Spontaneous generation vs. Biogenesis

Generatio spontanea (SPONTANEOUS GENERATION)

Robert Hooke	- 1664, the cells as structural units
Francisco Redi	- 1668, maggots screening
Antoni van Leeuvenhoek	- 1674-1723 -- *infusoria*
John Needham	- 1745 - (growth after boiling)
Lazzaro Spalanzani	- 1795 - (seals after boiling) v. *Vis vitalis*
Antoine Laurent Lavoisier	- 1743-1794 - oxygen
Theodor Schwann & Mathias Schleiden	- 1838

Omnis cellula ex cellula (ALL CELLS COME FROM CELLS)

Rudolf Virchow	- 1858 concept of **Biogenesis**
Louis Pasteur	- 1861

Germ Theory of Disease

Agostino Bassi	- 1835 - silkworm deseased by fungi
Charles Cagniard de la Tour	- 1838 - fermentation caused by yeast
Louis Pasteur	
Ignaz Semmelweiss	- 1848 - puerperal fever (childbirth)
Joseph Lister	- 1865 - Carbolic acid (phenol)

Louis Pasteur

1857 - lactic fermentation spoils milk -
1860 - milk sterilized at 125 centigrades and 1.5 Atm. pressure
1861 - anaerobic bacterial spoilage documented
1865 - silkworm deseased by protozoa
1872 - classic paper on microbial fermentation published
1890 - pasteurization obigatory in the US

1881 - vaccine to prevent anthrax
1885 - The first anti-rabies vaccine
1888 - Institut Pasteur is founded at Paris

Robert Koch (1843-1910)

Discovered causative agents of:
1876 - anthrax, *Bacillus anthracis*
1882 - tuberculosis, *Mycobacterium tuberculosis*, staining
1883 - conjunctivitis
1884 - cholera, *Vibrio cholerae*
1881 - pure culture techniques
Fannie & Walter Hesse: - Agar
1887 - Richard Petri
tuberculin as a test of tuberculosis, US 1892
1905 - Nobel Prize in physiology or medicine.

1884 Koch's postulates:

1. Microorganism always present in diseased animal
2. Isolated, Identified and grown in pure culture
3. Inoculated into a healthy animal: same symptoms,
4. Microorganism re-isolated and identified as the same.

Vaccination

Ancien China: smallpox pustules ground & applied
1798 - Edward Jenner
1880 - Louis Pasteur - virulent and avirulent chicken cholera
1881 - anti-anthrax vaccine
1885 - anti-rabies vaccine
1889 - Paul Ehrlich and Emil von Behring: diphtheria vaccine

"Magic Bullets"

1909 - Paul Ehrlich - Salvarsan (As) vs. Syphilis
1935 - Gerhard Domagk - D.D. Woods. p-aminobenzoic A.
growth factor analog
1929 - Alexander Fleming - penicillin
1939 - Howard Florey. Anti-pathogens,
1941 - mass production, USA -- other antibiotics

Transmission Light microscopy (TLM)

Microscope parts: top-to-bottom:

Oculars	project the image w/o changing resolution
Objectives	magnify AND resolve the image (100x w.oil)
Specimen	on slide w. cover slip
Stage	mechanical (X & Y coordinates + nonius)
Condenser	projects the cone of light on the specimen
Condenser iris diaphragm	balances resolution vs. contrast+depth-of-focus
Condenser centering screws	center the light cone
Condenser focusing knob	focus on the field iris diaphragm
Specimen focusing knob course	focus on specimen at low magnification
Specimen focusing knob fine	same on higher magnifications (Z coordinate)
Field iris diaphragm	Koehler monitoring device
Light source	tungsten bulb 6 or 12V, 3200 K
Rheostat	regulates light intensity (but not color)
(Neutral density filters)	same w/o affecting color

How to Koehlerize your microscope?

1. The specimen is the center of your concern. Place it on the stage and bring it in focus with 10x objective
2. open condenser iris diaphragm and close the field iris diaphragm
3. bring the image of the field diaphragm in focus (superimposed on focused specimen) and center it
4. open the field diaphragm out of the field of vision
5. move to higher magnifications (40x dry; 100x oil immersion)
6. optimize image by using rheostat and condenser diaphragm (open iris = maximum resolution; closed iris = maximum contrast and depth of focus)
7. observe, draw, measure, photomicrograph

Important notes:

- Take care of the microscope.
- Adjust the oculars to YOUR vision: 1. adjust interpupilar distance, 2. focus specimen with the fixed ocular first, then follow up with adjustible ocular. If astigmatic, wear glasses.
- Observe continuously moving from plain eye to higher and higher magnifications, so as to stay well oriented in the world of the small.
- After using oil immersion, DO NOT return to 40x directly – only via 10x (avoid soiling the 40x high dry objective with oil).
- Wipe the 100x lens only with clean and dust-free lens paper.

