LO3a

Recap L02

Core Concept

01: Evolution, Thermodynamics, Habitat diversity, Ecology, Physiology their integration define Microbiology

02: Unique goal of microbial life: survival, maintenance, generation of ATP, growth of new cells

03: Planet's habitat diversity results in genetic, molecular, metabolic and physiological microbial diversity

LIVING vs NON living

You are alive if you have:

1. A membrane subsystem for compartmentalizing the functional network components

2. An autocatalytic metabolic subsystem that functions out-of-equilibrium by capturing energy and material resources

3. An information-based subsystem for processing and transferring genetic information to the progeny via self-replication

Ganti, T. in The Principles of Life (ed. Szathmary, E. and Griesemer, J.) Ch. 3(Oxford Univ. Press, Oxford 2003)

How can microbes grow?

How did microbes invent biochemistry?

Key concepts for Microbial Life

- **Metabolic diversity**: cellular processes that support growth

  Energy is conserved from chemical reaction or from light

  ★**Energy is conserved, reducing power is obtain during catabolic reactions and cells growth by decoupling this power to anabolic-biosynthetic reactions (modularity)**

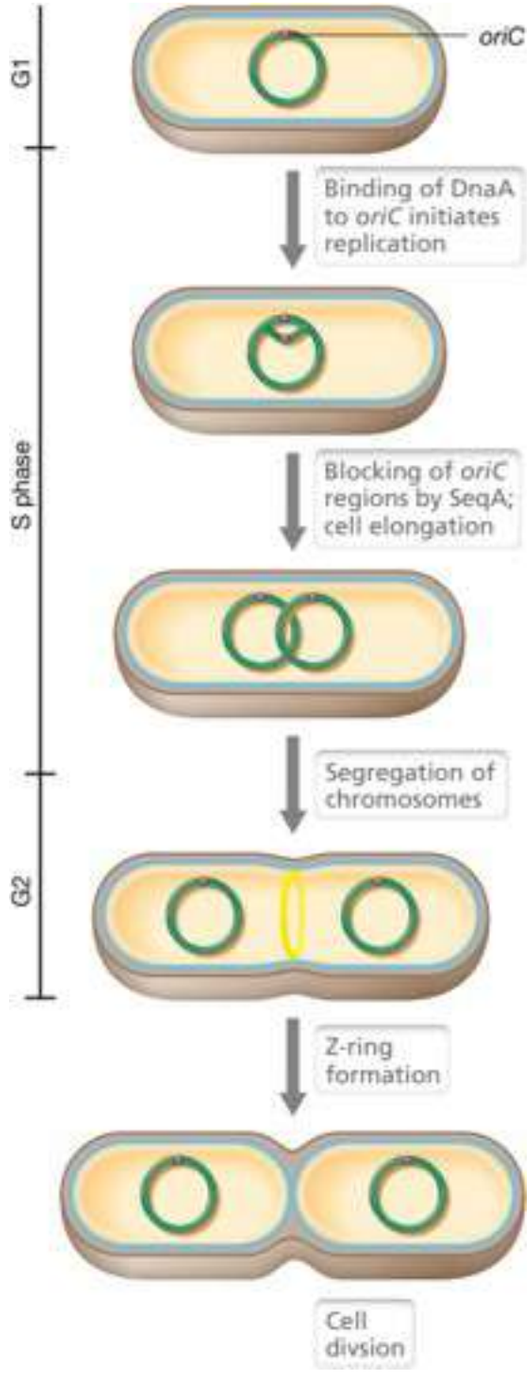
  ★**Electron flow via redox provide energy for ATP synthesis via:**

  1. **Substrate level phosphorylation**
  2. **Oxidative phosphorylation**
  3. **Photophosphorilation**

- **Ecological diversity**: interactions between organisms and their environments

- **Phylogenetic diversity**: evolutionary relationships between organisms

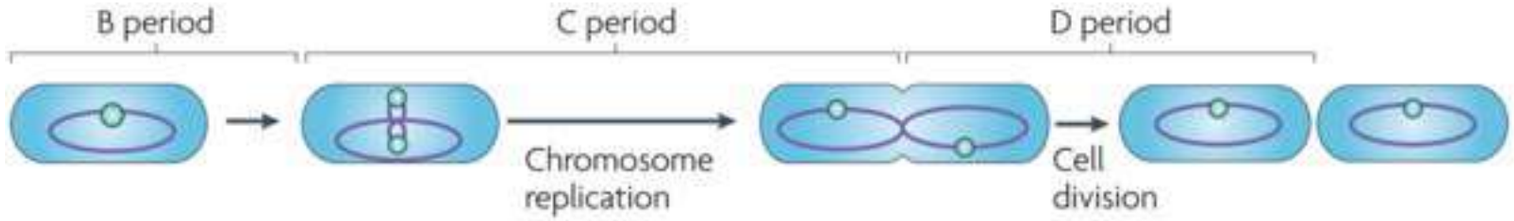
Cellular growth



Madigan et al. 2018

Growth is intimately connected with nutrient availably and energy status of the cell

Spatial and temporal coordination between the DNA replication and cell elongation, DNA segregation into new cells

