

BI-311

General Microbiology

Handout #2

DNA - Structure

Sugar backbone + Nucleotides
Antiparallel - complementary
Phosphate-diester bonding
Deoxi-nucleotide triphosphate units:
 dATP, dTTP, dGTP, dCTP
Code - Codon - Anticodon
Gene
Genome

PROKARYOTES

DNA circular
Supercoil: + and -

EUKARYOTES

DNA linear
Nucleosome-histones

REPLICATION	--	TRANSCRIPTION	--	TRANSLATION
together				separate (2 trnsctpts)
All codes				Intron - Exon
polycistronic				monocistronic

Restriction

Modification

Palindrome recognition

Methylation

REPLICATION

Origin of Replication -- Theta structure
Template -- Complement: 5' to 3' directionality
DNA-polymerases: I, II, III.

Topoisomerase I -- Helicase (ATP-hydrolysis)

Leading strand: 3' Polym-III
Lagging strand: Primase -- RNA primer -- Polym-III
-- Polym-I -- Ligase (Okazaki fragments ca 1kb each)

Topoisomerase II (Gyrase) ---- proofreading.

RNA

1. Ribose
2. Uracil for Thymin
3. Single strand
4. Separate enzymes

mRNA --- tRNA --- rRNA
(unstable) (stable)

1. Genetic information
2. Structure (Ribosomes + tRNA)
3. Enzyme function (Ribozymes - self-splicing introns - Ribonucleoproteins).

RNA-Polymerase **DNA region recognition**
Promoter ----- **Terminator**
Operator (DNA) 1. Palindromic loop
Operon (RNA) 2. Protein-RNA binding
Gene sequence (polycistronic)

Eukaryotes:	RNA-polym I: rRNA	INHIBITORS:
	RNA-polym II: mRNA	Rifamyucin (p)
	RNA-polym III: tRNA	Amanitin (e)
		Actinomycin

RNA-enzyme roles:
SPLICING: O
CAPPING: (-CH)
TAILING: AAAA

Codon (mRNA) ----- Anticodon (tRNA)

tRNA

- 1) **Recognition:** Aminoacyl-tRNA-synthetase (Anticodon)
- 2) **Activation of AA:** AA + ATP --- Aminoacyl-AMP
- 3) **Charging:** (1) + (2) + release from enzyme

Ribosomes

ribonucleoproteins

prokaryotes

eukaryotes

70S

80S

50S: 5S+23S RNA + 34 proteins
+ 30S: 16S RNA + 21 proteins

Sites on ribosome: **A** cceptor -- **P** eptide -- **E** xit

- 1) **Initiation:** Methionine (AUG) on mRNA
- 2) **Elongation:** Shift of sites + peptide bonding
- 3) **Termination-Release:** Stop codon
- 4) **Folding:** Chaparonin

O pen **R** eading **F** rame

CONTROLS

0) Constitutive enzyme (no control)

1) Activity control

Feedback inhibition: (product ----> 1st enzyme)

Allosteric enzymes

Branched pathways

Isozymes

2) Synthesis control

DNA-binding a) nonspecific
 b) specific

Controls:

a) Negative: 1. - Repression
 2. - Induction

b) Positive: Activator protein + Inducer
 Contact: a) direct, b) via Loop

c) Attenuation: Tryptophan Operon -
 Stalling downstream.

3) Global Controls:

(50 proteins response to P deficiency)

Environmental Signal (Stimulon)

Catabolite Repression

Di-Auxic Growth: 2 substrates one at a time

Catabolite Activation Protein --- CAP

CAP+C-AMP Cyclic AdenosineMonoPhosphate

Transformation: Free DNA transfer.

Requires competence factor

Transduction: Virus mediated transfer

Generalized: Accidental DNA fragments packed in the virion(random)

Specialized: Genes next to prophage are transduced

Conjugation: Plasmid-stimulated transfer

F- Recipient w/o plasmid

F+ Plasmid only is transferred

Hfr Plasmid is integrated in the chromosome and transferred

F' Plasmid w. chromosomal genes transfers. Duplicate gene

Mutations: spontaneous vs. induced

Spontaneous:

Point mutations: pairing error 10^{-6}

Base Pair: substitution --- insertion --- deletion

Frame-shift --- Back-mutation

Sense - missense - nonsense 10^{-4} (degenerate T)

RNA-mutation 10^{-3}

Sequence mutations:

Sequence: insertion --- translocation --- inversion

Transposable elements --- Transposons

Integron: transposon + integrase

Induced: mutagens - radiation - biological: SOS, transposons,
lysogenic, carcinogenic (Ames test)

Recombination:

homologous: same sequence-different source ---

partial heteroduplex vs. x-over

Plasmids: mobilize external receptors & pilus

F- no plasmid (competent vs. incompetent)

F+ High frequency *tra* --- separate circular

Hfr Low frequency *tra* --- integrated

F' plasmid + chromosomal genes

Transposable elements: transposase, inverted repeats

Conservative

Replicative

Integrations

Inversions

Genetic Engineering and Biotechnology

DNA Base Ratio: GC proportion to AT

$$\frac{G + C}{A + T + G + C} \times 100$$

DNA-DNA hybridization

1. Melting (separation of strands, denaturing) by high temperature control UV absorption
2. Reannealing (restoration of dsDNA by lowering T). When organism different,
3. Hybridization