Chapter 5

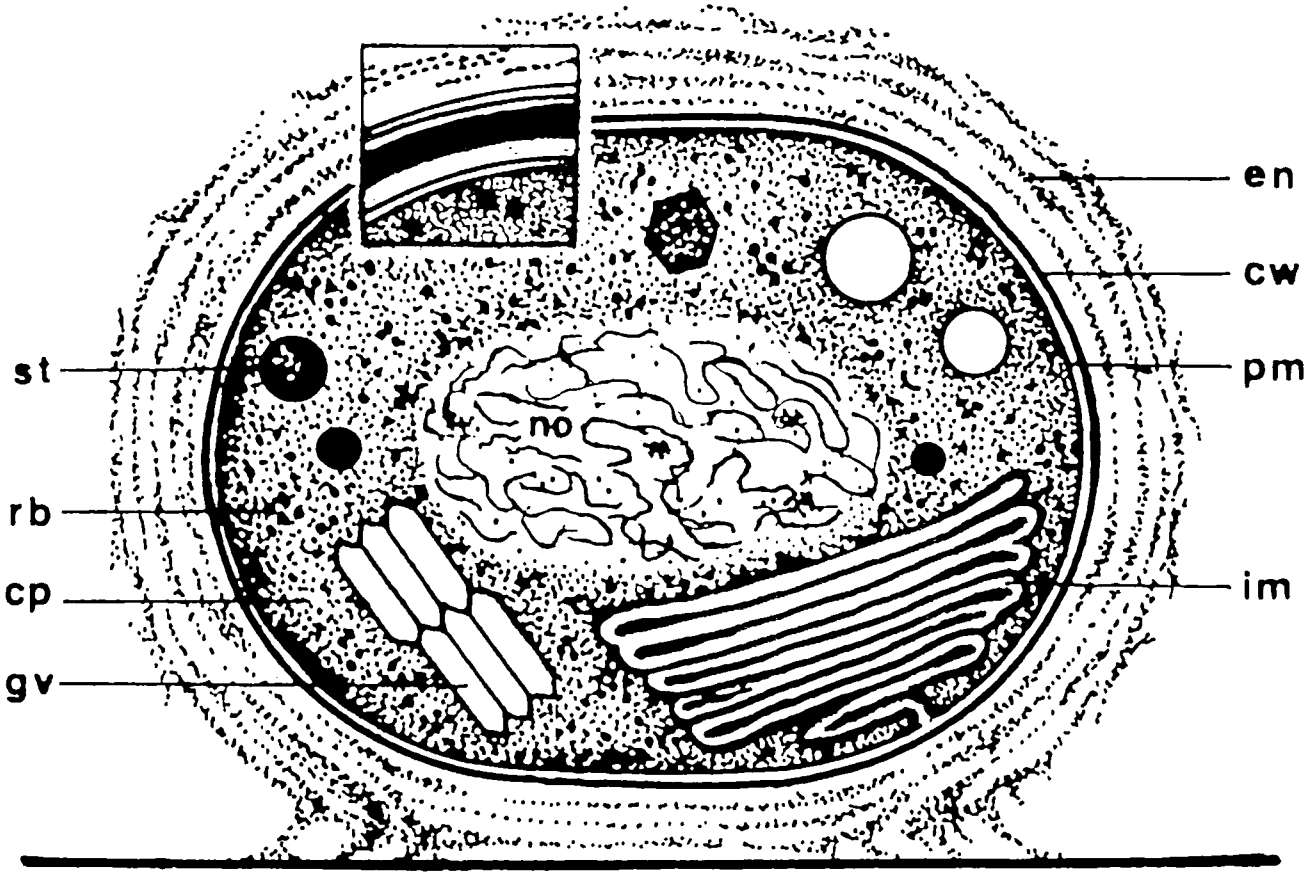


Figure 5.3 Ultrastructure of a prokaryotic cell: no, nucleoid (area with DNA); en, polysaccharide envelope (glycocalyx); cw, cell wall; pm, plasma membrane; im, intracytoplasmic membranes (e.g., thylakoids); gv, gas vesicles; cp, cytoplasm; rb, ribosomes; st, storage granules (e.g., lipids, polyphosphate, cyanophycine, sulfur, glycogen, PHB). (Modified from Schlegel HG. *Allgemeine Mikrobiologie*. Stuttgart: G. Thieme Verlag, 1985.)