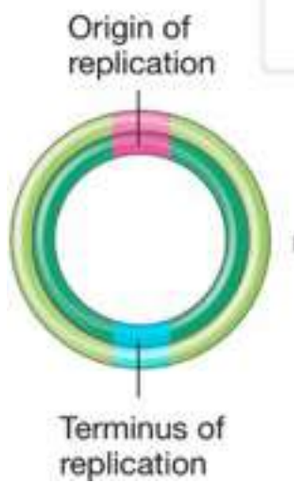


Wang & Levin, 2009

oriC in green

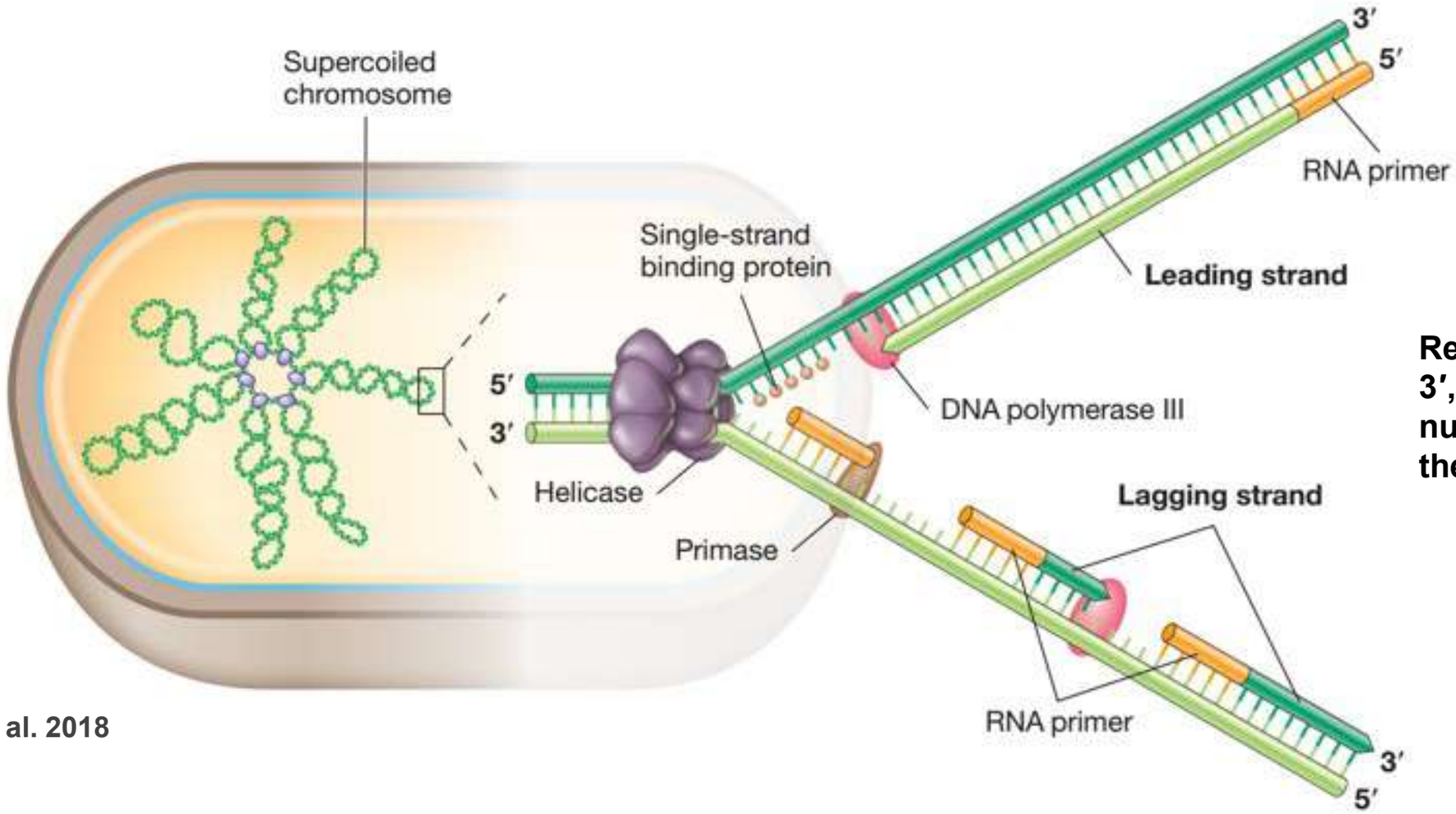
B period: time between division (birth) and the initiation of chromosome replication
C period: time window for chromosome replication
D period: time between the completion of chromosome replication and cell division

Protein Tus binds to terminus site and stop replication



Madigan et al. 2018

Zooming into DNA replication

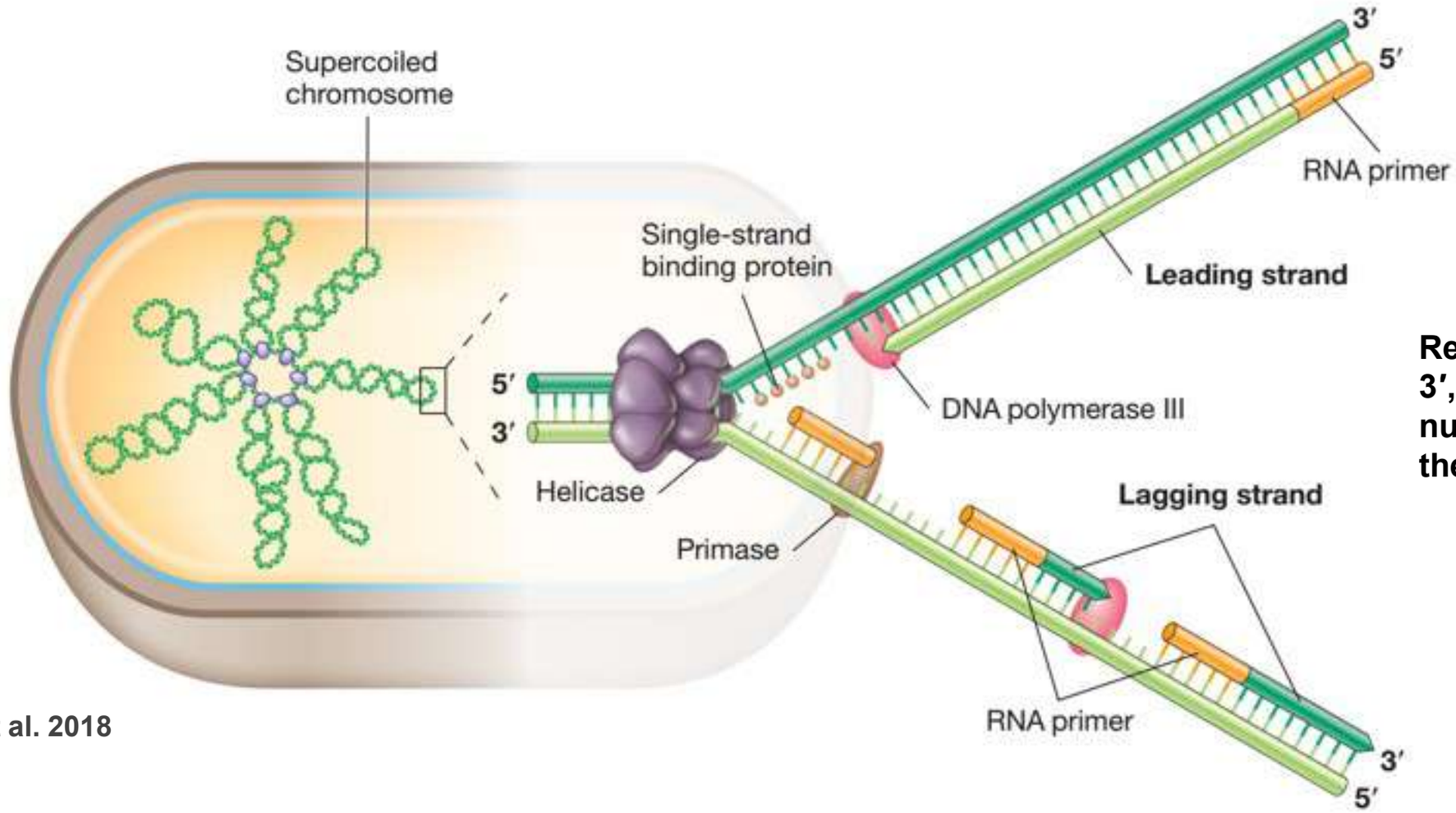


Madigan et al. 2018

Replication always 5′ to 3′, adding a new nucleotide to the 3′-OH of the growing chain

1. DNA synthesis begins at a single site on chromosome, origin of replication (**oriC**), where DnaA binds and opens up double helix
2. Stabilization of strands by **helicase** (DnaB), and its **helper loader protein** (DnaC). Two helicases are loaded, one onto each strand, facing in opposite directions