PCR – Polymerase Chain Reaction Ingredients: (1) Target DNA (organismal), (2) Oligonucleotide primers flanking the target sequence, (3) DNA polymerase of a hyperthermophile (*Taq* – *Thermus aquaticus*)

1. Heat denaturation of the target dsDNA
2. Cooling – annealing of the Primers
3. Primer extension by polymerase in both directions
4. Repeat of the cycle to separate the newly formed fragment
5. Accumulated sequence $10^{-6} - 10^{-9}$

DNA - sequencing (Maxam-Gilbert and Sanger dideoxy methods)

1. DNAPolymerase copies the ssDNA using dideoxy-nucleoside analogs (w/o OH) to form short chains from each of the four bases: ddGTP, ddATP, ddTTP, and ddCTP, which are color-coded with different fluorescent dyes
2. Electrophoretic separation on acrylamid gel
3. Detected by spectrophotometer and recorded and analyzed by computer.

Genomics:

Science of mapping, sequencing and analyzing genomes

E. Coli: 70% Single genes (not in operons)
 24% Operons
 6% Operons w. polycystronic RNA

Gene families: (a) sequence: similar
 (b) function: similar
 (c) origin: duplication + experimenting

	ORF	Mbp	
"Minimum organism":	470	0.58	(250 proteins)
<i>Methanococcus jannaschii</i>	1,738	1.67	
<i>E. coli</i>	4,288	4.64	
<i>Streptomyces</i>		8.00	
<i>Myxococcus xanthus</i>		9:00	
<i>Saccharomyces cerevisiae</i>	6,034	12:00	haploid
<i>Homo sapiens</i>			

YEAST

haplont (16Ch)

diplont (32Ch)

haploid ← meiosis ← diploid
 mitosis
 gametes → fertilization → zygote
 mating types: α vs. a

Plasmid: 2 μ m circle (in nucleosomes non-integrated)
 Chromosomal inheritance (Mendelian)
 Mitochondrial inheritance (Non-Mendelian)

Molecular Cloning

1. Source DNA
2. Cloning Vector
3. Host Organism
4. Detection & Purification
5. Mass Production

1. - **Source DNA:** Genomic DNA, Derived DNA, Synthesized DNA **Procedure:** Restriction enzyme fragmentation
2. - **Cloning Vector:** Plasmids, Viruses, Expression vectors, Secretion vectors, Shuttle vectors. **Procedure:** Joining DNA fragments with Vector DNA (Ligase, DNA linkage adapters)
3. - **Host Organism:** *E. coli*, *B. subtilis*, *S. cerevisiae*.
Procedure: Transformation (electroporation, particle gun).
Result: Gene library of diverse clones (shotgun cloning)
4. - **Right Clone Detection and Purification:**
Procedures: Gene expression – protein formed and active; antigen-antibody reaction, screening not selection. Nucleic acid probes. Reading the message
5. - **Mass production:** (1) Microbial fermentation: antibiotics, peptides & amino-acids; (2) Virus vaccines; DNA vaccines (3) Mammalian proteins: haemoglobin, insulin, relaxin, interferon; (4) Transgenic plants and animals: genetic farming, herbicide & insect resistance: *Bacillus thuringiensis*; (5) Environmental agents: biodegradation, detoxification; (6) Regulation of gene expression, gene therapy. -

Processes involved:

sequencing – cloning – restriction digest – replication – transformation – transduction – conjugation – lysogeny - transcription – reverse transcription – translation

0	Phanerozoic	0 - 0.54
	Rhynie Chert, Devonian, England	0.3-0.4
	Neoproterozoic	0.54 - 1.0
	Doushantuo, China	0.5-0.6
	Draken Conglomerate, Spitsbergen	0.7-0.8
	Eleonore Bay Group, Greenland	0.7-0.8
	Bitter Springs, Australia	0.8
1	Mesoproterozoic	1.0 - 1.6
	Wumishan, China	1.2-1.3
	Belt Supergroup, USA	1.4
	Gaoyuzhuang, China	1.4
	Billyakh, Russia	1.3-1.6
	Paleoproterozoic	1.6 - 2.5
2	Dahongyu, China	1.7
	Balbirini, Australia	1.7
	Gunflint, Canada	2.0
	Belcher Supergroup, Canada	1.9-2.1
	Duck Creek, Australia	1.9-2.1
	Late Archean,	2.5 - 3.0
	Transvaal Supergroup, South Africa	2.5
	Fortescue Group, Australia	2.8
3	Early Archean,	3.0 - 3.5
	Warrawoona Group, Australia	3.4-3.5
	Onverwacht & Fig Tree, South Africa	3.4-3.5
4	Planet formation	

BACTERIA**Proteobacteria** α , β , γ , δ , ϵ ,**Gram +**

Low G+C

Gram +

High G+C

Cyanobacteria**Chlamydia****Planktomyces****Bacterioid./Flavob.****Green sulfur
bacteria****Spirochaetes****Deinococc./Thermus****Green non-sulfur
bacteria****Thermotoga****Aquifex****ARCHAEA****Euryarchaeota**