Table 1. Major Differences Between Prokaryotes and Eukaryotes

PROKARYOTES	EUKARYOTES
ORGANISMAL GROUPS Archaeobacteria, Eubacteria	Protists (protozoa & algae), Fungi, Plants, Animals
CELL ORGANIZATION Simple, all cell functions take place within a single intracellular space bounded by a unit membrane. Cells (0.2-)0.5-2(-80) µm wide.	Intracellular space is compartmentalized into membrane-bounded organelles performing specialized functions. Cells (0.5-)10-50(-200,000) µm wide.
DEVELOPMENT Mostly unicellular and microscopic forms. Differentiation limited.	Uni- and multicellular, micro- and macroscopic forms. Differentiation can be extensive.
CELL WALL Contains peptidoglycan only in Eubacteria. Glycoproteins only in Archaeobacteria. Cell wall absent in mycoplasmas.	Contains chitin or cellulose. Glycoproteins common. Cell wall absent in protozoa and animals.
DNA A single molecule of DNA is in a closed-loop chromosome (nucleoid), attached to plasma membrane. Additional DNA in circular plasmids.	DNA distributed in several linear chromosomes, complexed with proteins (histones), within a membrane-bounded nucleus which also contains RNA.
SEXUAL SYSTEMS Absent or unidirectional (from donor to recipient). Genetic transfer and recombination by transformation, transduction, or conjugation	Regular, involving equal participation of both partners. Diploid and haploid forms alternate betweem fertilization and meiosis
RIBOSOMES All ribosomes with a sedimentation constant of 70S (Swedberg units, with subunits of 50S & 30S)	Ribosomes in cytoplasm with sedimentation constant of 80S (subunits 60S & 40S), those in mitochondria and plastids of 70S or variable
CELL DIVISION Cell division by fission, following DNA duplication and separation along plasma membrane.	Cell division by various forms of mitosis, involving microtubules and mitotic spindle in chromosome separation.
MOTILITY Simple, rotating bacterial flagella composed of flagellin protein. No cytoskeleton, intracellular motility or phagocytosis. Gliding motility common. Gas vesicles present in some forms.	Complex, flexing 9+2 flagella and cilia, composed of tubulin and other proteins. Cytoskeleton, amoeboid movement and phagocytosis based on actin-like proteins. Gliding motility common. Gas vesicles absent.
SPORES	

Endospores containing dipicolinic acid, heat-resistant. Actinospores, conidia, myxospores, akinetes.

Endospores absent. Various reproductive and resting spores following mitosis, meiosis, or fertilization (zygospores).

METABOLISM----------

Extremely diverse. Obligately and facultatively anaerobic, microaerophilic, and aerobic forms.

Almost all are aerobic; exceptions are few and mostly secondary.

GLYCOLYSIS AND RESPIRATION----------

Several glucose metabolism pathways. Respiration enzymes bound to plasma membrane or mesosomes. Not packaged separately.

Embden-Meyerhof glucose metabolism, followed by Krebs (CTA)-cycle, and cytochrom-based electron transport. Respiration enzymes packaged within mitochondria

PHOTOSYNTHESIS----------

Anoxygenic and oxygenic photosyntheses, with one or two photosystems. Various electron donors including H_2O. Enzymes bound to plasma membrane, chromatophores, thylakoids or vesicles, not packaged separately.

Only oxygenic photosynthesis involving two photosystems. H_2O is used as electron donor. Enzymes for photosynthesis in thylakoids, packaged within membrane-bounded plastids.

LIPIDS AND SECONDARY PRODUCTS---------

Vaccinic and oleic acids, and hopanes common. Archeobacterial lipids ether-linked. Steroids rare. Various antibiotics common.

Linoleic acid common, Steroids and alcaloids common.