Zooming into DNA replication

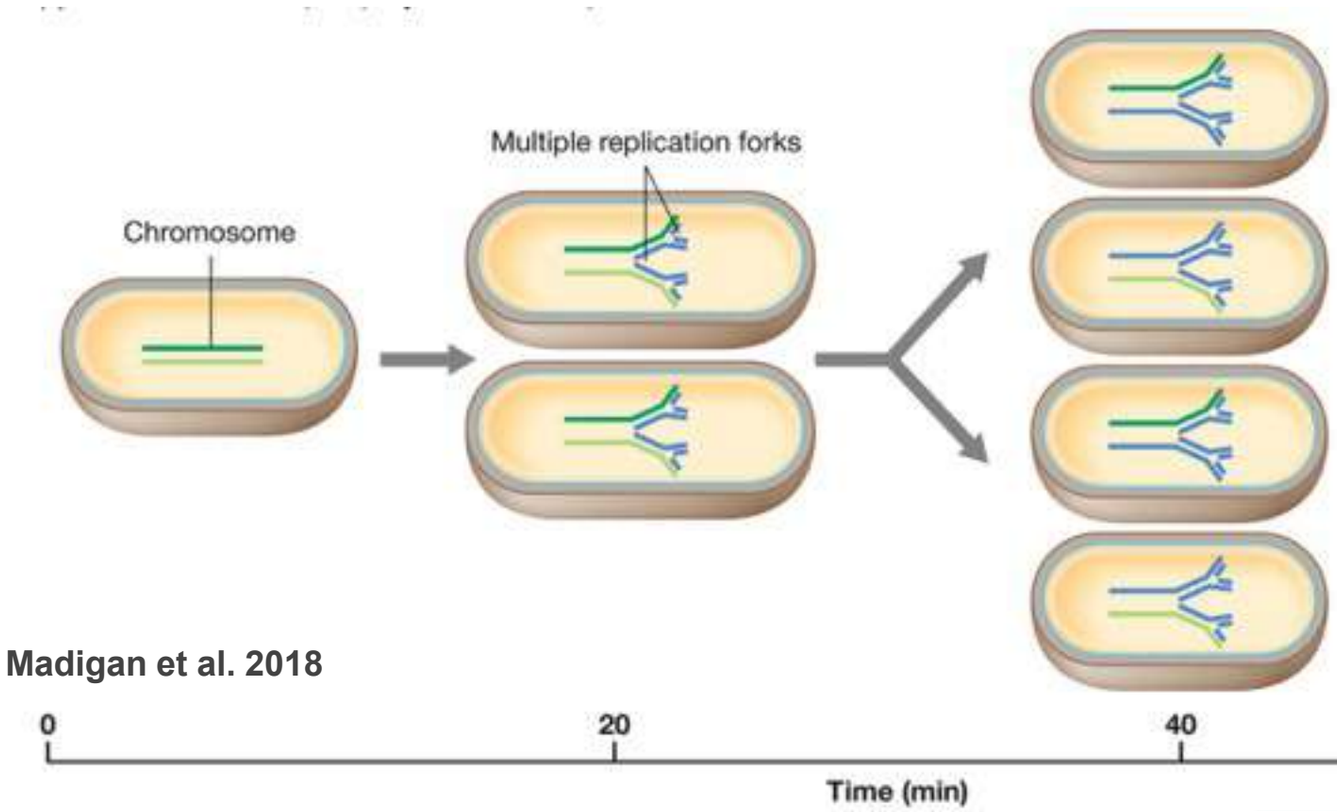


Madigan et al. 2018

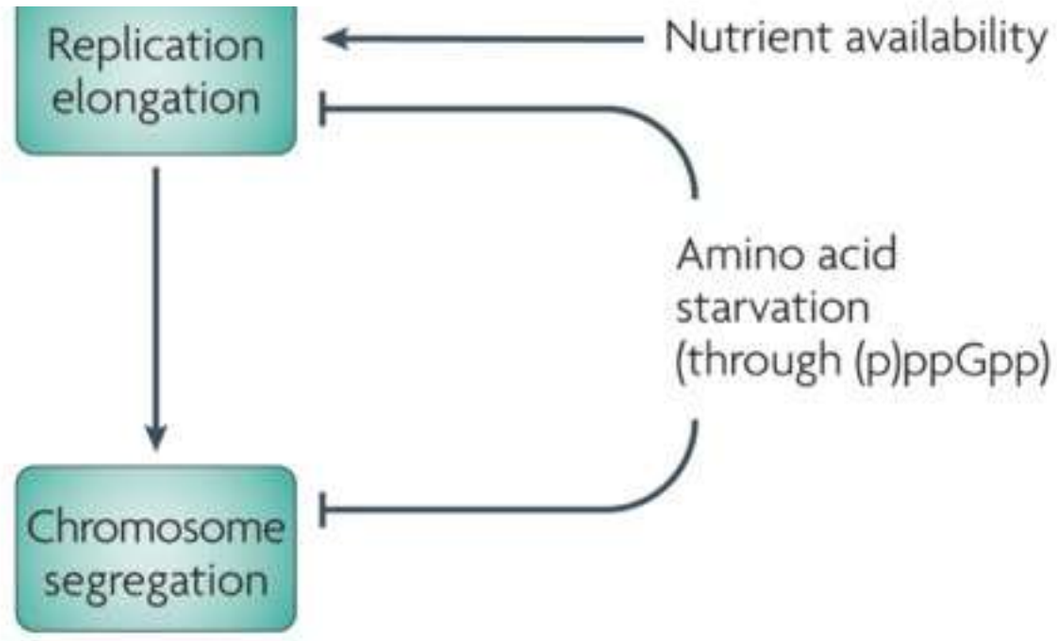
Replication always 5′ to 3′, adding a new nucleotide to the 3′-OH of the growing chain

3. Two **primase and two DNA polymerase III** enzymes are loaded onto the DNA behind helicases and initiation of DNA replication begins
4. As replication proceeds, replication fork appears to move along the DNA

Nutrient & energy status controls growth rate



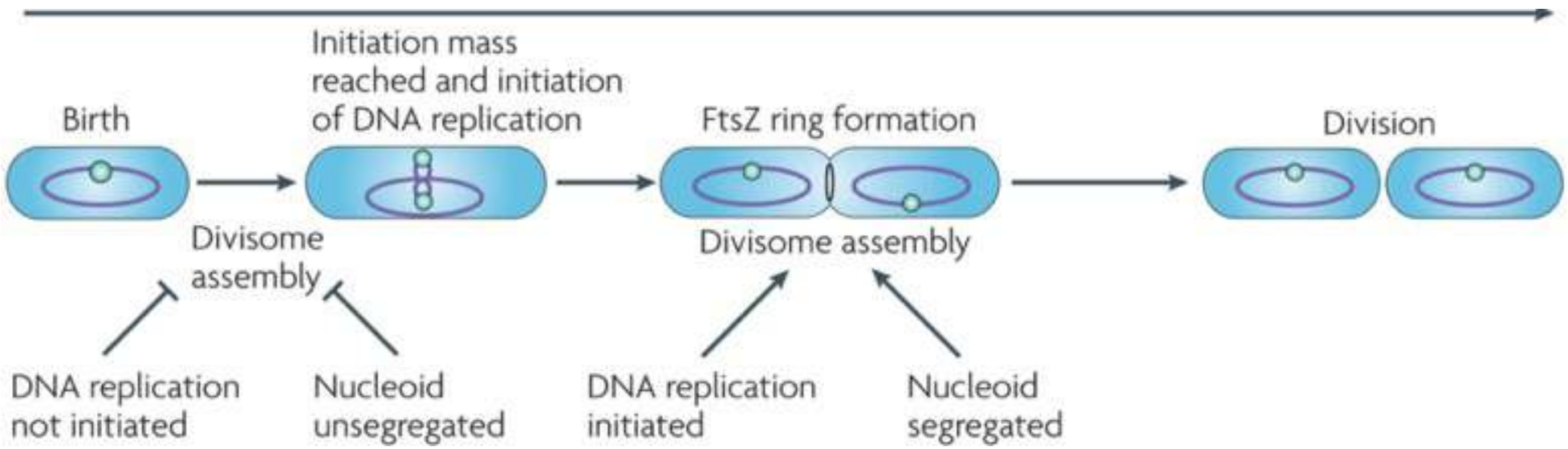
- From 40 to 20 min replication in *E.coli*
- Multiple replication forks
- P replete cells
- ATP cellular pool is essential for DNA replication
- A.A. pull rich



