradiance response curves: E_c, irradiance compensation point; E_k, saturating irradiance; and P_{max}: maximum photosynthetic rate. (a) typical plot; (b) comparison of two curves with different slopes: keeping constant the number of photosynthetic units, but increasing the functional absorption cross-section, the slope increases; and (c) comparison of two curves with different maximum photosynthetic rate: increasing the number of photosynthetic units, P_{max} increases.

Measurement of photosynthetic production

Methods:

- gravimetric
- turbidimetric
- fluorometric
- gasometric
  - IRGA; O_2 measurement

Physiology and Cultivation of Algae and Cyanobacteria

4.

Biogechemical role of algae

- Energy flow in the environment
- Limiting nutrients
  - Liebig's Law of the Minimum
  - $\mu = \mu_{max}$ [LM] / [LM] + K_m
- Cycles of nutrients
  - Phosphorus
  - Nitrogen
  - Sulphur
  - Carbon & Oxygen
- environmental factors

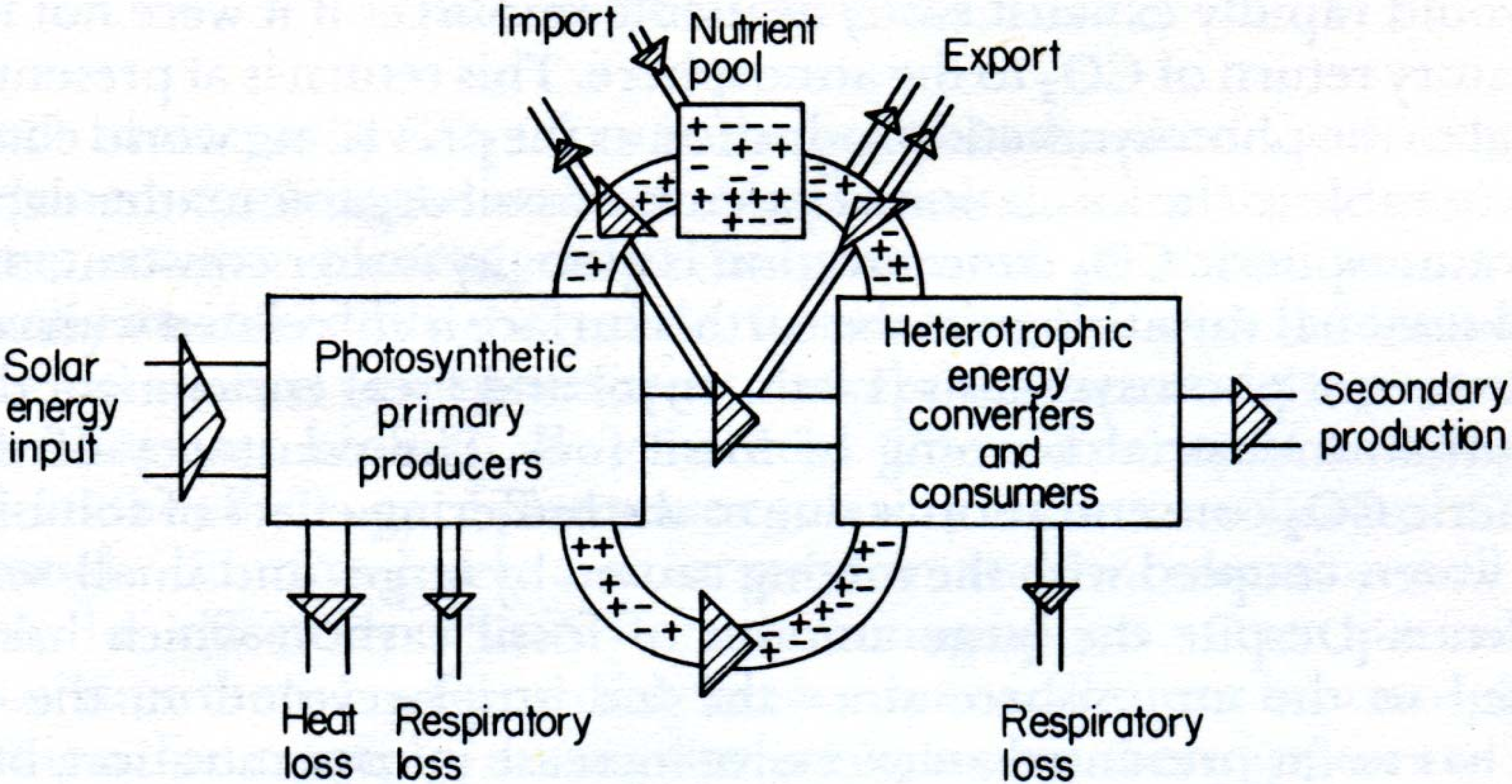


Figure 9.2 The relationship of the ecosystem nutrient cycle to energy flow. After E. P. Odum, *Fundamentals of Ecology*, 3rd. Edn., Saunders, Philadelphia, 1971, p. 87.

Phosphorus

- PO_4^{3-}
- membranes, coenzymes, DNA, RNA, ATP
- orthophosphate; metaphosphate (polyphosphate); “organic” phosphate

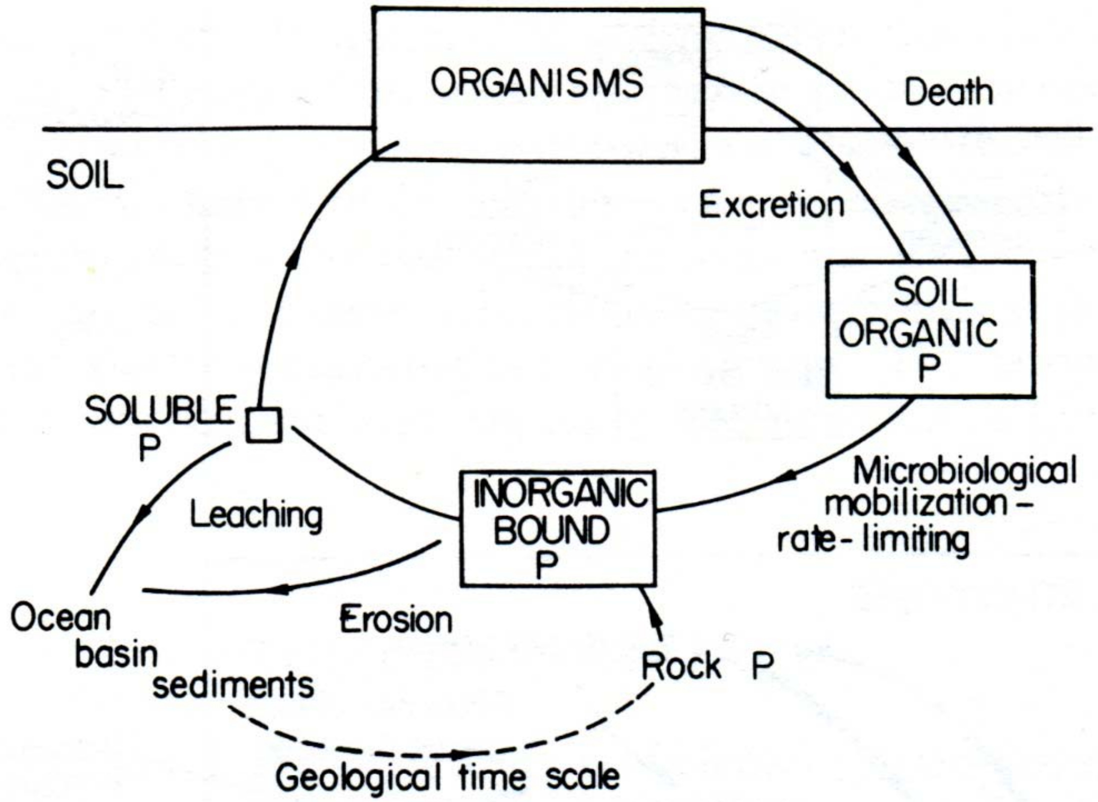


Figure 9.5 The local cycle of phosphorus. Both the organic and inorganic pools of soil phosphorus are essentially rather immobile with the result that the mobilization of organic P into the plant-available phosphate form is rate-limiting to the cycle, and also that the leaching loss of soil P is fairly slow. In many cases it is physical loss by soil erosion which removes P from natural ecosystems and agricultural land. Loss by these two mechanisms is only counteracted on a geological time-scale when ocean basin sediments become elevated to form new land surfaces. Soil P is normally very insoluble but the addition of fertilizer P causes a great increase in the rate of leaching to ground water or water courses.