Energy & Biocatalysis

$G^{0'}_f$ = free Energy of Formation

for elements $G^{0'}_f = 0$

$\Delta G^{0'}$ = Change in free Energy in reactions

reactants products

$\Delta G^{0'}$ of A + B ---> C + D equals

$\Delta G^{0'}$ [C+D] - $\Delta G^{0'}$[A+B]

products - reactants

if + ENDERGONIC (E-input needed)

if - EXERGONIC (E-yielding reaction)

$\Delta G^{0'}$ does not affect the rates of reaction,

Enzyme catalysis does -- by a factor up to 10^{20}

(1) specific substrate-binding

(2) substrate orientation to active site

(3) lowering the activation energy

Reduction Potential

E_0' = Reduction Potential ("Red-Ox potential", Eh)

Potential of an oxidized compound to become reduces under standard conditions (pH 7, T=25°C); it is a two-step process:

step I. **Ox/Red = half reaction** e^- **donor**

↓

step II. **Ox/Red = half reaction** **acceptor**

---

e.g.

I.	**$2H^+/ H_2$**	at	**- 421 mV**
II.	1/2 **O_2/ H_2O**	at	**+ 816 mV**

$$H_2 + 1/2\ O_2 \Rightarrow H_2O$$

ELECTRON TRANSPORT CHAIN
(Separation of protons and electrons)

	receives	donates
1. NADH + H^+	**H**	**H**
(NADH-dehydrogenase) - Inside membrane attached		
2. Flavoprotein (FMN, FAD) (transmembrane)	**H**	**e^-**
3. Non-heme Fe-S protein (Ferredoxin, Fe_2S_2; Fe_4S_4)	**e^-**	**e^-**
4. Quinones (hydrophobic, intramembrane)	**H**	**e^-**
5. Cytochromes (Fe-heme proteins)	**e^-**	**e^-**

Cyt. bc_1 complex (transmembrane)
Cyt. c (outside membrane attached)
Cytochrome oxidase (Cyt. a; transmembrane)