Wang & Levin, 2009

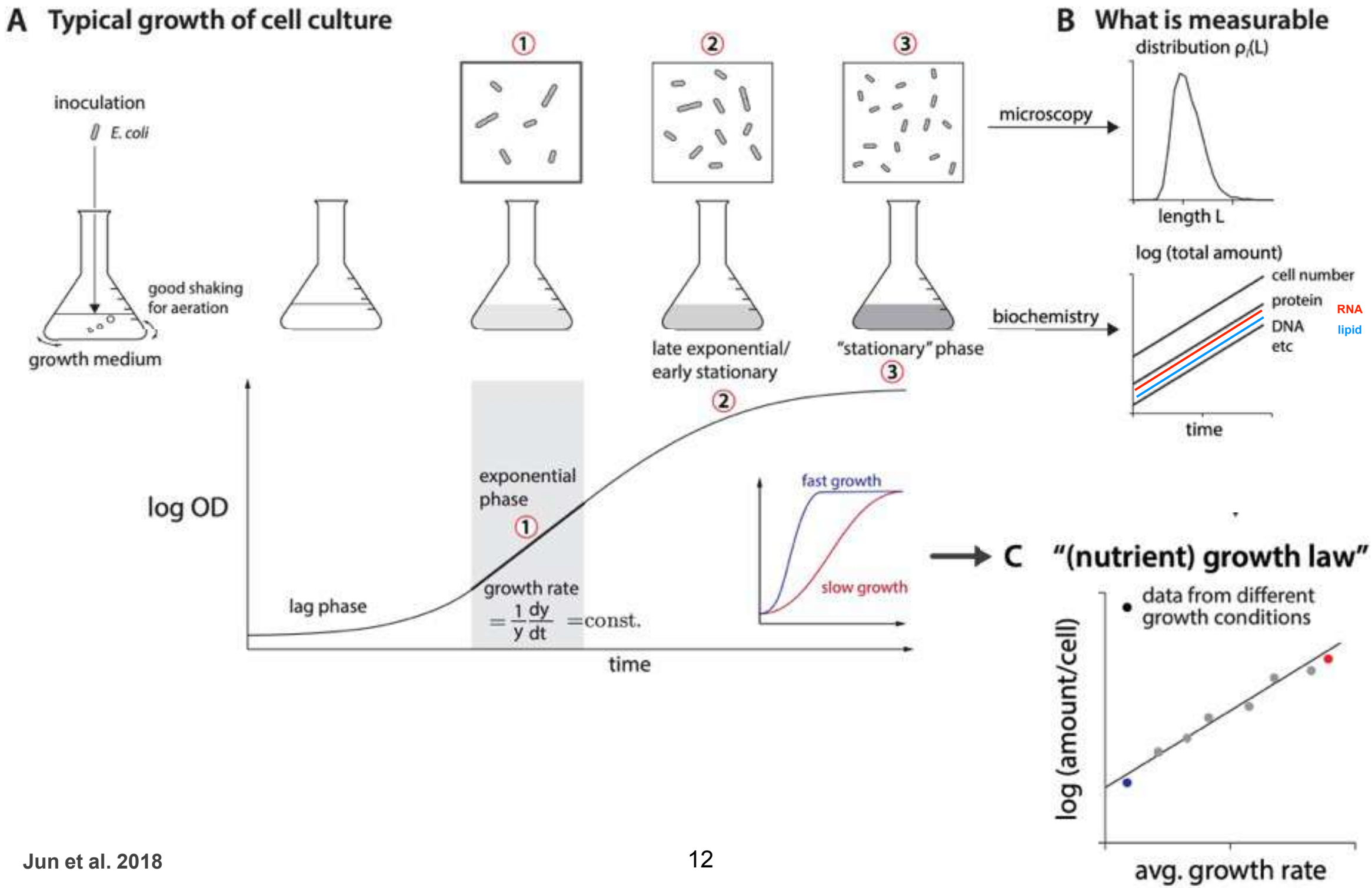
Chromosome segregation and Divisome complex

- ***E.coli* daughter chromosomes must still be segregated prior to cell division**
- After replication the resulting **circular chromosomes remain interlinked**
- Linkage is broken by the structural maintenance of chromosome complex, which is composed of a **topoisomerase (IV) and MukBEF protein**
- MukBEF proteins move to discrete locations within the nucleoid and recruit a topoisomerase to separate replicated sister chromosomes (a process called **decatenation) prior to segregation**
- **Divisome complex: Fts proteins interact in cell to form a division apparatus**
- Precise and temporally coordinate cleavages for growth