Nitrogen

- diazotrophs
  - N-fixation (N_2 >> NH_3)
  - assimilation (NO_3^-&NH_4^+ >> organic N)
  - mineralization (organic N >> NH_4^+)
  - nitrification (NH_4^+&NO_2^- >> NO_3^-)
  - denitrification (NO_3^- >> NO, N_2O, N_2)
- nitrogenase; MoFe(V)-protein; PS I;
- *Nostoc*, *Anabaena*, *Trichodesmium*, *Katagnymene*

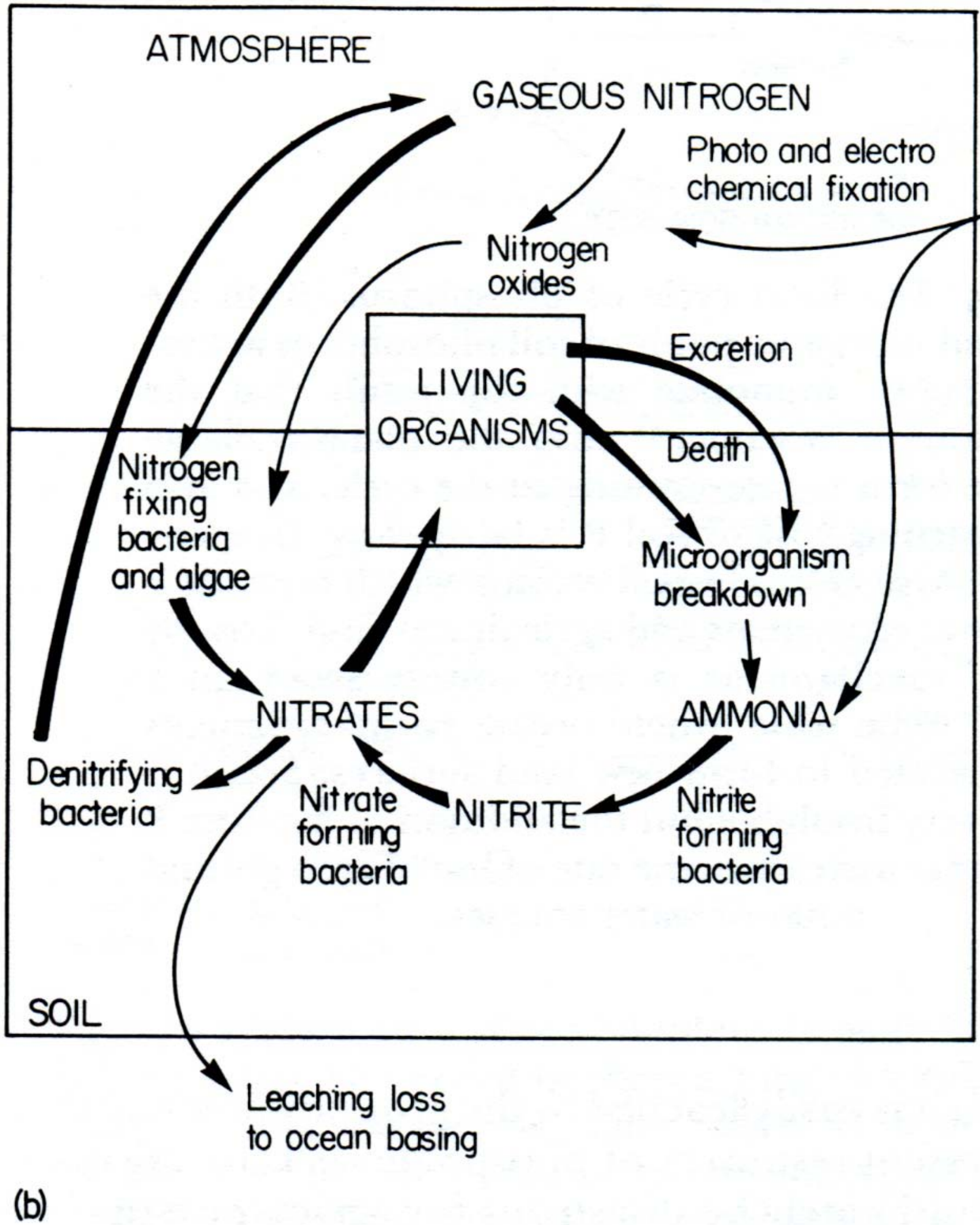


Figure 9.4 The nitrogen cycle. (a) Major relationships between the very large atmosphere pool of gaseous nitrogen and the biosphere. (b) The complex interrelationships of the soil-based portion of the cycle.

Silicon

- $[Si(OH)_4]$; $(SiO_2 \cdot nH_2O)$
- silica-forming organism
    - Chrysophyceae
    - Bacillariophyceae
    - Dictyochlorophyceae
- cell wall; sediments

Sulphur

- aminoacids (Cys, Pro, cystine)
- SO_2, SO_4^{2-}, H_2S,
- APS (adenosine-5’-phosphosulphate)
- PAPS (3’-phosphoadenosine-5’-phosphosulphate)
- DMS (dimethyl sulphide)
- DMSP (dimethylsulphonium propionate)

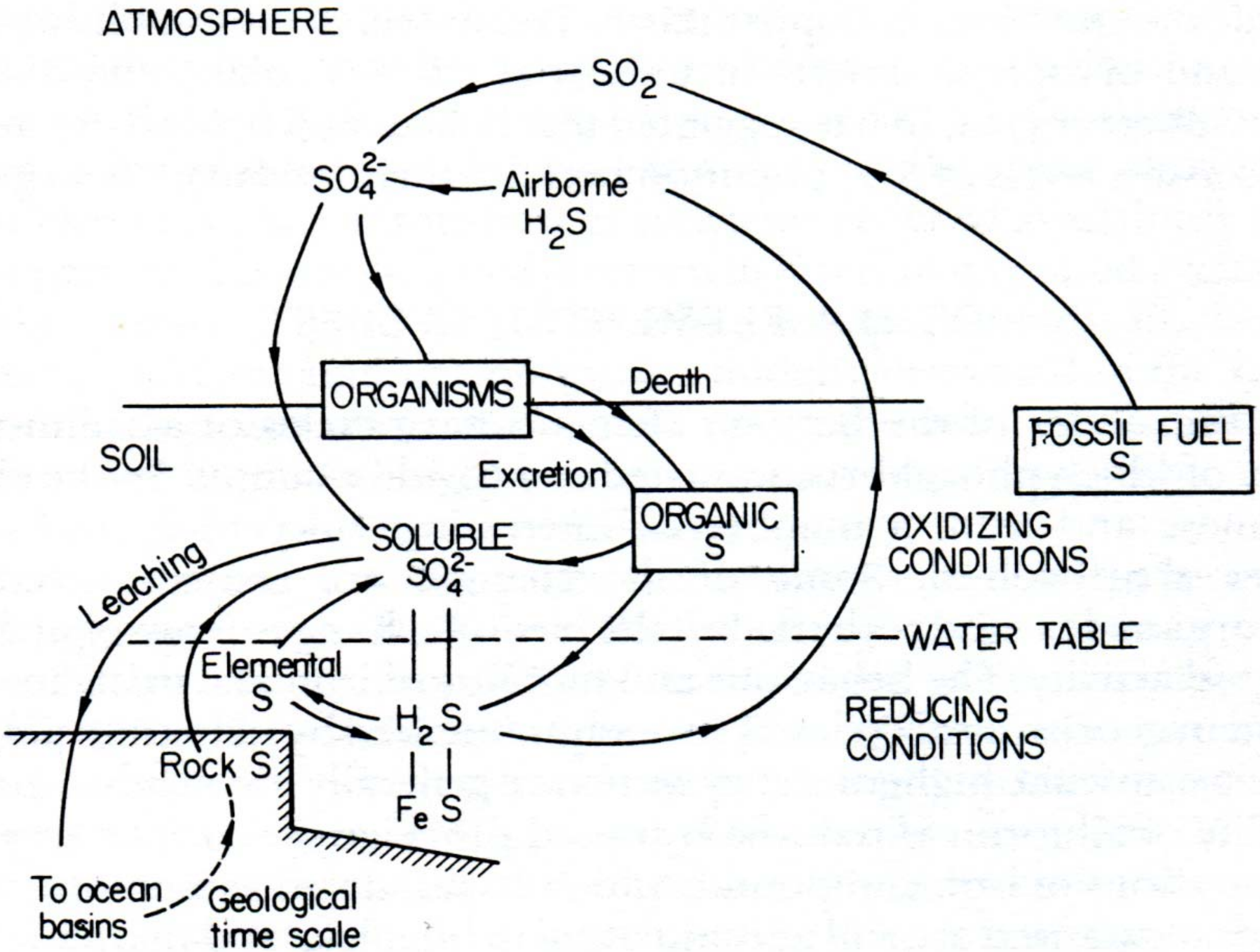


Figure 9.6 The hybrid cycle of sulphur. Though the cycle is essentially local it may be opened by the leaching of soluble sulphate into a reducing zone below the water table. If hydrogen sulphide is produced it may be liberated to the atmosphere where it rapidly oxidizes to sulphate. The conversion of organic sulphur to the plant-available sulphate and the various interconversions of sulphur in wet soil are all bacterially mediated processes.