6. Oxygen (intracellular)

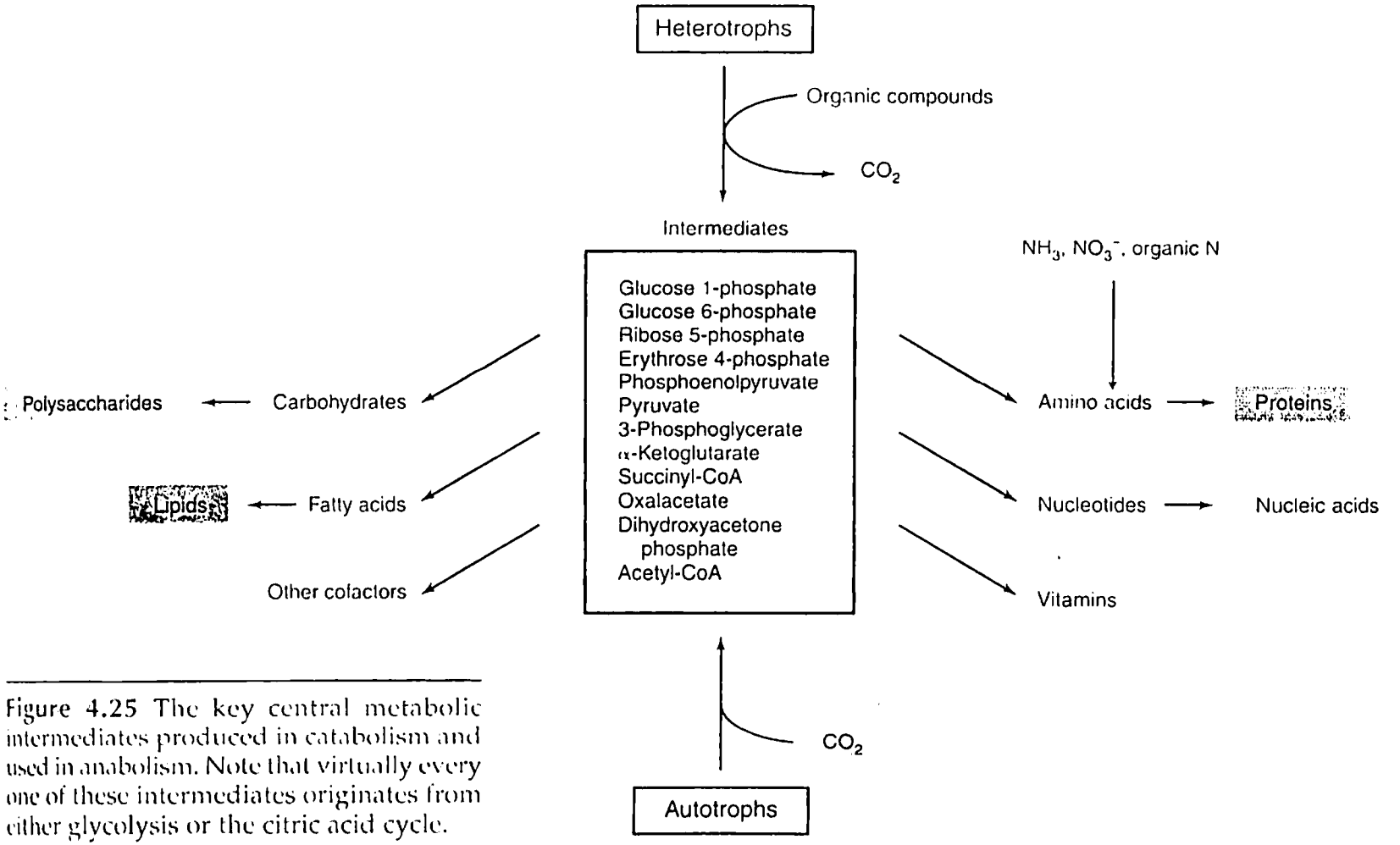


Figure 4.25 The key central metabolic intermediates produced in catabolism and used in anabolism. Note that virtually every one of these intermediates originates from either glycolysis or the citric acid cycle.

Exponential growth

GENERATIONS :	0	1	2	3	4	5	6 ... ... n
CELL NUMBER:	1	2	4	8	16	32	64 ... ...
AS BASE OF 2:	2^0	2^1	2^2	2^3	2^4	2^5	2^6 ... ... 2^n

If at the time 0, *the population consists of* N_0 *bacteria, then after* n *generations the number* N_n *of bacteria will be:*

$$N_n = N_0 \cdot 2^n$$

hence: $\log N_n = \log N_0 \cdot n \log 2$ *from here:*

$$n = \frac{\log N_n - \log N_0}{\log 2}$$

division rate $v = \frac{n}{t} = \frac{\log N_n - \log N_0}{\log 2 (t - t_0)}$

the number of generations $n = tv$

and generation time $g = \frac{t}{n}$ *or* $\frac{1}{v}$

Microbial Growth Control

Pasteurization - reduction of number of viable bacteria (active)
Heat Sterilization - Autoclave: **121°C 15 lb/squ.inch** ($1.1 kg/cm^2$)

Decimal Reduction Time
Thermal Death Time
Radiation Sterilization: Ionizing -
radiation unit: **1 roentgen**
absorbed radiation dose: **1 rad = 100 erg/g;**
1 Gy (Gray) = **100 rad**
Filter Sterilization:
a) Depth (fiber), b) Milipore, c) Nucleopore

BacterioSTATIC - Inhibits microbial growth
BacterioCIDAL - Kills bacteria
BacterioLYTIC - Kills and lysis (destroys) bacteria

Minimum Inhibitory Concentration (MIC)

Antiseptics - Disinfectants - Sterilants
Lipid solvent - Protein denaturant - Oxidizing - Alkylating
e.g. ethanol, isopropanol, formaldehyde, glutaralndehyde,
hydrogen peroxide, phenol, copper sulfate, iodine, chlorine

(a) Synthetic agents (Chemotherapy)
Growth factor analogs - Salvarsan -
Sulfaninamid - p-amino-benzoic A. (PAMA). S ----> O
F, Br, added ----> AA - nucleotides
Quinolones ----> DNA-gyrase
(b) Antibiotics - β - Lactam group: Penicillins & Cephalosporins
----> transpeptidization. Aminoglycosides: Streptomycin -
Macrolides: Erythromycine, Tetracyclines

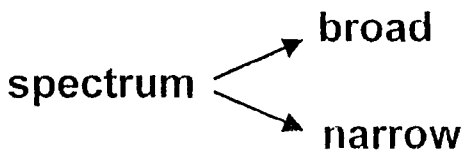
Sources of natural antibiotics:

Fungi: *Penicillium chrysogenum, Aspergillus fumigatis, Cephalosporium acremonium*

Actinomycetes: *Streptomyces griseus, S.* spp.