Wang & Levin, 2009

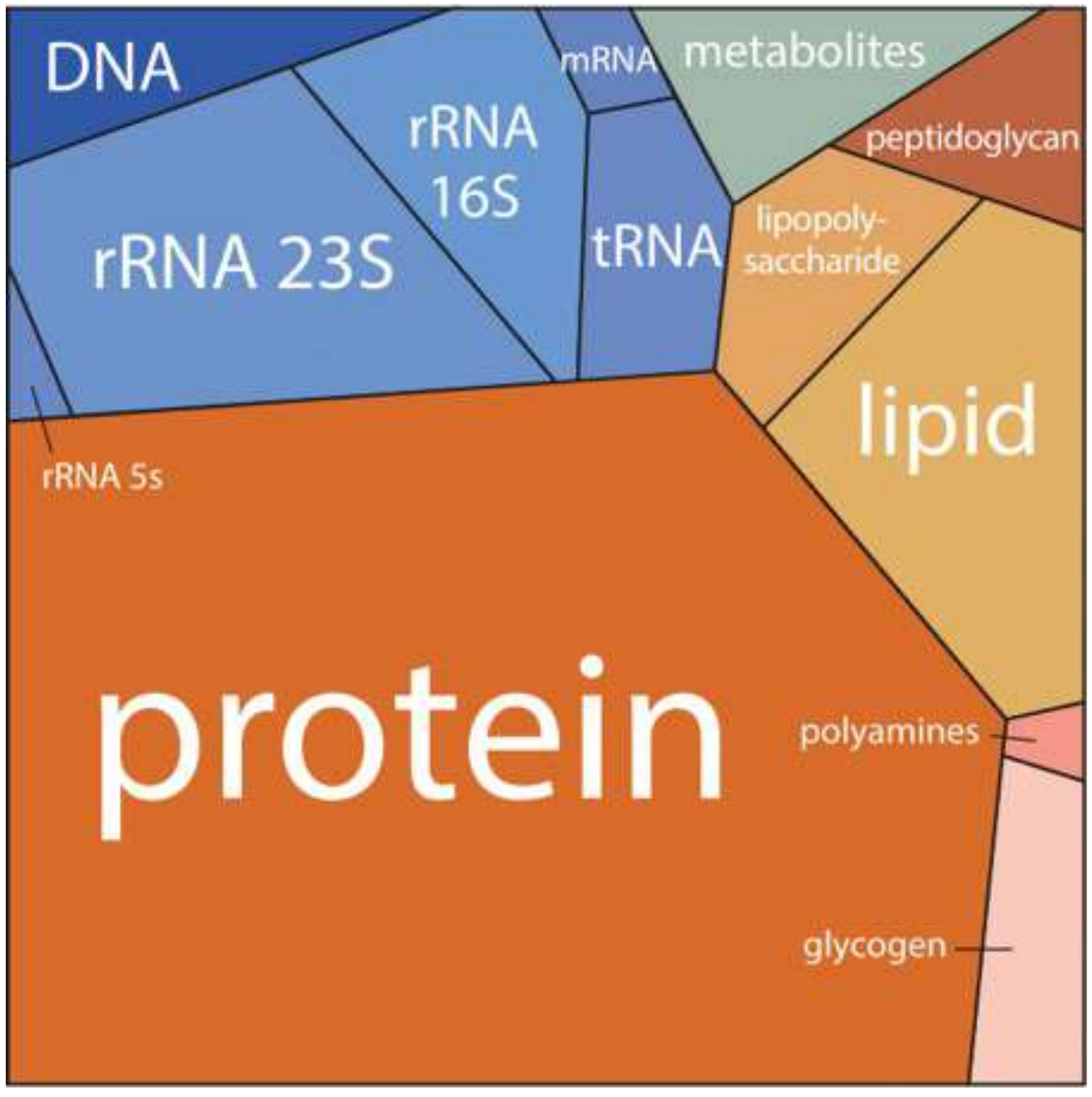
Growth in an homogeneous-predictable-low diversity environment, I



Jun et al. 2018

Growth in an homogeneous-predictable-low diversity environment, II

A Voronoi tree diagram of *E.coli* composition



http://book.bionumbers.org/

40 min

- Each polygon area is the relative fraction of the corresponding cellular constituent (dry mass)
- Similar colors = related functional role
- Steady-state mean cell size (large circles) scales exponentially with nutrient-determined growth rate

Defined growth conditions
What is missing?

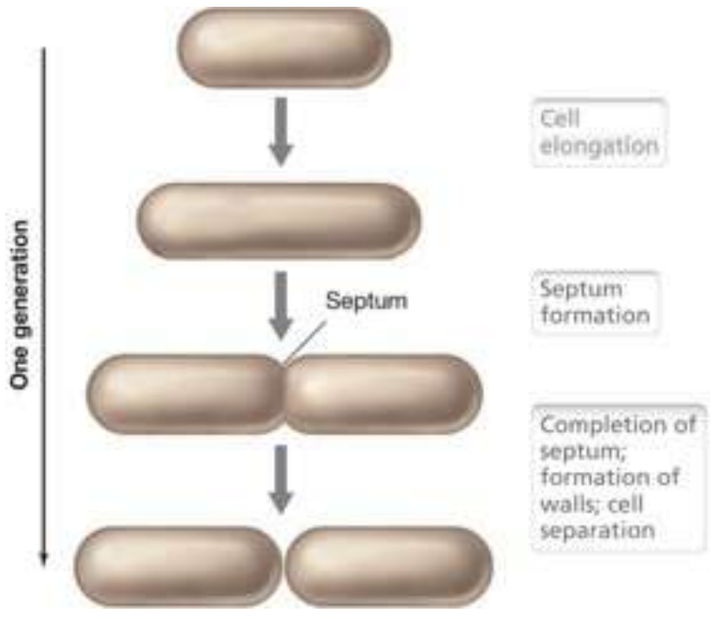
Growth

- A fixed relationship exists between initial cell number in a culture and cell number present after a period of exponential growth: **$N = N_0 2n$** where N is final cell #, N_0 is initial cell #, and n # of generations during period of exponential growth

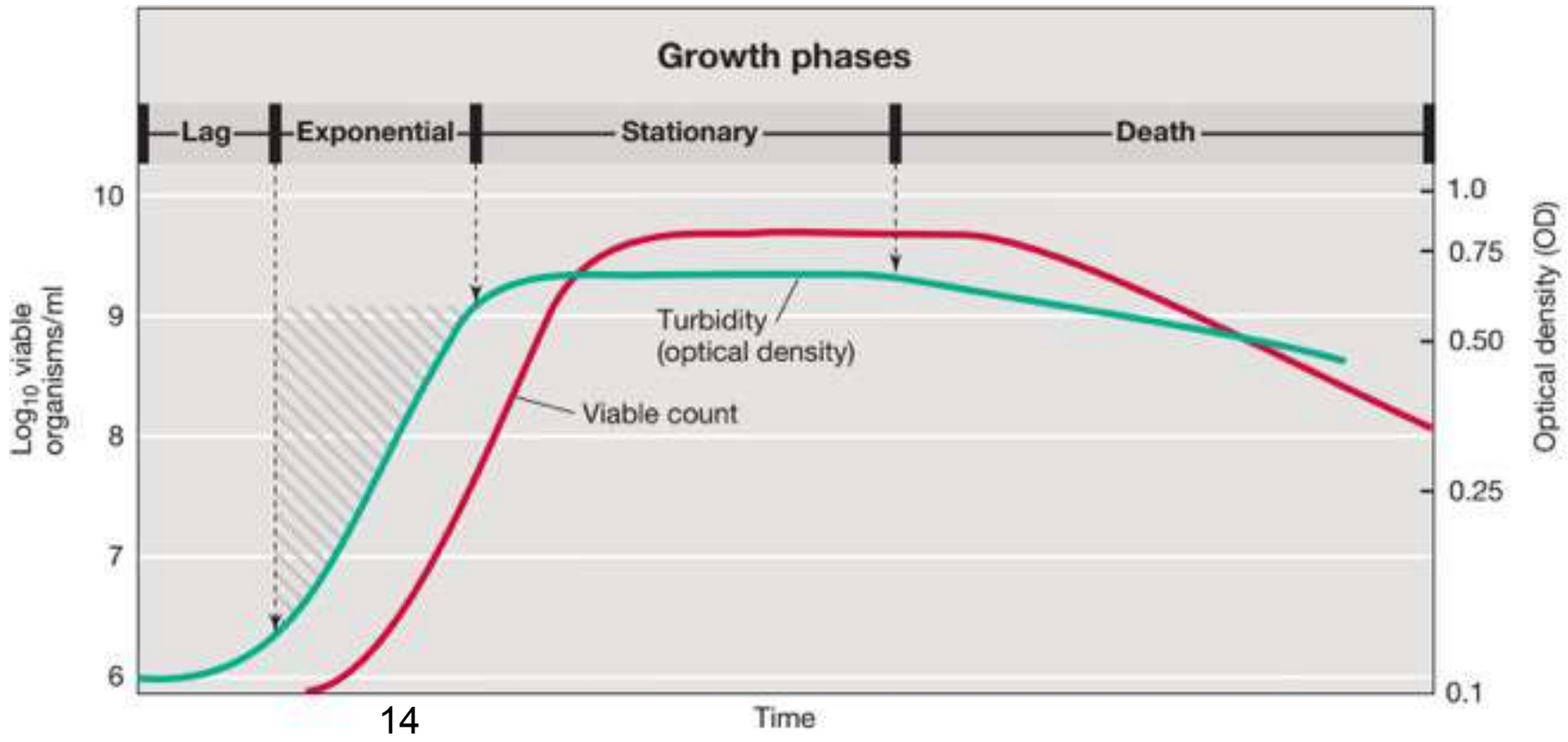
- **Generation time** (**g**) of the exponentially growing population is **t/n**, where t is the duration of exponential growth in days, hours, or minutes **(g is the time from 1 cell to 2 cells)**