Halophiles

Methanogens

Hyperthermophiles

Crenarchaeota

Hyperthermophiles

Korarchaeota**EUKARYA****Animals**

Metazoa, Sponges,

Choanoflagellata

Plants

Vascular plants,

Bryophyta,

Chlorophyta

Haptophyta

Stramenopiles

Chrysophyta,

Diatoms,

Brown algae,

Oomycetes,

Labyrinthula

Alveolates

Ciliata

Dinoflagellata

Slime molds**Flagellates,****Euglenids,****Trypanosoma****Trichomonads****Microsporidia****Diplomonads**

BACTERIA

Proteobacteria:

- α *Rhodospirillum*, *Acetobacter*, *Rickettsia*, *Caulobacter*,
Agrobacterium, *Hyphomicrobium*, *Rhodomicrobium*, *Nitrococcus*. ☐
- β *Neisseria*, *Leptothrix*, *Sphaerotilus*, *Zoogloea*, *Nitrosomonas*, *Gallionella*. ☐
- γ *Chromatium*, *Thiocapsa*, *Thiospirillum*, *Ectothiorhodospira*, *Thiothrix*,
Thioploca, *Beggiatoa*, *Escherichia*, *Vibrio*, *Salmonella*, *Yersinia*. ☐
- δ *Desulfovibrio*, *Bdellovibrio*, *Mixococcus*
- ϵ *Thiovulum*, *Helicobacter*.
-- Mitochondria

Low G+C Gram + : *Lactobacillus*, *Heliobacterium*, *Clostridium*, *Bacillus*,
Streptococcus, *Staphylococcus*, *Mycoplasma*, ☐

High G+C Gram + : *Actinomyces*, *Arthrobacter*, *Corynebacterium*,

Cyanobacteria:

Chroococcales: *Gloeobacter*, *Synechococcus*, *Gloeotheca*.
Pleurocapsales: *Dermocarpa*, *Pleurocapsa*.
Oscillatoriales: *Oscillatoria*, *Lyngbya*, *Microcoleus* – *Pseudanabaena*.
Nostocales: *Nostoc*, *Anabaena*, *Scytonema*, *Calothrix*.
Stigonematales: *Stigonema*, *Fischerella*.
Prochloron, *Prochlorothrix* – Chloroplasts. ☐

Chlamydia: *C. psittaci*, *C. trachomatis*

Planctomyces: *Pirella*, *Isosphaera*

Bacterioides/Flavobacteria: *Cytophaga*

Green sulfur bacteria: *Chlorobium*, *Prostechochloris*, ☐

Spirochetes: *Spirocheta*, *Treponema*

Deinococcus / Thermus: *D. radiodurans*, *T. aquaticus* (Taq)

Green non-sulfur bacteria: *Chloroflexus aurantiacus*, *Heliothrix*,
Oscillochloris. ☐

Thermotoga: sheathed thermophile (-90°C)

Aquifex: H₂ – chemolithotroph

*photosynthesis: ☐

oxygenic photosynthesis: ☐

Proteobacteria

1.- Phototrophes anoxygenic:

- a – Purple sulfur: *Chromatium*, *Ectothiorhodospira*, *Thiocapsa*
- b – Purple non-sulfur: *Rhodospirillum*, *Rhodomicrobium*

2.- Chemolithotrophs:

- a - Nitrosifiers & nitrifiers: *Nitrosococcus*, *Nitrobacter*
- b - Sulfur oxidizers: *Thiobacillus*, *Beggiatoa*, *Thioploca*
- c - Iron oxidizers: *Leptothrix*, *Gallionella*
- d - Hydrogen oxidizers
- e - Methane oxidizers

3.- Chemoorganotrophs:

- a - Aerobic respirers: Pseudomonads, Acetic A., N-fixers:
Azotobacter, *Photobacteria*
- b - Anaerobic respirers: S - reducers, *Desulfovibrio*
- c - Facultative aerobes: Enteric bacteria, *E. coli*
- d - Fermenters: *Zymomonas*
- e - Pathogens: *Neisseria*, *Campylobacter*, *Salmonella*

Vibrios, Spirilla, Prostecate bacteria, Myxobacteria

Gram-positive bacteria

Gram-positive – Low GC

- 1 - Nonsporulating - Lactic Acid Bacteria: *Streptococcus*,
Staphylococcus, *Lactobacillus*
- 2 - Sporulating: *Bacillus*, *Clostridium*, *Heliobacterium*
- 3 - Cellwall-less: *Mycoplasma*, *Spiroplasma*

Gram-positive – High GC

- 4 - Coryneforms: *Arthrobacter*, *Propionibacterium*
- 5 - Mycobacteria: *Mycobacterium tuberculosis*
- 6 - Actinomycetes: *Streptomyces*

ARCHAEA

Euryarchaeota

Marine euryarchaeota

Halophiles: *Halobacterium*, *Halococcus*, *Natronococcus*

Na/K compatible solution - bacteriorhodopsin Na⁺/H⁺ antiporter

Methanogens: *Methanobacterium*, *Methanococcus*,

Methanosarcina

Habitats: Anoxic mud: swamps -- Animals: rumen, coecum, colon, termite hind guts, endosymbionts in ciliates --

Environments: sewage digestors, Geothermal vents.