Carbon & Oxygen

- CO_2, H_2O, CO, O_2, HCN, CH_4, $CaCO_3$, CFC, HCFC
- photosynthesis
- oxidizing atmosphere
- coccoliths

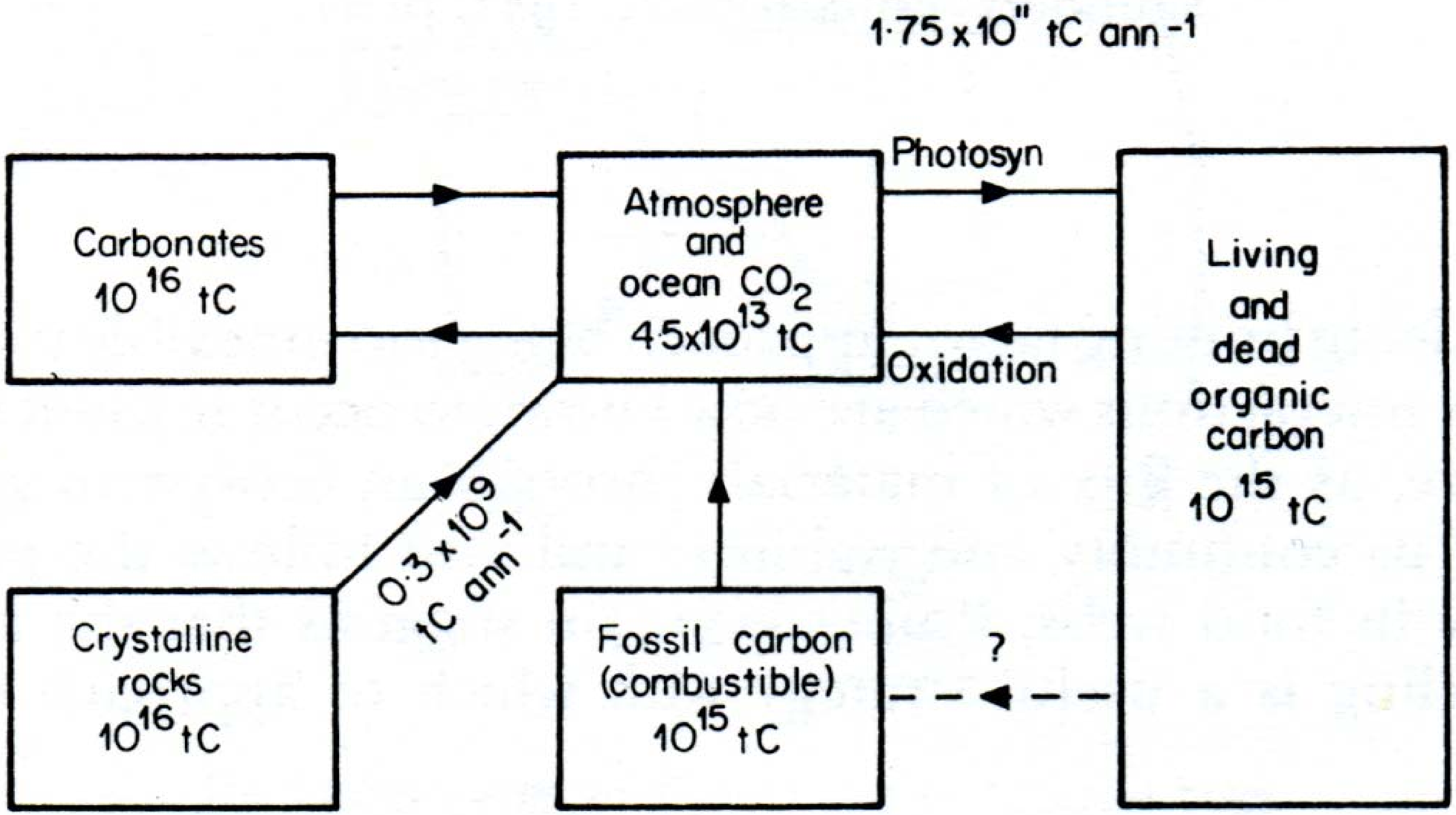


Figure 9.3 The global carbon cycle. Data from Nichiporovich (1969), Wassink (1968) and Revelle and Fairbridge (1957).

Algae & Men

- macroalgae (commerce - 42 countries)
  - food
    - *Laminaria* (China, N.,S.Korea, Japan, Philipines, Chile, Norway, Indonesia, U.S., India)
    - *Porphyra, Kappaphycus, Undaria, Euchema, Gracilaria*
    - **Nori** (*Porphyra yezzoensis*) – 13mil. t/y
- microalgae
  - carotenoids, pigmenst, proteins, vitamins, …
    - nutraceuticals, pharmaceuticals, animal feed additives, cosmetics, fertilizers
    - *Dunaliella, Haematococcus, Arthrospira, Chlorella*

Physiology and Cultivation of Algae and Cyanobacteria

5.

Overview

- Collection of the samples
- Isolation & purification of algal culture
- Culturing
  - Methods
  - Equipments & material
  - Conditions
  - Culturing media

Collection of the samples

- purpose specific
- sample specifics (nature & environment)
- time
- concentration
- type of vessel/container
- removal of the unwanted organisms (filter)
- transfer conditions & storing

Isolation & purification of algal culture

- Equipments & suplies
  - Microscopes
  - Filters & sieves
  - Glasseare, Plasticware, Utensils
- Methods
  - Sterile manipulation
  - Isolation techniques

Equipments & suplies

- Microscopes
  - dissecting (80x,..)
  - inverted
  - lighting
    - dark-field
    - fiber optic light source
    - fluorescent lamp
    - epifluorescence
- Filters & sieves
  - woven screens
  - nylon netting
  - membrane filters (material)
  - differential filtration
- Flow-box

Glasseare, Plasticware, Utensils

- borosicate glassware
- plasticware (ready-to-use, culture-grade)
- sterilization technique & sterility
- dust-proof cabinet / clean containers
- caps & splips
- sterile filtration apparatus
- sterile spatula
- pens, labels
- parafilm

- growth chambers
  - test tubes, flask, culture flask, Erlenmeyer flask, Petri dish, plugs, two-steps screw
  - cultivator, tank, bioreactor

Sterilization & sterile manipulation

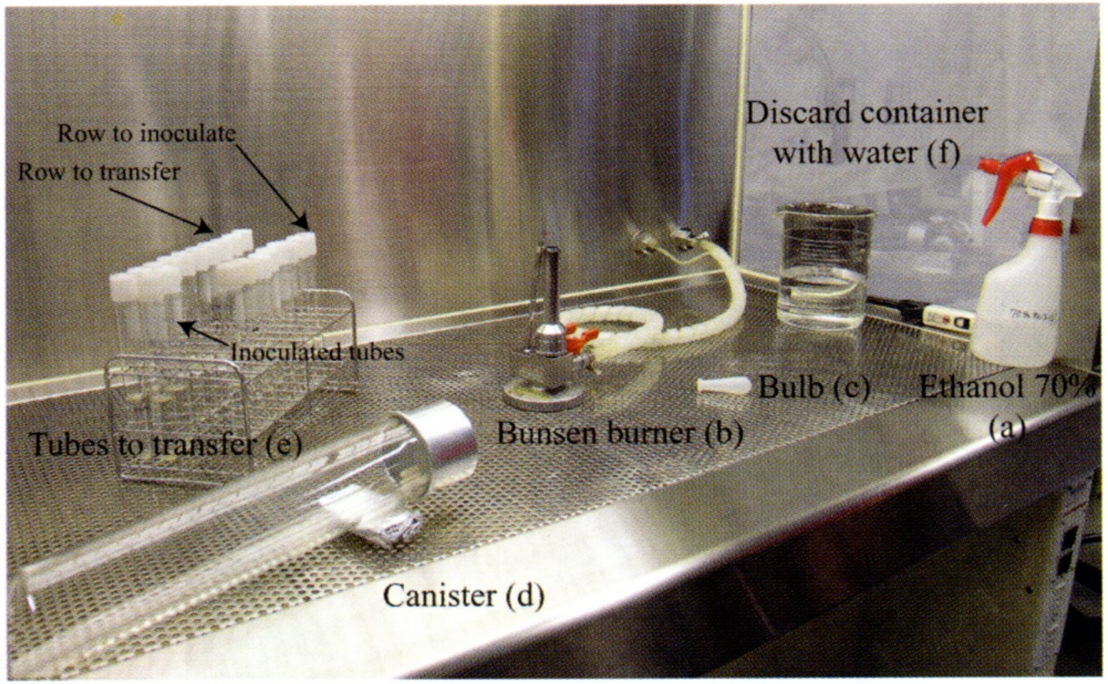


FIGURE 5.10. A laminar flow hood with Bunsen burner and supplies organized for use by a right-handed person. Placement and use order of the materials is indicated with the letters (a) to (f). Note the stopper for the cylindrical canister. Test tube arrangement is to facilitate handling and to avoid mistakes during manipulations.