- Equation $N = N_0 2n$ can be expressed in terms of n by taking the logarithms of both sides: **$n = [3.3(\log N - \log N0)]$**

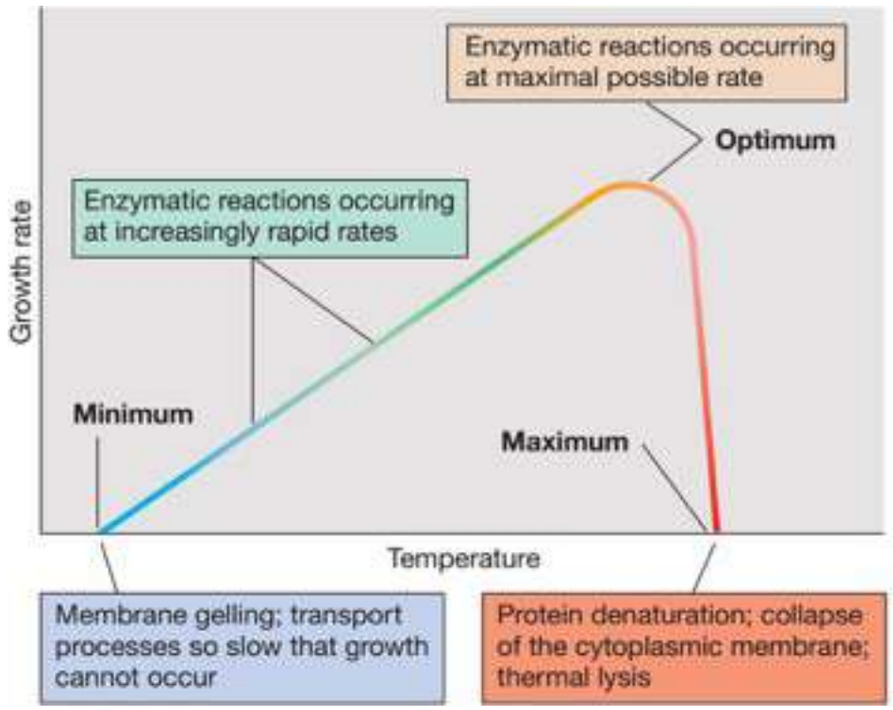
- I**nstantaneous growth rate constant** expresses the rate at which the population is growing at any instant (by contrast, g is the mean time required for the cell population to double); **k** is expressed in units of reciprocal hours (h-1): **$k = 0.693/g$**