Substrates: CO₂, CO, formate, methanol, methylamine, methylmercaptan, methylsulfide, acetate.

Hyperthermophiles: *Thermoplasma*, *Thermococcus*, *Methanopyrus*

Crenarchaeota

Marine euryarchaeota

Hyperthermophiles: *Sulfolobus acidocaldarius*, *Thermoproteus*,

Pyrodictum,

Korarchaeota

The most ancient Archaea

Hyperthermal adaptations:

Membrane: Glycerol diether, Diglycerol tetraether (dibiphytanyl) in phospholipid monolayer, Tetraether lipoglycan.

Proteins: "Salt bridges" = ionic cross connections in proteins ---

Chaperonins = heat-shock repair proteins (re-folding), e.g.

Thermosomes. ---

DNA stability: K-cyclic diphosphoglycerate --- reverse DNA gyrase (positive supercoiling) --- Archaeal histons: nucleosomes. --- 150 centigrade max ATP limit. --- Life on Earth and elsewhere

EUKARYA

Animalia

Plantae

Vascular plants



Bryophytes



Chlorophytes



Fungi

Basidiomycetes

Ascomycetes

Stramenopila

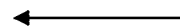
Phaeophyceae



Diatoms



Chrysophyceae

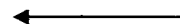


Oomycetes

Alveolata

Ciliata: *Paramecium*, *Stentor*, *Blepharisma*, *Coleps*

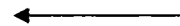
Dinoflagelates: *Peridinium*, *Ceratium*, *Goniaulax*,



Apicomplexans: *Plasmodium falciparum*

Rhodophyceae

Bangiophyceae



Florideae



Myxomycetes (slime molds)

Entamoebae

Euglenoids

← chloroplast acquired ←

Tripanosoma

← mitochondrion acquired

Microsporidia

Trichomonads

← hydrogenosome acquired

Diplomonads

Giardia

Photosynthesis

Reaction centers: Chlorophyll a, Bacteriochlorophylls

Accessory pigments:

1. Carotenoids - Caroten, Xanthophyll, Lycopene, Zeaxanthin
2. Phycobilins (phycobilisomes): Phycocyanin. Pycoerythrin

Respiration

Dissimilative reductions of oxidized compounds of:

Aerobic respiration: Oxygen

Anaerobic respirations:

1. N
2. Fe
3. N - denitrification
4. S - sulfite, thiosulfate, S - disproportionation
5. C - methanogenesis - acetogenesis

Chemolithotrophy (heterotrophic, autotrophic)

Oxidations of reduced compounds of:

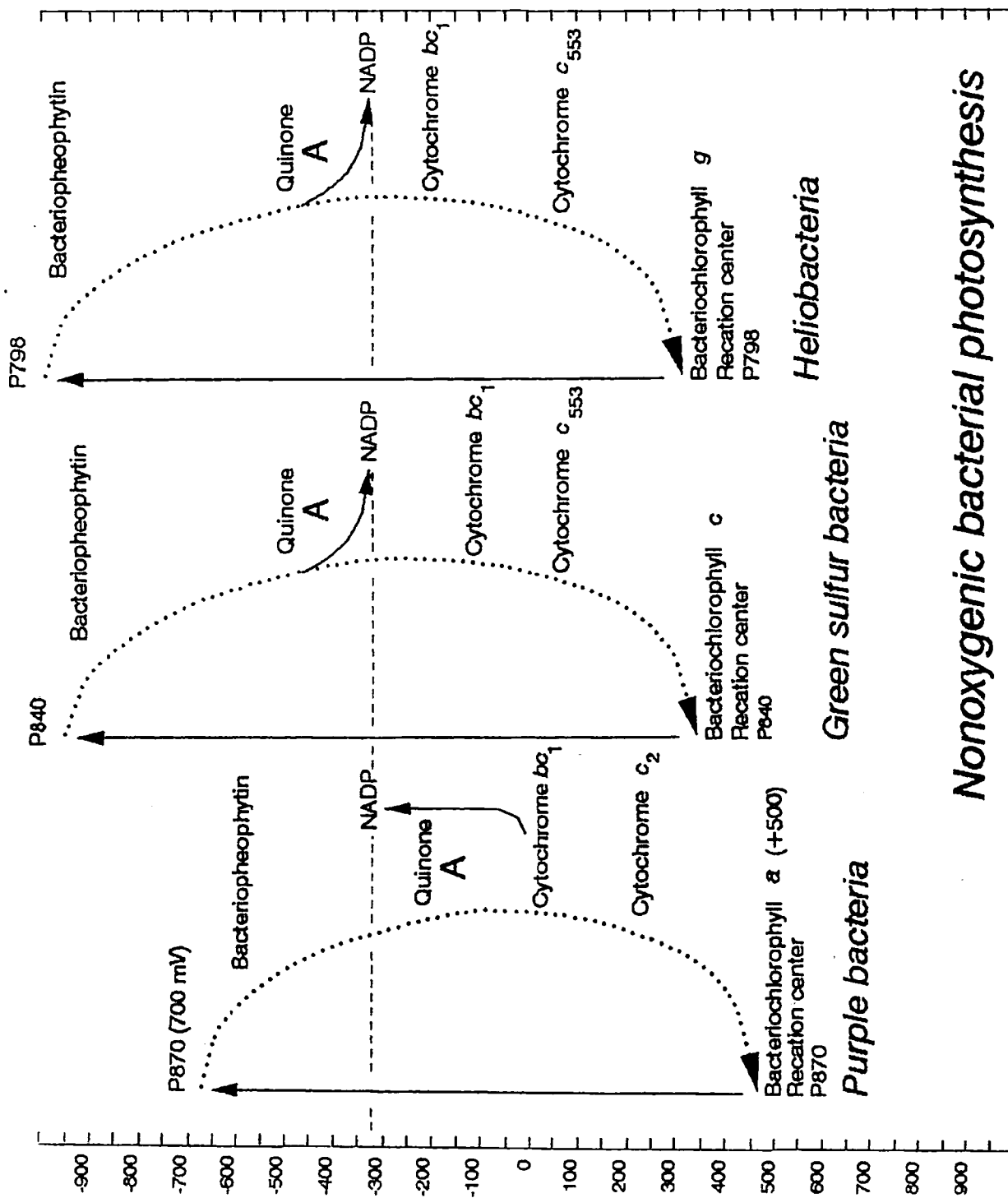
1. H - Hydrogen bacteria
2. C - Methan oxidizers
3. S - Hydrogen sulfide
4. N - Ammonia, nitrification
5. Fe - Iron bacteria