TABLE 5.1. Summary of sterilization types, including applicability and limits

Category	*Sterilization method*	*Effective method*	*Application*	*Limitation*
Heat	Flame	Direct heat with fire (Bunsen burner)	Surface sterilization (test tube openings, transfer loops, glass pipettes)	Non–heat-resistant materials (e.g., most plastics)
Heat	Autoclaving	2 atm (steam pressure), 121°C; time varies (10, 20 min for small liquid vol; 1 h for large vol)	For general use: liquids and agar, glass and metal vessels, equipment	Non–heat-resistant materials; pH change; metal contamination
Heat	Dry heat	250°C, 3 to 5 h; current protocol at 150°C for 3 to 4 h	Dry goods: glass and metal vessels and equipment	Non–heat-resistant materials; liquids
Heat	Pasteurization	66–80°C for at least 30 min, followed by quick cooling (4–10°C)	Liquids with heat-labile components	Not complete sterilization (originally for killing food germs)
Heat	Tyndallization	60–80°C, 30 min, followed by quick cooling; cycle repeated 3 times in 3 d	Liquid with heat-labile components	Requires time
Filtration	Filtration	≤0.2 μm pore size filter	Liquid with heat-labile components	Small volumes, high-viscosity liquids, viruses not eliminated
Electromagnetic waves	Microwave	10 min at 700 W; 5 min with intervals at 600 W. For dry goods: 20 min at 600 W with water, 45 min without water	Liquids: small volume of media; dry goods: glassware, vessels	Small liquid volumes; dry goods with water require elimination of water
Electromagnetic waves	Ultraviolet radiation	260 nm, 5–10 min	Surface of materials, working area	Ultraviolet-sensitive plastics
Chemical	Bleach (sodium hypochlorite)	1–5 mL for 1 L water, several hours	Large volume of water for aquaculture	Cysts may survive; neutralization required (e.g., sodium thiosulfate, 250 g · L^{-1} stock solution; 1 mL for 4 mL of bleach)
Chemical	Ethanol	50–70% solution	Popular, general disinfection	Some resistant microorganisms
Chemical	Ethylene oxide	Airtight room or pressure cabin	Plastic and rubber products, non–heat-resistant products	Explosive; chemical residue is problematic or toxic
Chemical	Corrosive sublimate, $HgCl_2$	0.1%; add same amount of NaCl and dissolve with distilled water	Antiseptic and disinfectant	Poison; not for materials contacting live cells
Chemical	Phenol (carbolic acid)	3% solution	Antiseptic and disinfectant	Poison; not for materials contacting live cells
Chemical	Saponated cresol solution	3–5% solution	Antiseptic and disinfectant	Poison; not for materials contacting live cells
Chemical	Formaldehyde (formalin)	2–5% solution	Antiseptic and disinfectant	Poison; not for materials contacting live cells

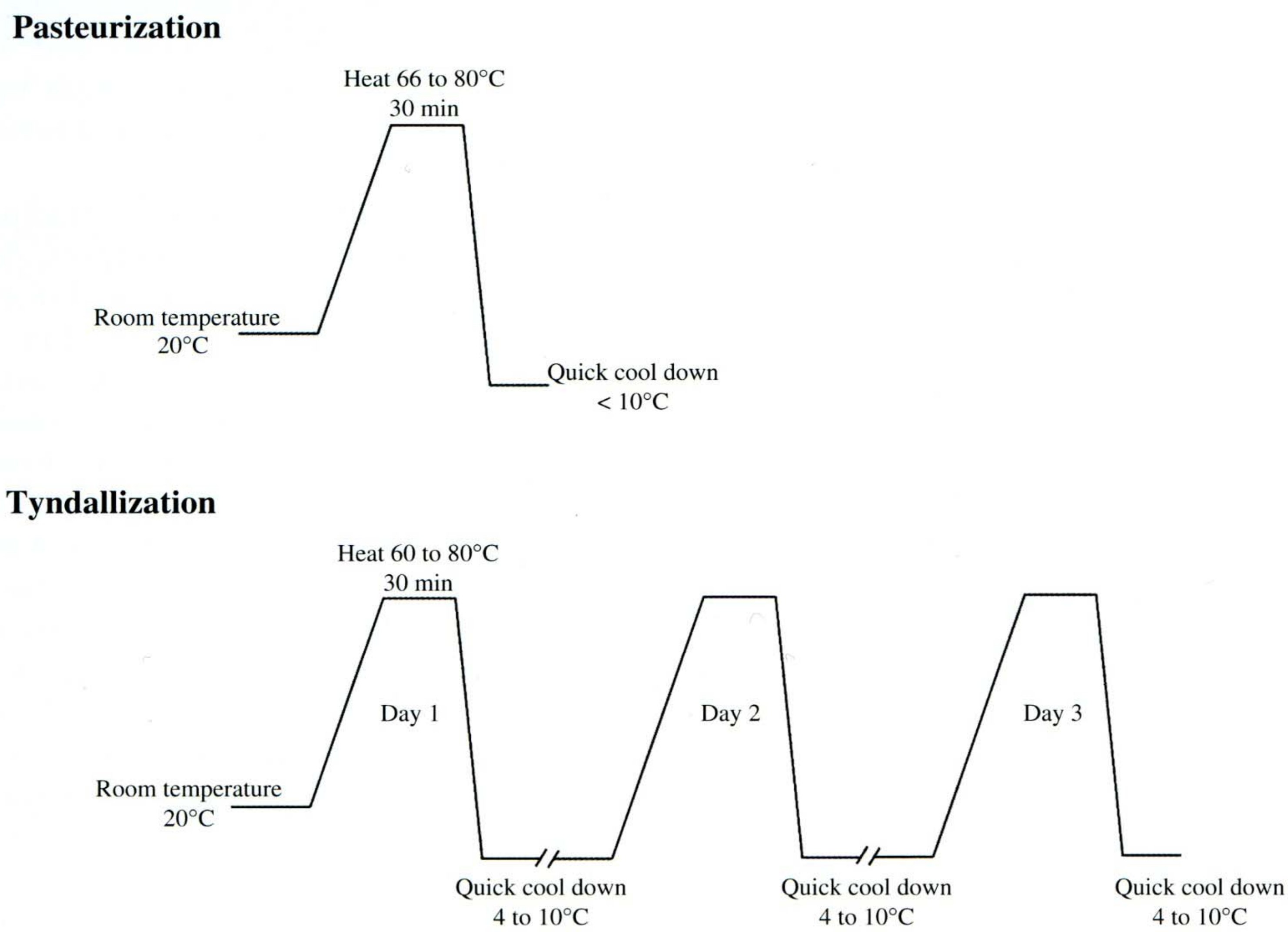


FIGURE 5.5. Schematic representation of the temperature cycles for pasteurization and tyndallization.

Methods of isolation & purification

- Enrichment culture
- Single-cell isolation
- Size separation >> filtering
- Density separation >> centrifugation
- Dilution
- Isolation with use of agar
  - streaking
  - spray
- Isolation with use of phototaxis
- Automatization (flowcytometer)