Madigan et al. 2018

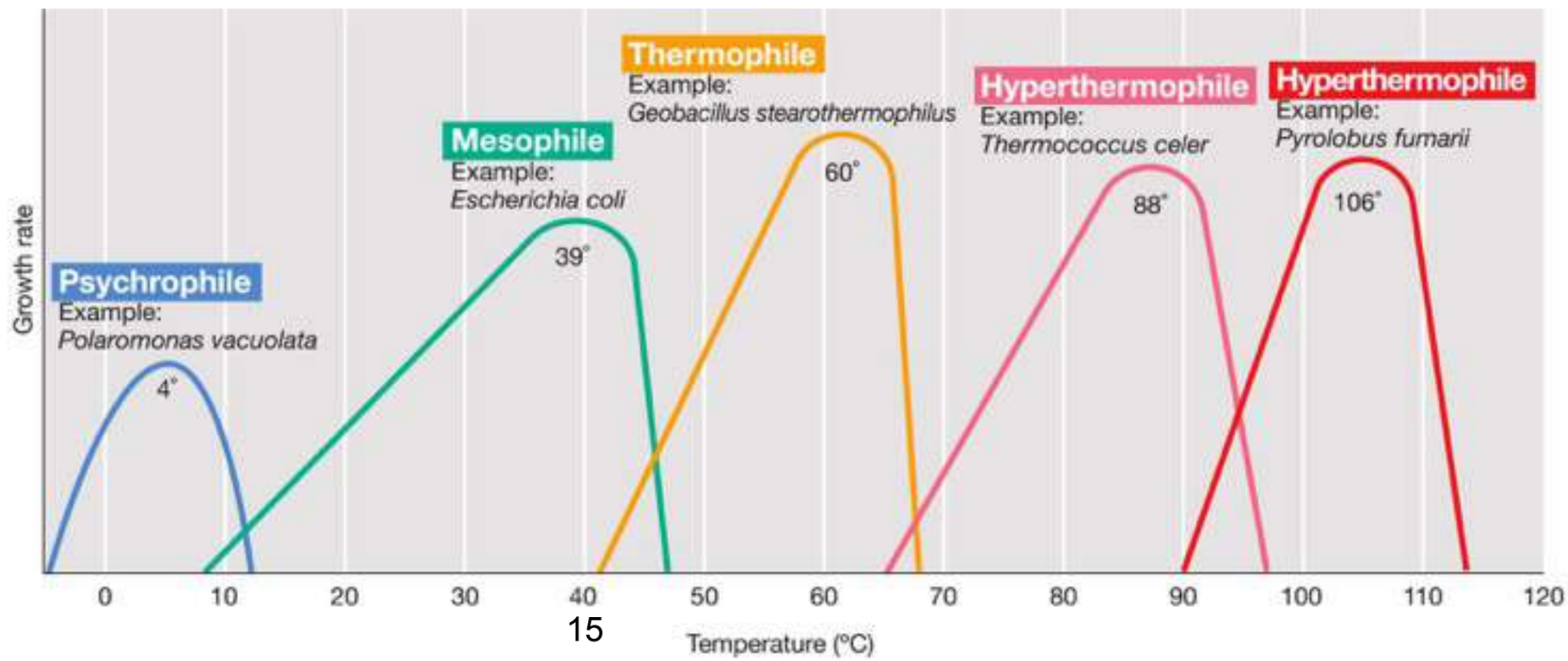


Temperature affects growth



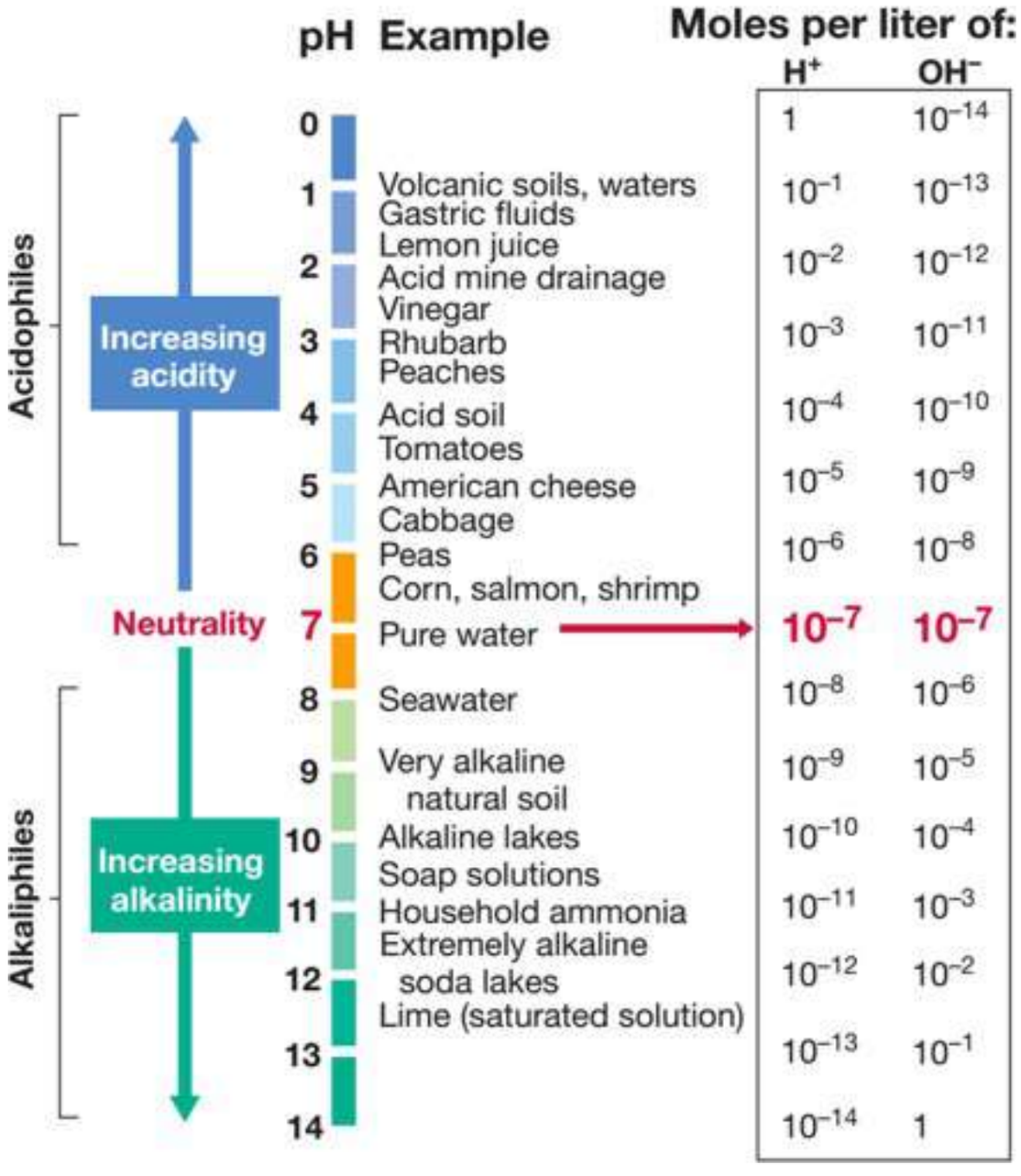
- **Reactions** occur **faster** at warmer temperatures b/c rate of collisions between molecules increases
- If temperature gets too hot the enzyme may denature and fail to function
- **Arrhenius activation energy**, which describes temperature effect **on catalytic rate constant**, kcat, and thermal stability, which describes temperature effect on **thermal inactivation rate constant**, kinact

Peterson et al., 2007



Madigan et al. 2018

pH affects growth



Madigan et al. 2018

- **Optimal pH** for growth refers to **extracellular environment only**
- **Intracellular pH** must be maintained in 5 - 9 range
- Extreme acidophiles and alkaliphiles maintain cytoplasmic pH values ~neutrality
- **Extreme pH** affects macromolecule **structures**
- H-bonds holding together strands of DNA break up at high pH
- Lipids are hydrolyzed by an extremely basic pH
- PMF responsible for production of ATP in cellular respiration depends on concentration gradient of H^+ across membrane

pH adaptive strategies

Active (**proton exclusion, exchange, pumping, consumption and neutralization**) and passive (**cytoplasmic buffering**) mechanisms of pH homeostasis as well as damage mitigation strategies (**DNA repair, synthesis of acid stable proteins**)

Quatrini & Johnson, 2018

- *Lactobacillus plantarum* is an anaerobic bacterium that **produces lactic acid** as metabolic product and thus lowers pH but also prefers low pH values
- *Corynebacterium ammoniagenes* produces **urease that cleaves urea into ammonia** and thus increases pH at the same time, it prefers higher pH values
- *Pseudomonas veronii* also **increases medium pH** but prefers low pH values for growth
- *Serratia marcescens* **strongly lowers pH** but better tolerates comparably higher pH values, with a slight optimum at around pH 8

In summary, we find that microbial growth often leads to dramatic changes in the pH of the environment, and this pH change can promote or inhibit bacterial growth

Ratzke & Gore, 2018

Osmotic pressure affects growth

- **Water availability** is expressed in terms of **water activity** (aw): vapor pressure of air in equilibrium with a substance or solution / the vapor pressure of pure water
- Values of aw vary between 0 (no free water) and 1 (pure water)

- **H_2O diffuses** from regions of high water concentration (**low solute concentration**) –> regions of lower water concentration (**higher solute concentration**) in the process of **osmosis**
- Cytoplasm has a higher solute concentration than the environment, so H_2O –> into cell
- Under such conditions, cell is said to be in **positive water balance**, <u>normal cell state</u>

TABLE 5.4 Water activity of several substances

Water activity (a_w)	Material	Example organisms[a]
1.000	Pure water	*Caulobacter, Spirillum*
0.995	Human blood	*Streptococcus, Escherichia*
0.980	Seawater	*Pseudomonas, Vibrio*
0.950	Bread	Most gram-positive rods
0.900	Maple syrup, ham	Gram-positive cocci such as *Staphylococcus*
0.850	Salami	*Saccharomyces rouxii* (yeast)
0.800	Fruit cake, jams	*Zygosaccharomyces bailii* (yeast), *Penicillium* (fungus)
0.750	Salt lakes, salted fish	*Halobacterium, Halococcus*
0.700	Cereals, candy, dried fruit	*Xeromyces bisporus* and other xerophilic fungi