Fermentations

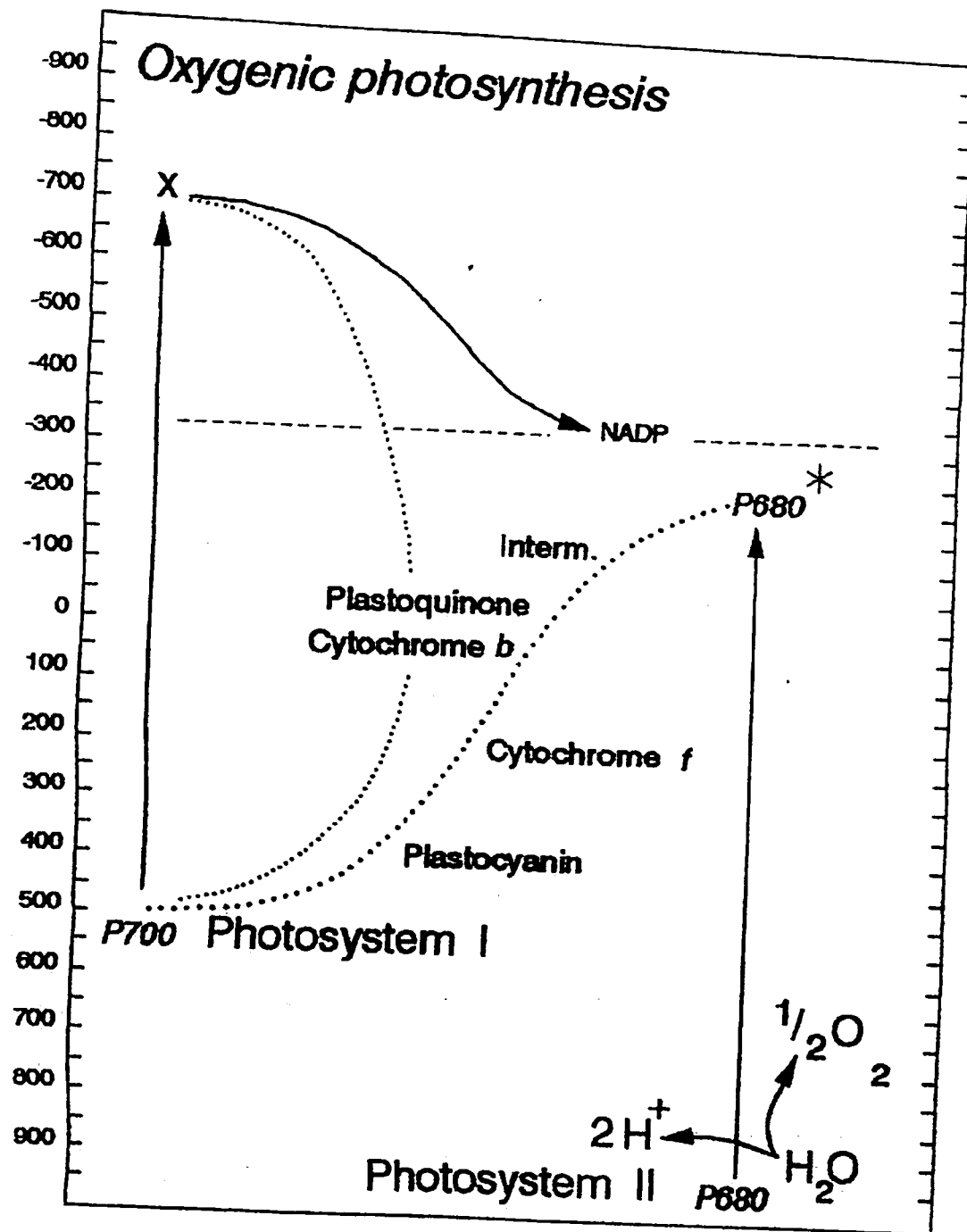
1. Alcoholic
2. Homolactic
3. Heterolactic
4. Mixed acid -
5. Butyric
6. Homoacetogenic
7. Methanogenic
8. Other (unusual - succinate, oxalate)