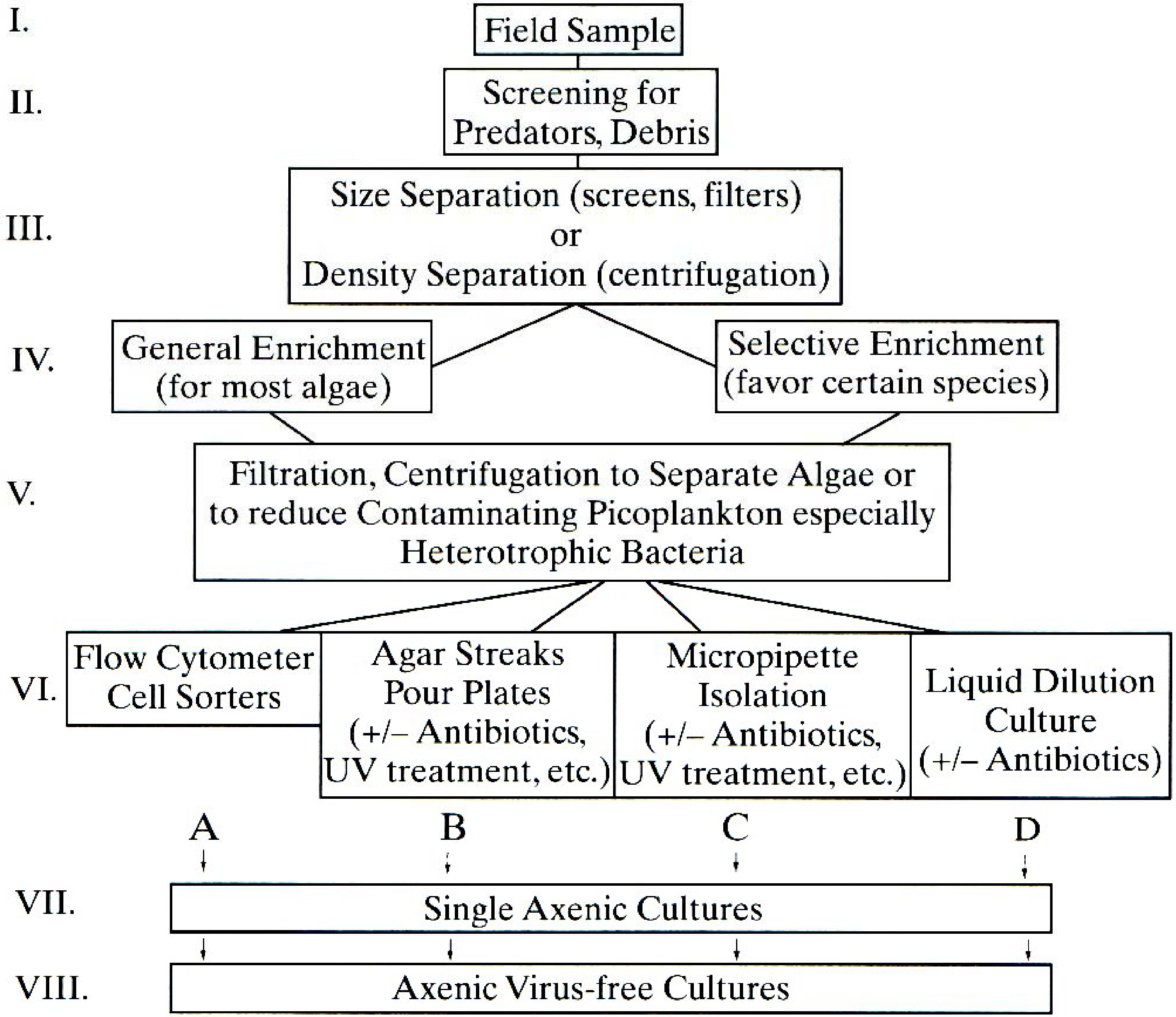


FIGURE 8.1. Flow diagram of purification steps of single-cell algae (after Guillard and Morton 2003).

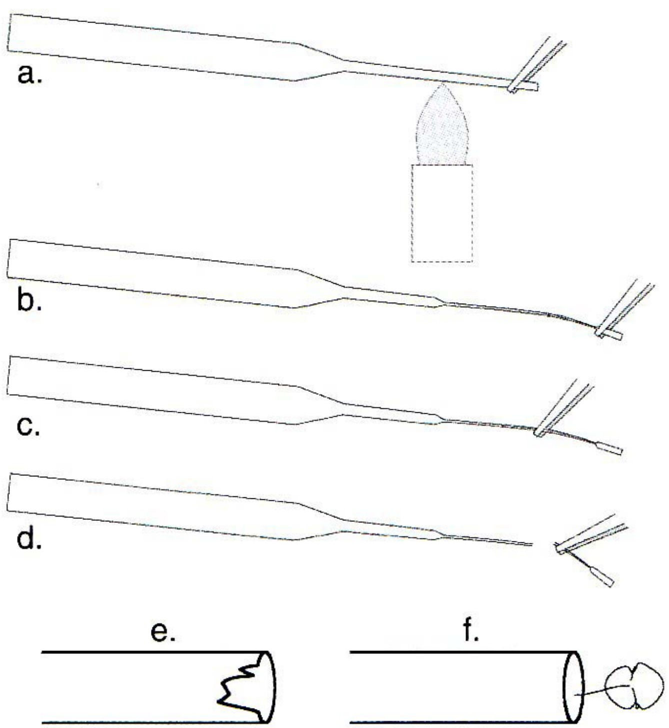


FIGURE 6.3. Preparation of a micropipette from a Pasteur pipette. **(a)** The Pasteur pipette is held in the hottest region of the flame, supported on the left by a hand and on the right by forceps. The pipette should be rotated as the glass is heated to a soft, pliable condition. **(b)** When the glass is soft, the pipette is quickly removed from the flame with a gentle pull to produce a thin tube. **(c)** The forceps is then relocated to the appropriate region of the thin tube. **(d)** The forceps is used to gently bend the thin area so that it breaks, forming a micropipette. **(e)** An enlarged tip of a micropipette, showing a jagged break; this tip is not suitable for use. **(f)** An enlarged tip with a very smooth break; this tip is suitable for use. Note that the diameter of the tip is larger than the flagellate cell (bearing microscopic scales), thus reducing the probability of shearing as the cell enters the micropipette during isolation.

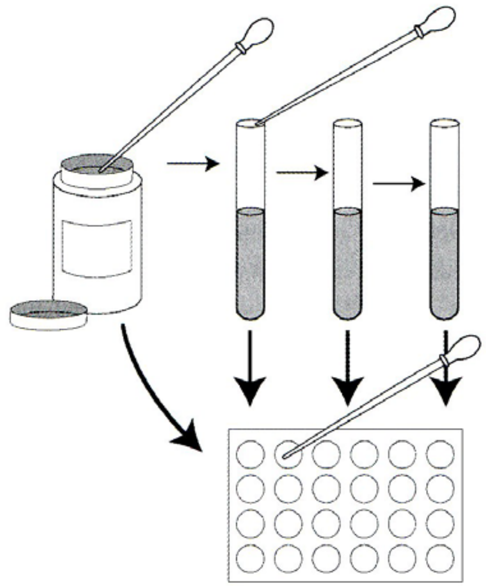


FIGURE 6.6. An illustration of the dilution technique. An aliquot is removed from the sample jar (*left*) and placed into a test tube containing sterile medium. After mixing, one aliquot is removed from the test tube and dispensed into multiwells containing sterile medium, and a second aliquot is removed and added to the middle test tube. After mixing, the process is repeated (i.e., dispensed into multiwells and added to the test tube on the right). Each cycle dilutes the original sample and increases the probability of single-cell isolation; the cycle stops when it is probable that no cell will be transferred.

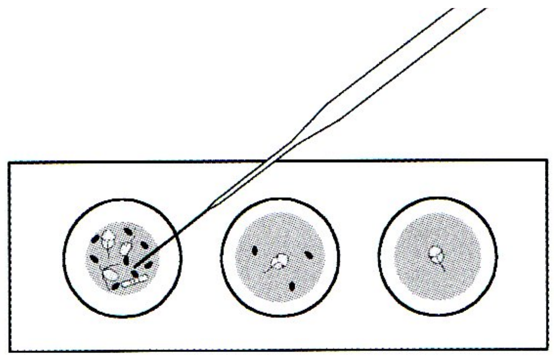


FIGURE 6.4. A pipette is used to remove other small cells (*left, middle*), leaving the target organism free of contamination (*right*). This procedure limits the handling of the target organism.