G+ bacteria: *Bacillus polymyxa, B. licheniformis*

Antibiotics:



Mode of action

DNA gyrase: (quinolones)

RNA polymerase: (actinomycin)

Protein synthesis: interference with ribosome functions

Protein chain initiation (streptomycin) - 30S

Protein elongation (chloramphenicol, cyclohexamide, tetracycline) - 50S

Cell membrane & wall synthesis: (penicillin, cephalosporin)

Chemical structure

ß-Lactams (penicillin, cephalosporin)

aminoglycosides (streptomycin, neomycin)

macrolides (erythromycin)

tetrecyclines in (chloro-, oxy-)

VIRUSES

Virion -- free virus particle
Infection
Host -- cell interior: (bacteria (phage, animals, plants)
Replication

Martinus Beijerinck 1892 - Tobaco Mosaic Virus (TMV) - filter test - *Contagium vivum fluidum* - Cell cytoplasm dependence - filterable viruses - viruses.

Virion: 5 - 200 kb = NucleoCapsid

Composition:

Capsid (coat, shell) - Capsomeres = self-assembling units
Shapes: isodiametric - Icosahedron 20 faces 3 axes
linear - Helical (TMV)

Naked: Nucleocapsid only
Enveloped: + Lipid bilayer (host)+glycoproteins (virion)
Complex: Head - Tail - Fibers (T4)

Life cycle:

Adsorption (attachment) - Penetration - Early Protein Synthesis
Replication - Late Protein Synthesis - Virion Assembly - Release

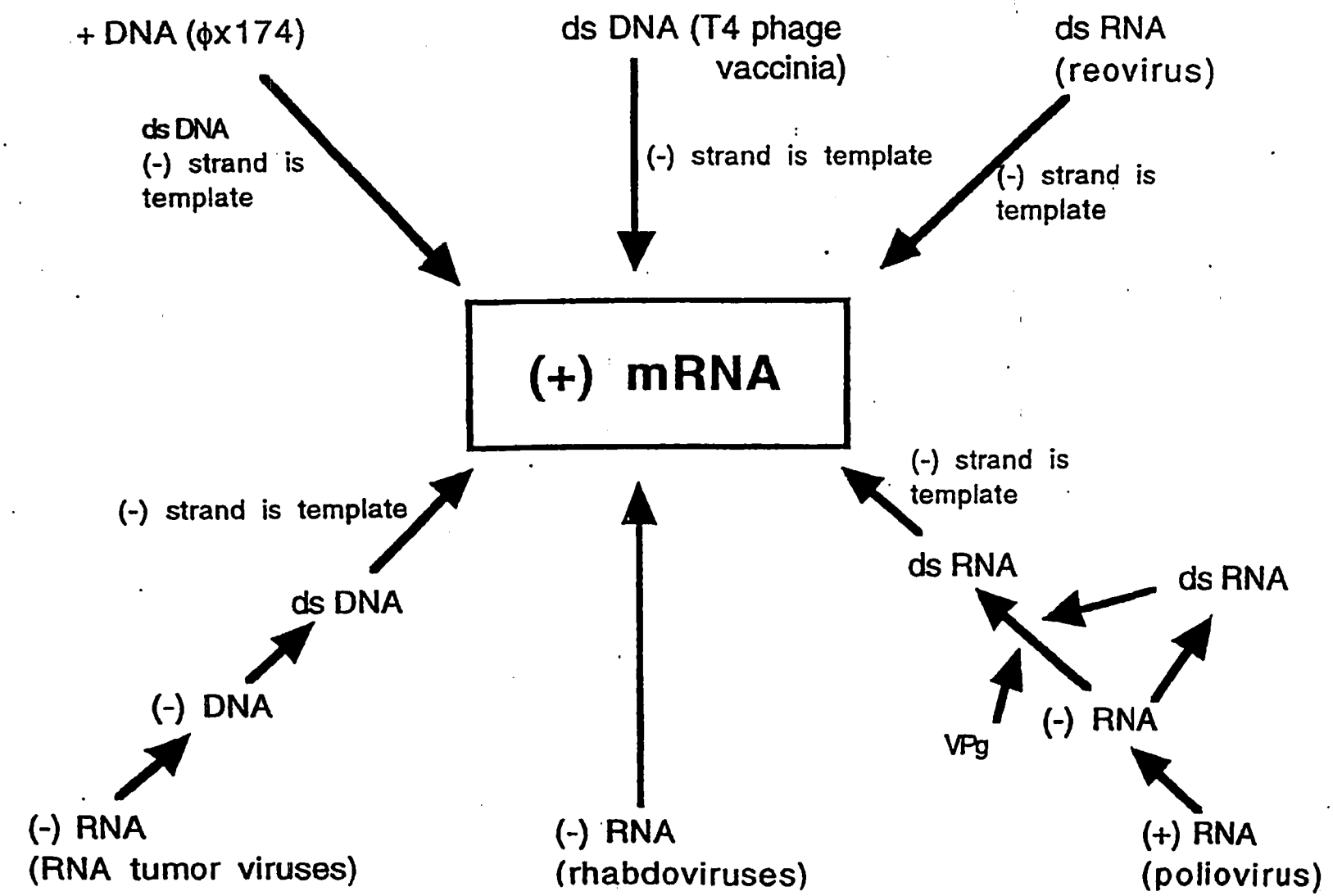
Enzyme synthesis:

a) Early: RNA-polymerase - Reverse transcriptase -
Neuraminidase - Lysozyme (bacteriophages)
b) Late: Coat Protein Synthesis

Parasite-Host Interactions:

VIRUS	HOST
Enzymes - Reorganization - Lysis	Restriction enzymes
Methylation + Glucosylation (T4)	
Enzyme blockade	Multiple Restrictions

14. A general diagram as originally envisioned by Baltimore may be used:



BacterioPhages

RNA

(1) **MS2** RNAss --- :Direct Protein synth.-Gene *Overlap*

DNA

(2) **fiX174** DNAss - O: Rolling Circle replication

(3) **M13** DNAss --- : Leaves the host live

(4) **T4** DNAds --- 170kbp : Lytic cycle 25 min - Sequential host RNA modification

Host RNA+ʃ-factor -> Early Enzymes -> Modify host RNA -> Middle Enzymes ->T4 ʃ-factor+DNA synthetase

(5) **LAMBDA** DNAds --- 48kbp:
with staggered ss-tails or 12 nucleotide at 5' (adhesive)
P_{left}: L1, L2; P_{right}: R1, R2; Regulation region

LYSIS (virulent)	vs.	LYSOGENY (temperate)
full gene expression		repressed expression
cro 3 - 2 - 1		Lambd-repressor 1 - 2 - 3
Antiterminators N+Q		blocks N + Q
DNA rolling circled		Int host DNA - Prophage
concatameres 12 nuctd tails		Host replication
late structural proteins		Induction (DNA damage)
Lysis	<	

---

(6) **MU** DNAds --- 39kbp: Mutator obligatory phage
Integration obligatory
with Transposable elements
Host DNA fragments incorporated

Animal Viruses

(7) **PicoRNA** RNAss --- + naked; 7.5kb - Polyo
cytoplasmic; monocystronic -> polyprotein -> 20 proteins
Polyomyelitis
Hepatitis-A
Common Cold

(8) **Rhabdo** RNAss --- - enveloped; Rabies
Virion Enzymes -> RNA+ -> Enzymes; Structural Proteins
capsomere + envelope - membrane (host) + Proteins (viral)

(9) **Influenza** RNAss --- - enveloped;
neuraminidase (destroys host's sialic acid) - segmented genome - antigenic shift (hybridized surface antigens) - shape: polymorphic.
1918 Pandemic Influenza

(10) **Reoviruses** RNAds --- naked - virion enzymes
Respiratory-enteric-orphan
Rota-v. Infant diarrhea
Orbi-v. Colorado tick-fever.

(11) **Herpes** DNAds --- 152kbp enveloped; nuclear
Circularization - Rolling circle - nuclear envelope wrap
H. simplex; Varicella-Zoster; Epstein-Barr - lymphoma

(12) **Retroviruses** 2xRNAss --- cytoplasmic

1. Entrance — HIV-AIDS
2. Reverse transcription — Rous sarcoma v.
3. Integration — Tumors
4. Transcription DNA -> mRNA -> progeny RNA
5. Encapsidation
6. Budding

(1) Entry: gp120 env.protein -> CD4 receptor -> membrane
(2) Rev.transciptase:
a - RNA->DNA; b - DNA->DNA; c - ribonuclease H
primer tRNA - Long Terminal Repeats (LTR) ->
(3) Integration: provirus (transposable element, cf. MU)