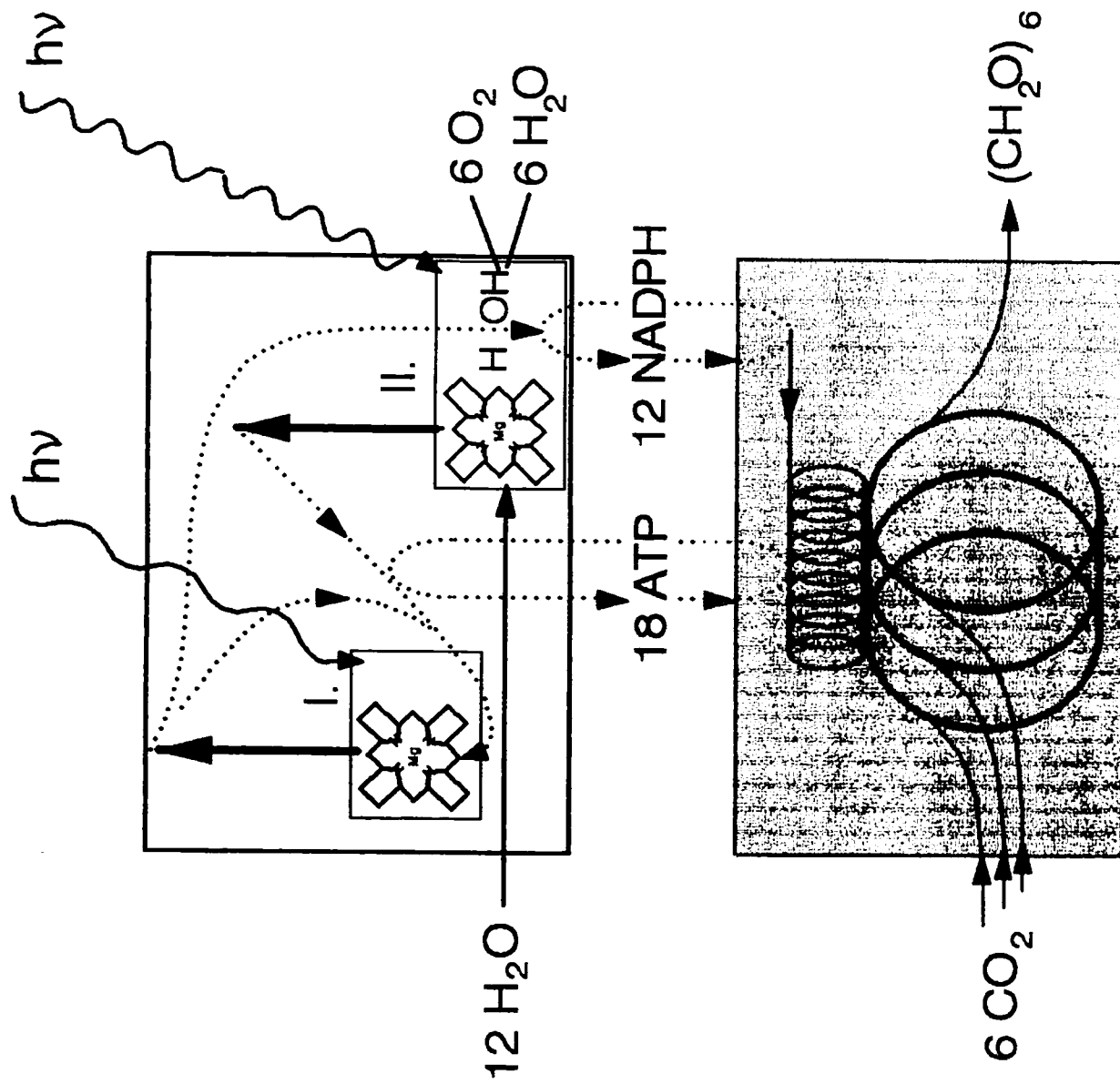
N-fixation

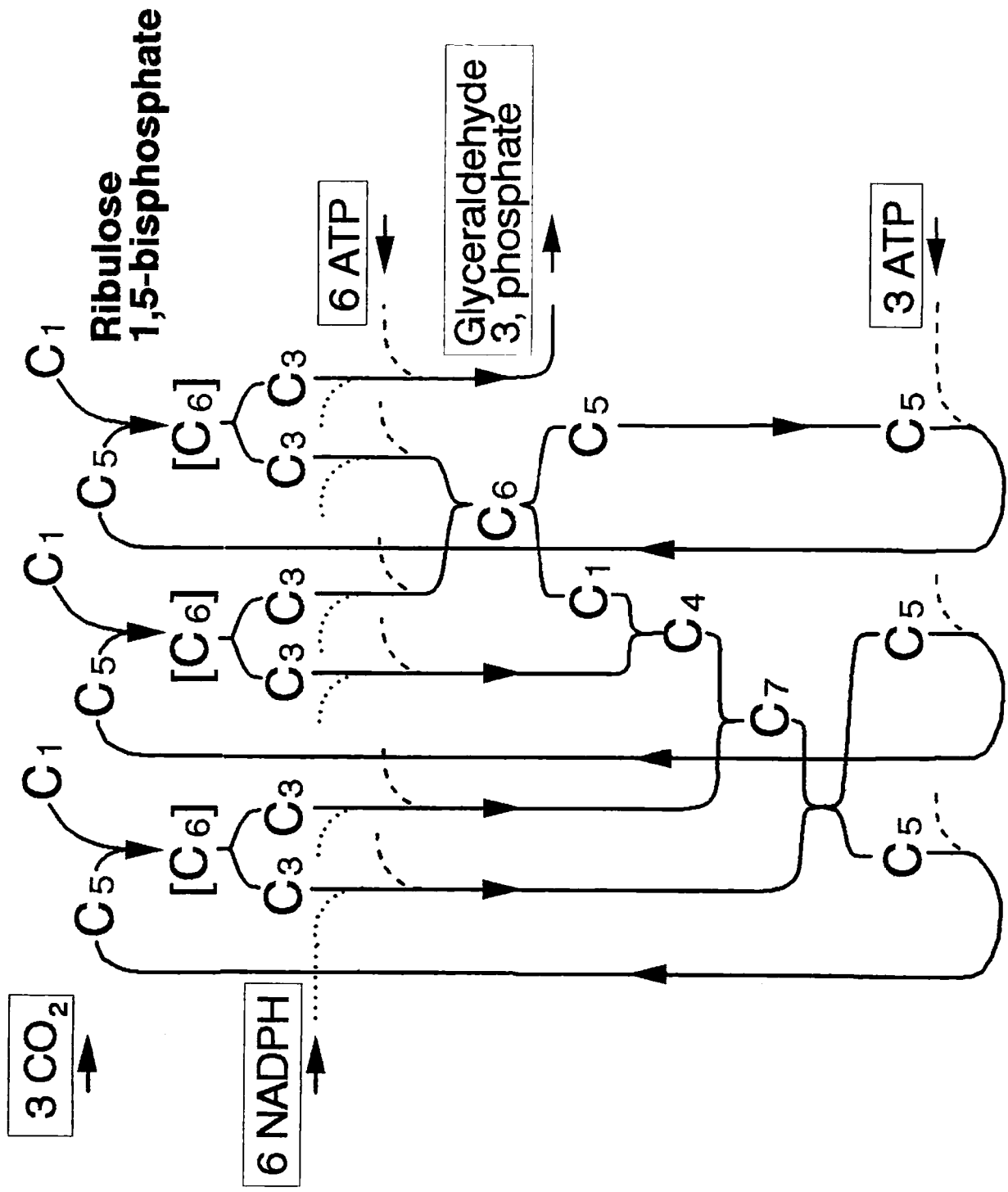
Syntrophy



Nonoxygenic bacterial photosynthesis