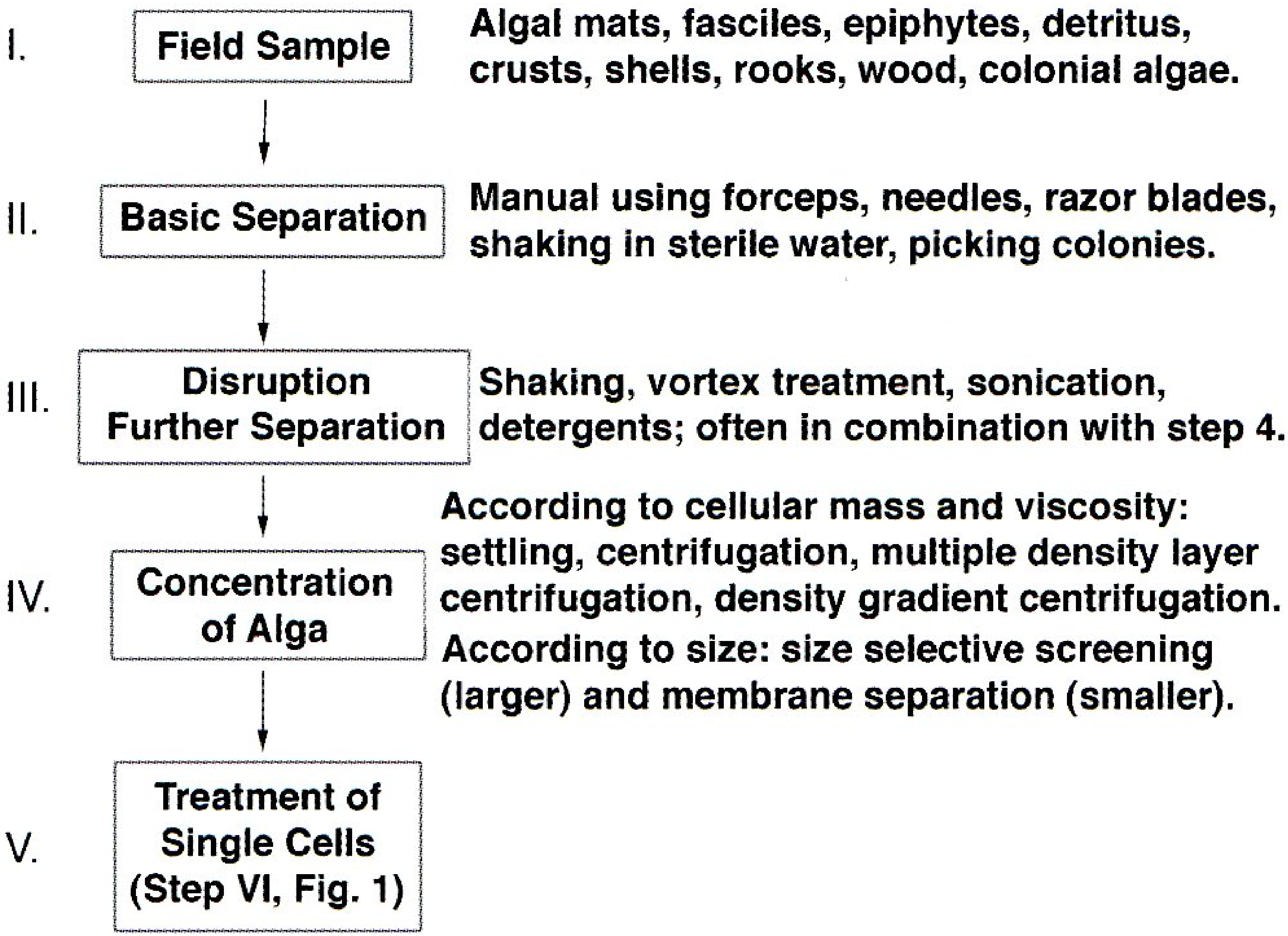


FIGURE 8.2. Flow diagram of purification steps for attached or colonial algae.

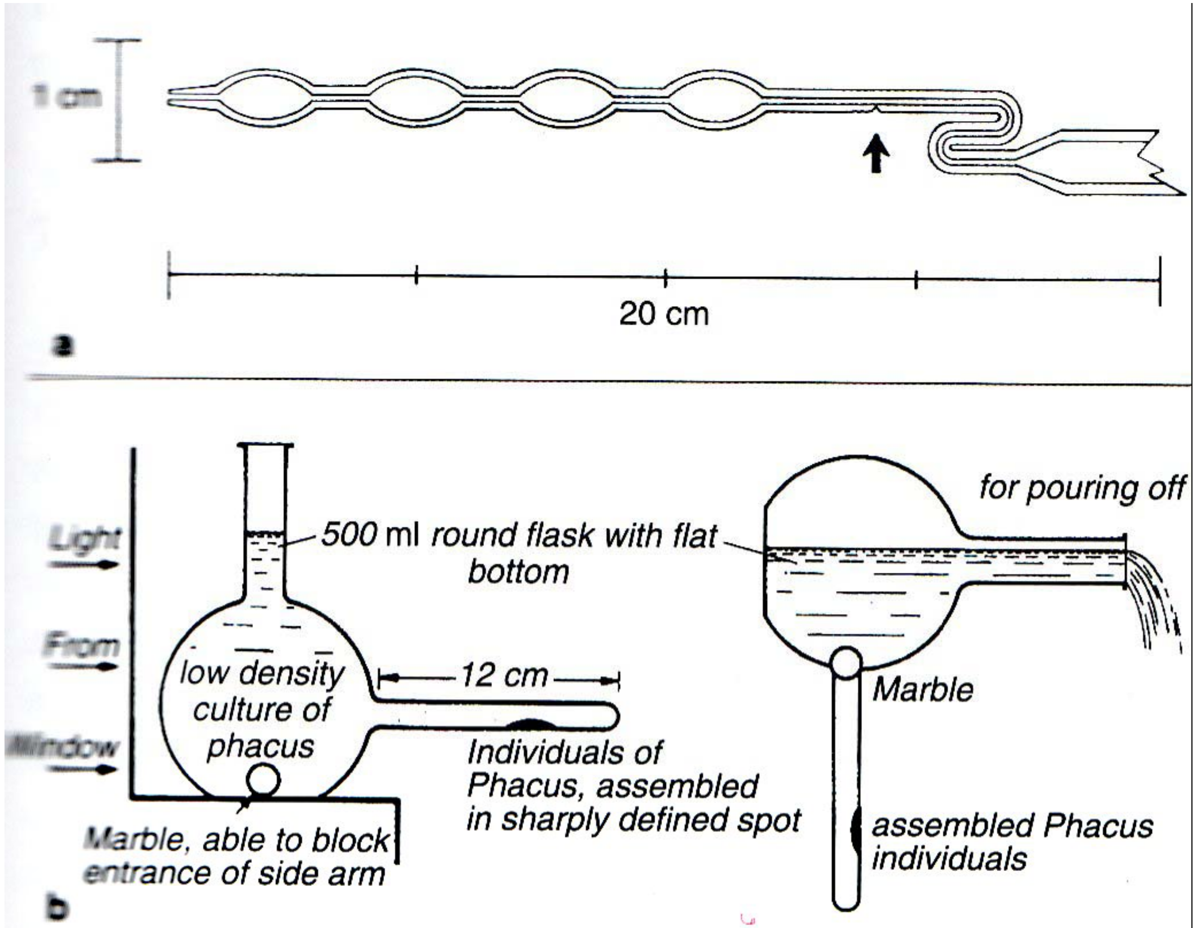


FIGURE 6.7. Phototaxis apparatuses. **(a)** Phototactic flagellates drawn into the tip of the pipette and then allowed to migrate through the cavities and into the pipette itself. The tip is broken at the arrow and discarded, and the cells in the pipette are then discharged into sterile culture medium (e.g., test tube, flask, or multiwell) (Paasche 1971, modified from Guillard 1973). **(b)** Negatively phototactic flagellates concentrated with bright light in the narrow arm of the flask (*left*) and then retained by decanting the original sample while the target cells are trapped in the arm by a glass bead (from Meeuse 1963).