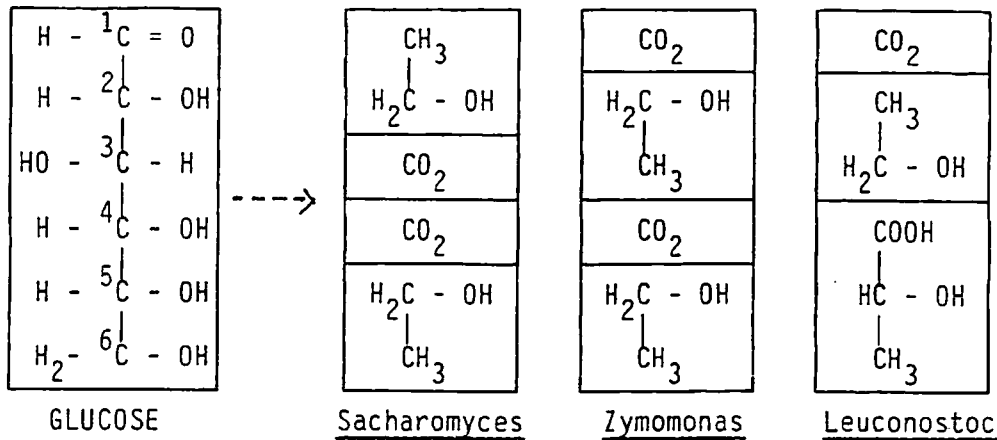






CALVIN CYCLE

Different ways in which microorganisms degrade glucose are illustrated below:

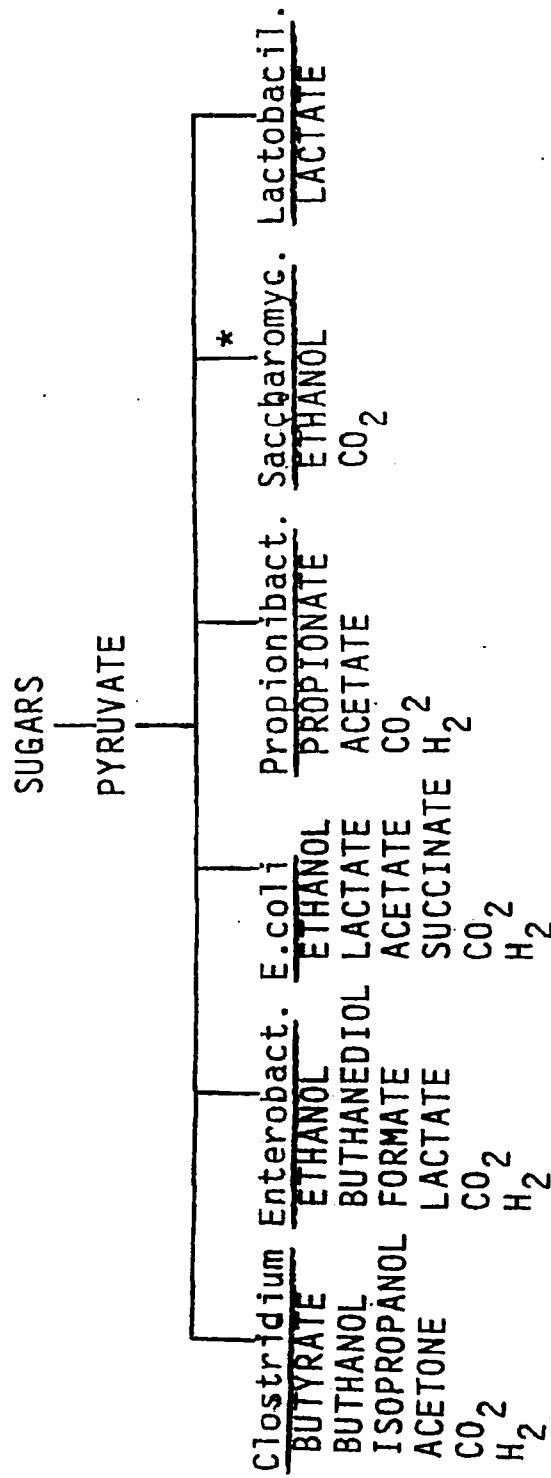


Fermenters like Sacharomyces and Zymomonas (alcohol fermenters) and Streptococcus lactis and Lactobacillus bulgaricus (lactic acid fermenters), which produce exclusively one product are called homofermenters, those like Leuconostoc, which produce two or more products are called heterofermenters.

Lactic acid fermentation has been empirically in use for centuries in food preparation and conservation. Preparation of sauerkraut and sour beets, for example, has been traditional way of preserving vegetables through the winter in German and Slavic countries. Lactic acid produced by Leuconostoc and Lactobacillus plantarum, the associated low pH, and possibly other antibiotic properties of these organisms keep the microorganisms of spoilage away. Oriental cultures have developed a variety of fermentation products, the best known of which are soy sauce and fermented black beans. Dairy industry has also started with empirical household use of microorganisms, and developed it to modern microbiological techniques and standardized products. Different strains and mixtures of Streptococcus, Lactobacillus and Leuconostoc are involved in production of sour cream (Streptococcus cremoris) and buttermilk, cottage cheese, yoghurt, kumiss and kefir. In the production of mature cheeses propionic bacteria are added (swiss cheese). Molds (P. roquefortii and Penicillium camembertii) and various bacteria are either inoculated into the cheese with a needle, or applied from the outside as a soft white coating, so that maturation i.e. partial microbial digestion of the cheese progresses from the outside inward.

In addition to the family lactobacteriaceae it is the family enterobacteriaceae which contains a vast variety of fermenters with an array of products:

Important fermenters and their products:



In addition to sugars, fermenters (Clostridium spp.) can degrade proteins (proteolysis) and ferment amino acids. Amino acid fermentation accomplished in two steps: 1. deamination and 2. decarboxylation. Fermentative redox reactions can also be carried between amino acids, whe alanine, leucine, isoleucine, valine and serine may serve as H-donors, glycine, prolin, arginin and tryptophan as H-acceptors.

Microbial Ecology

Cells ---> Populations ---> Guilds ---> Communities
+ Environment

Ecosystems

Methods:

A. Diversity

1. Direct observations - Acridine orange stain - Autofluorescence
2. Enrichment cultures
3. Immuno-probes - DNA probes

B. Activities -- Guilds -

1. Microelectrodes: pH, O₂, N₂O, H₂S.
2. Radioisotopes: C
3. Stable Isotopes: $\frac{^{13}\text{C}}{^{12}\text{C}}$ sample - $\frac{^{13}\text{C}}{^{12}\text{C}}$ standard

 $\frac{^{34}\text{S}}{^{32}\text{S}}$ $\frac{^{13}\text{C}}{^{12}\text{C}}$ standard

Habitats: Aquatic vs. Terrestrial

Marine - Deep sea

Freshwaters - Interior waters - Lakes - Rivers

Soil - Deep Subsurface