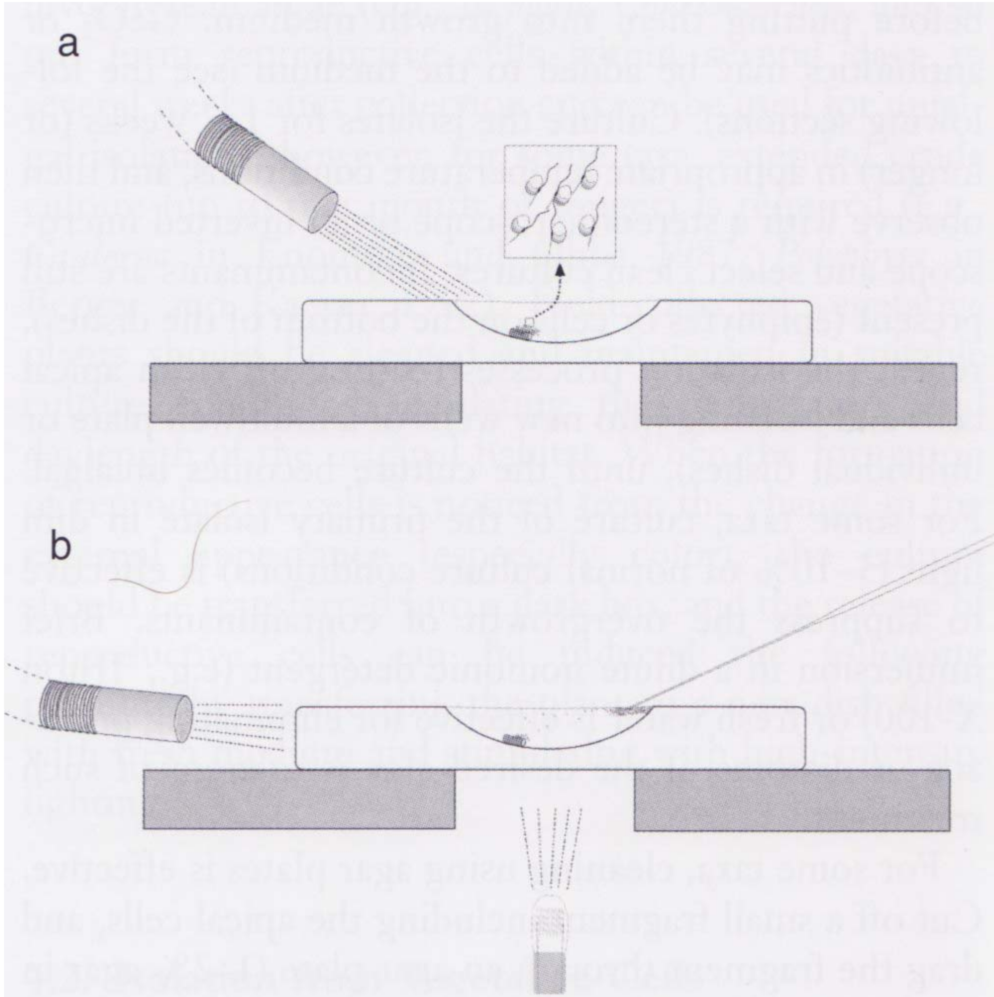


FIGURE 9.10. Isolation of swimming zooids using phototaxis. **(a)** Unilateral illumination from a fiber-optic light source to stimulate zooid release; **(b)** simultaneous lateral and bottom illumination to accumulate zooids at one upper edge of the depression slide well.

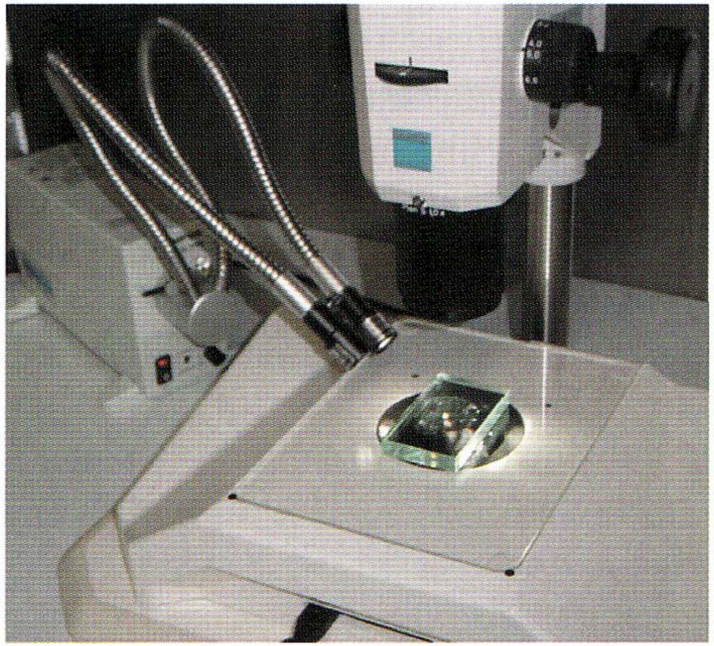


FIGURE 9.8. Isolation of phototactic zooids under observation with a stereomicroscope: Fertile algal tissue is placed at the bottom of a depression slide and the release of zooids is induced by intense illumination from a fiber-optic light source. Released zooids are accumulated at the surface of the medium (lighting from the upper side when they are positively phototactic, and from the lower side when negatively phototactic), and distant from the algal tissue.

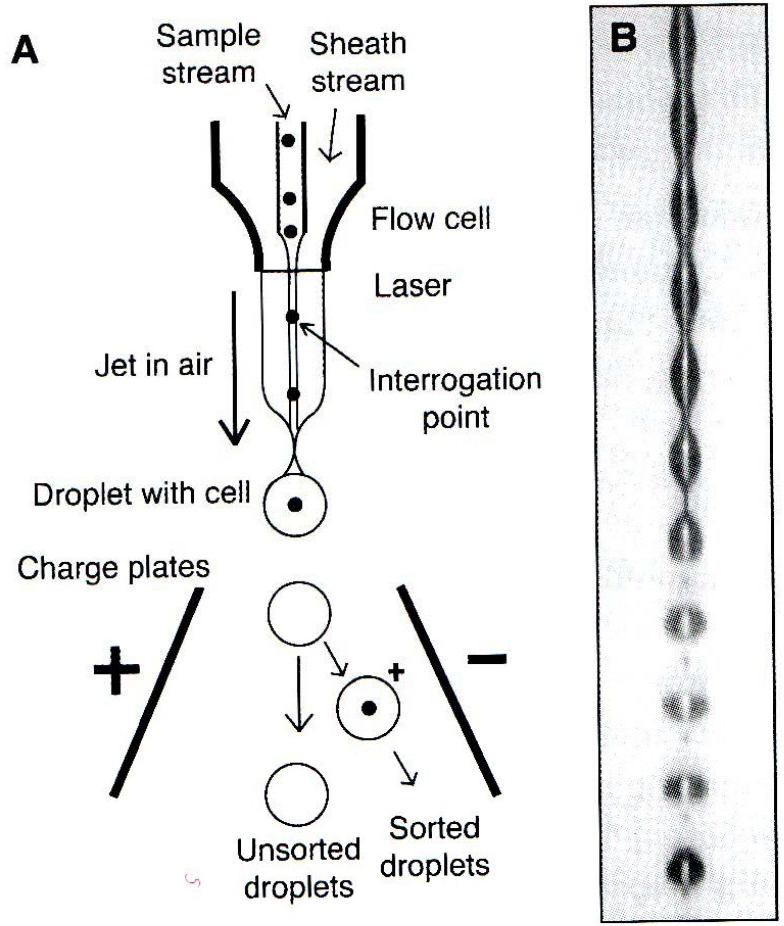


FIGURE 7.1. **(a)** Schematic diagram of droplet cell sorting, showing the joining of the sample stream and sheath fluid stream in the flow cell, forming the jet-in-air flow stream with the cells in single file. The laser intercepts the cells at the interrogation point, where measurements are made. If a cell meets the criteria of the sort logic, then the flow stream is charged just before the break-off point of the droplet containing the target cell. The droplet retains the charge and is deflected by the charge plates toward the collection tube or plate. **(b)** Image of flow stream at the droplet break-off point with use of a 70-µm tip.

Culturing techniques

- chemicals
- equipment – balance, ph-meter, autoclave, filtration, ultrasonic washer, refrigerator, cultivator
- conditions – ph, temp, irradiation, photoperiode,..
- glasware – E. flask, reagent bottles, pipetes, flask, tubes, P. dishes, spatulas, funels, filter holder, syringe, ..
- water
- agar
- soil

Media

- stock solutions
  - macronutrients
  - trace elements
  - vitamins
  - chelators
  - soil extracts
- preparation of media
- synthetic media
- enriched media
- soil water media
- solidified media – agar
- Freshwater media
- Seawater media
    - natural & artificial

Physiology and Cultivation of Algae and Cyanobacteria

6.

Culture methods

- Bath cultures
  - common, simple, low cost, closed system, volume-limited
  - any flow of nutritions & products
  - Erlenmeyer flasks, tubes, Petri dishes
  - growth curve phases – lag, acceleration, exponential, retardation, stationary, decline

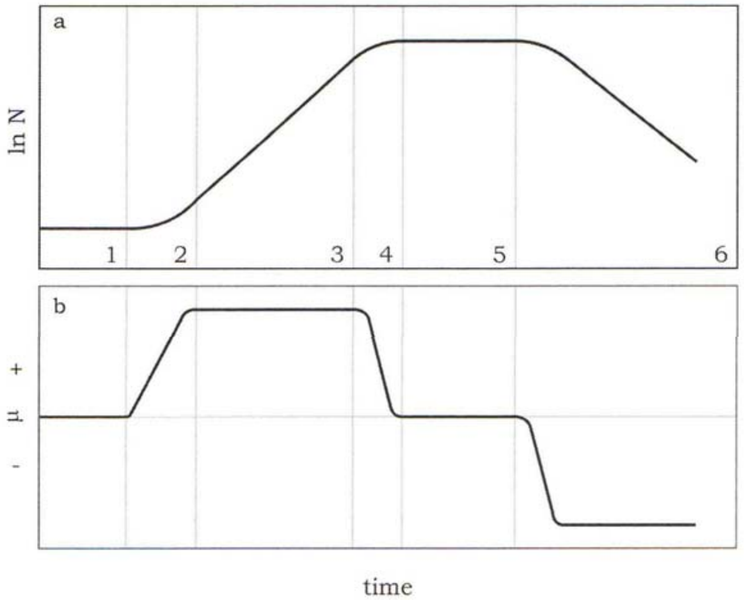


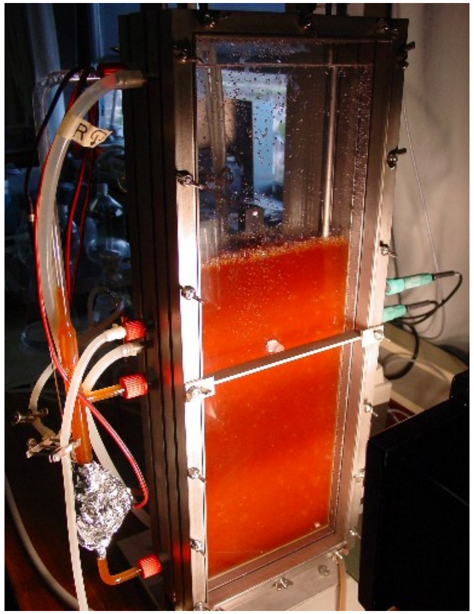
FIGURE 6.1 Growth curve of an algal population under batch culture conditions (a) and corresponding variations of the growth rate (b).

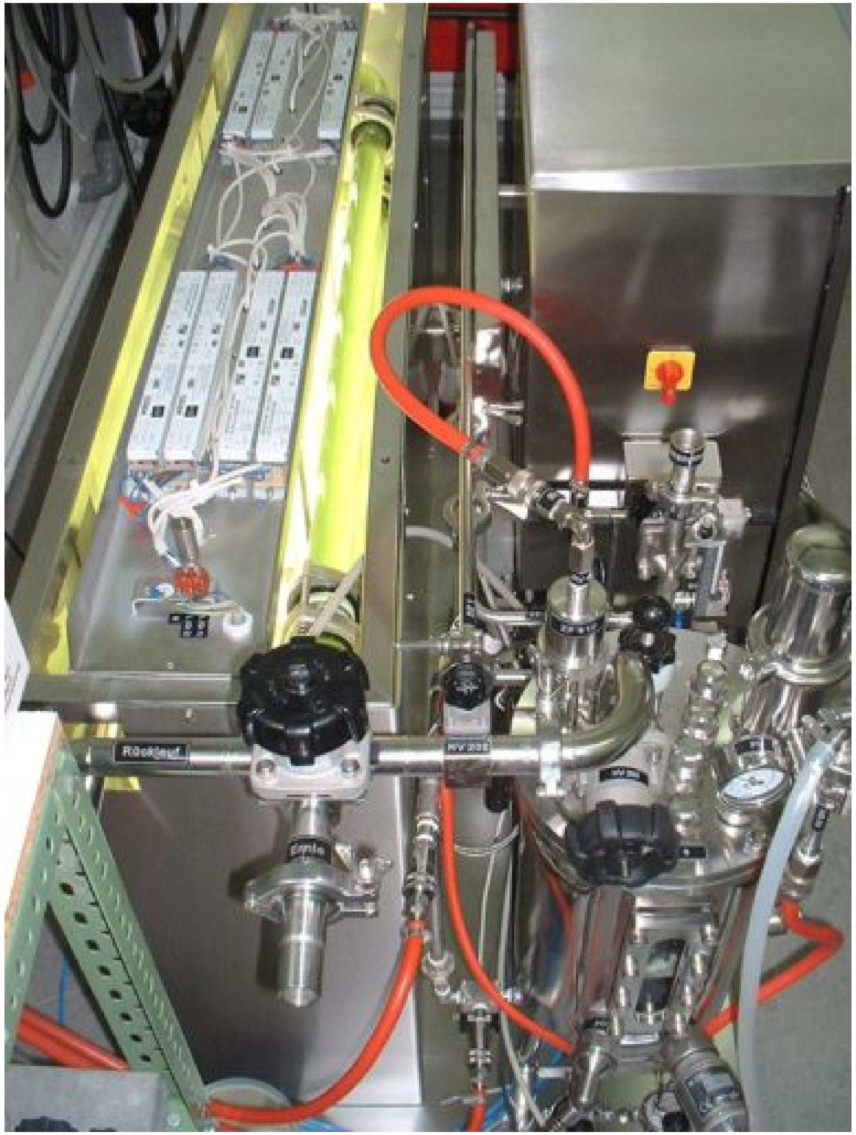
TABLE 6.19
Description of the Six Successive Phases of Growth for an Algal Population under Batch Culture Conditions

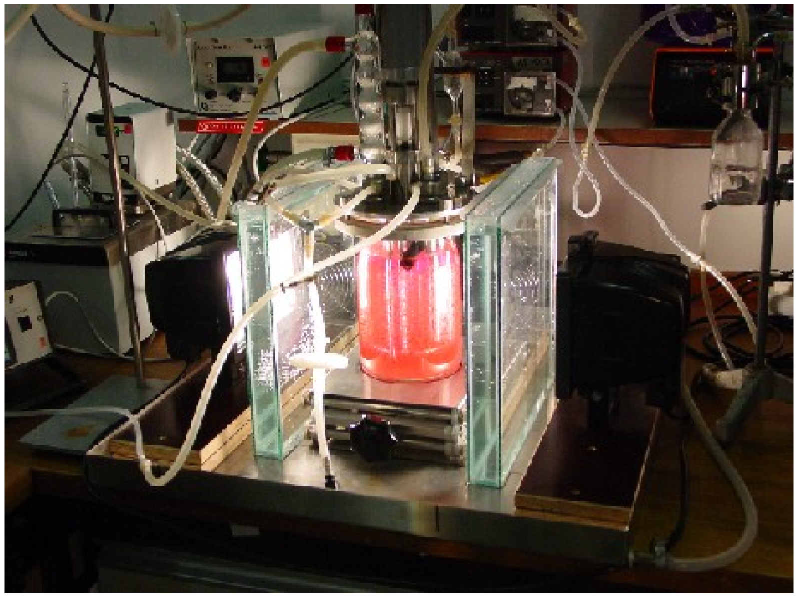
Phase	Growth	Growth Rate Interpretation	Description
1	Lag	Zero	Physiological adaptation of the inoculum to changing conditions
2	Acceleration	Increasing	Trivial
3	Exponential	Constant	Population growth changes the environment of the cells
4	Retardation	Decreasing	Effects of changing conditions appear
5	Stationary	Zero	One or more nutrients (or light) are exhausted down to the threshold level of the cells
6	Decline	Negative	The duration of stationary phase and the rate of decline are strongly dependent on the kind of organisms

Culture methods

- Continuous cultures
  - resources are potentially infinite
  - cultures are maintained at chosen point on the growth curve by regulated addition of fresh medium
  - air pump – CO_2 source, mixing
  - categories of contin. cult.:
    - turbidostat cultures
    - chemostat cultures
- Semi-continuous cultures
  - periodic fresh medium addition & harvesting




Rücklauf



Culture methods

- <u>Commercial-scale cultures</u>
  - volume of cca. $10^2 - 10^9$ l
  - large open ponds, circular ponds with rotating arm, raceway ponds, large bags, tube system
  - factors to be considered:
    - biology of alga; the cost of land; labor; energy; water; nutrients; climate (if outdoors); type of product
    - light utilization efficiency; ability to control temp.; hydrodynamic stress; ability to maintain culture unialgal or axenic; scale up ability
  - *Chlorella*, *Spirulina*, *Dunaliella*








Methods used for algal culture growth evaluation

- direct
  - fresh/dry mass determination
  - counting – number of cells (colonies)
  - cell volume
  - protein content
  - calorific value
  - flow-cytometry & epifluorescence microscopy
- indirect
  - turbidity; optical density
  - chlorophyll content

Endogenous rhythms

- circadian rhythms
  - molecular feedback loop ~ 24h~ environmental light-dark cycles ~ positive/negative phase shifts according to phase-response curve of particular response
  - necessity to transfer sunlight-sensitive processes into the night (cell division in night)
  - lost under certain conditions *(e.g.* constatnt light, bright light, growth of *Euglena* on organic medium)
    - phototaxis
    - timing of cell division
    - photosynthetic capacity
    - bioluminiscence
    - gene expression
    - sensitivity to UV

Endogenous rhythms

- biweekly (circa-semilunar) rhythms
  - wave action (sea may reduce gamete concentration of marine org. with external fertilization)
  - *e.g.:*brown alga Dictyota dichotoma releases eggs twice a month in the field; synchronization signal every second full-moon light
- day-length effect (photoperiodism)
  - circadian timer measures length of night and triggers photoperiodic response
  - LD (12:12~light:dark) – induce upright thali formation
  - SD (8:16)– induce reproductive organs formation
- circannual rhythmicity
  - sequence of short and long days over the years results cyclic reproductive stages formation (e.g. Ascophyllum, Laminaria)
  - signal probably temporal sequence of different physiological stages

Documetation

- Microcopy (light & fluorescence)
  - drawings
  - photography – classic & digital

- algal culture collections
    - Web pages of culture collections in the world: http://wdcm.nig.ac.jp/hpcc.html
- macroalgae ~ herbarium