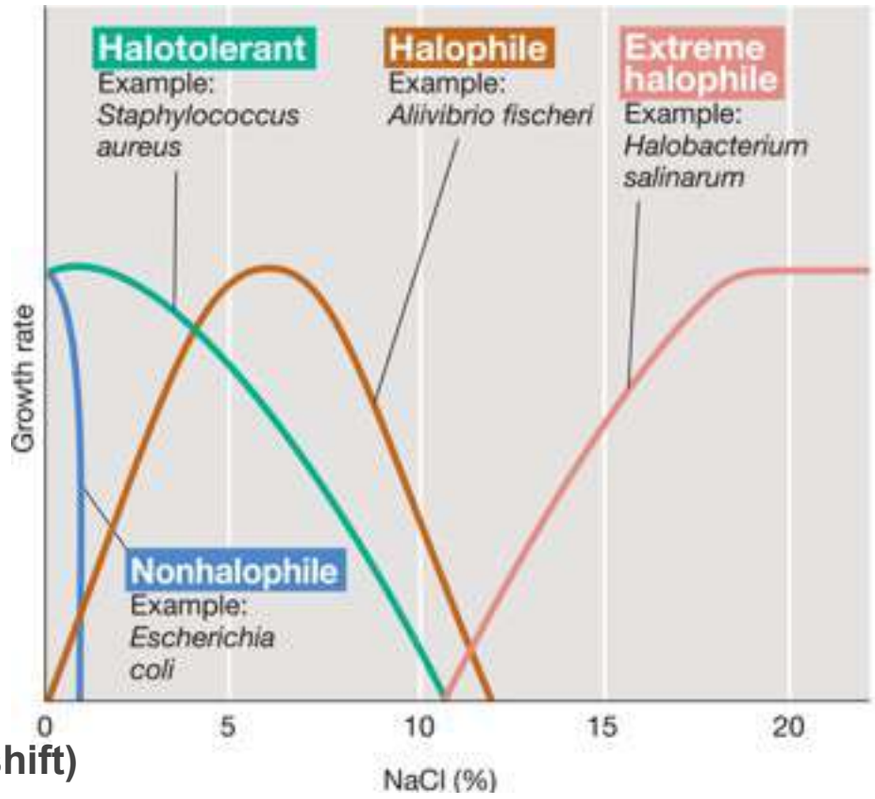
Madigan et al. 2018

Osmotic pressure strategies

Madigan et al. 2018

From high aw –> low aw: cells maintains positive water balance **by increasing its internal solute** (**compatible not interference with metabolism**) concentration:

1. Pumping solutes into the cell from the environment
2. Synthesizing cytoplasmic solutes



Compatible solutes are highly water-soluble organic molecules & electrolytes: sugars, alcohols, and amino acid derivatives
Glycine betaine, an analog of the amino acid glycine, is widely distributed among halophilic bacteria
Other common compatible solutes include sugars such as sucrose and trehalose, dimethylsulfoniopropionate (produced by marine algae)

H_2O OUT of cells as their medium becomes more concentrated (an osmotic upshift)
H_2O INTO cells as their medium becomes more dilute (an osmotic downshift)

Bacterial membranes have high water permeabilities, so **cellular hydration is altered within seconds** of an osmotic shift

Approximately **0.5 g of wate**r is bound **per gram of cytoplasmic macromolecules**

At low osmolality, phosphate, the predominant inorganic anion, is present at a concentration of approximately 10 mM. Most metabolites are maintained at comparable or lower concentrations

At high osmolality, the amount of K+ exceeds that of nucleic acid phosphate and glutamate accumulates as K+ counterion (the concentration of glutamate rising from approximately 0.05 to 0.50 M). The trehalose concentration rises from approximately 0.04 to 0.4 M

Wood, 1999 & 2011

O_2 affects growth

- Molecular oxygen (O_2) is not toxic
- O_2 can be converted to toxic oxygen by-products:
    A. superoxide anion ($O2^-$)
    B. hydrogen peroxide (H_2O_2) —> damage cell components
    C. hydroxyl radical ($OH\cdot$) —> oxidation macromolecules & other organic compounds

- All by-products of the reduction of O_2 to H_2O in respiration
- Flavoproteins, quinones, and iron–sulfur proteins, electron carriers found all cells also catalyze some of these reductions

Reactants		Products	
$O_2 + e^-$	→	O_2^-	(superoxide)
$O_2^- + e^- + 2\,H^+$	→	H_2O_2	(hydrogen peroxide)
$H_2O_2 + e^- + H^+$	→	$H_2O + OH\bullet$	(hydroxyl radical)
$OH\bullet + e^- + H^+$	→	H_2O	(water)

Outcome:

$$O_2 + 4\,e^- + 4\,H^+ \rightarrow 2\,H_2O$$

Madigan et al. 2018

O_2 adaptive strategies

TABLE 5.6 Oxygen relationships of microorganisms

Group	*Relationship to O_2*	*Type of metabolism*
Aerobes		
Obligate	Required	Aerobic respiration
Facultative	Not required, but growth better with O_2	Aerobic respiration, anaerobic respiration, fermentation
Microaerophilic	Required but at levels lower than atmospheric	Aerobic respiration
Anaerobes		
Aerotolerant	Not required, and growth no better when O_2 present	Fermentation
Obligate	Harmful or lethal	Fermentation or anaerobic respiration

Specific niche

Metabolic machinery to detoxify

$H_2O_2 + H_2O_2 \rightarrow 2\,H_2O + O_2$

(a) **Catalase**

$H_2O_2 + NADH + H^+ \rightarrow 2\,H_2O + NAD^+$

(b) **Peroxidase**

$O_2^- + O_2^- + 2\,H^+ \rightarrow H_2O_2 + O_2$

(c) **Superoxide dismutase**

$4\,O_2^- + 4\,H^+ \rightarrow 2\,H_2O + 3\,O_2$

(d) **Superoxide dismutase/catalase in combination**

$O_2^- + 2\,H^+ + \text{rubredoxin}_{\text{reduced}} \rightarrow H_2O_2 + \text{rubredoxin}_{\text{oxidized}}$

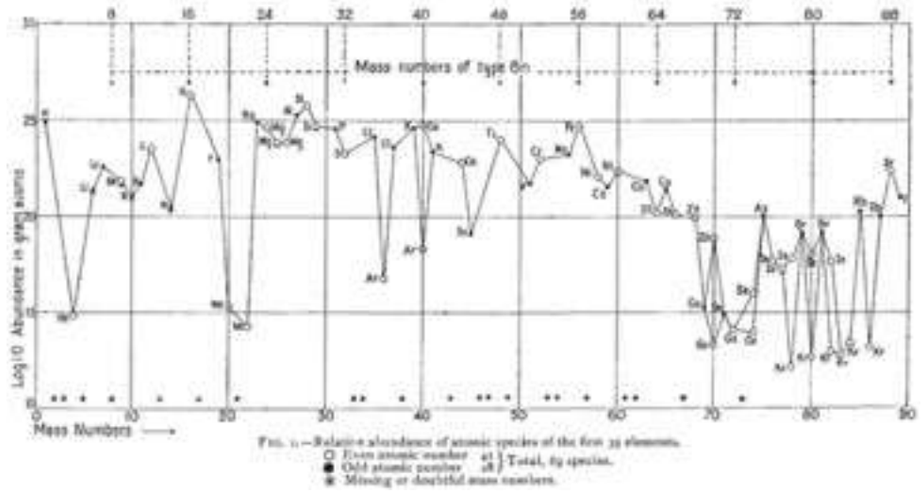
(e) **Superoxide reductase**

Madigan et al. 2018

Elemental composition of Earth and microbes

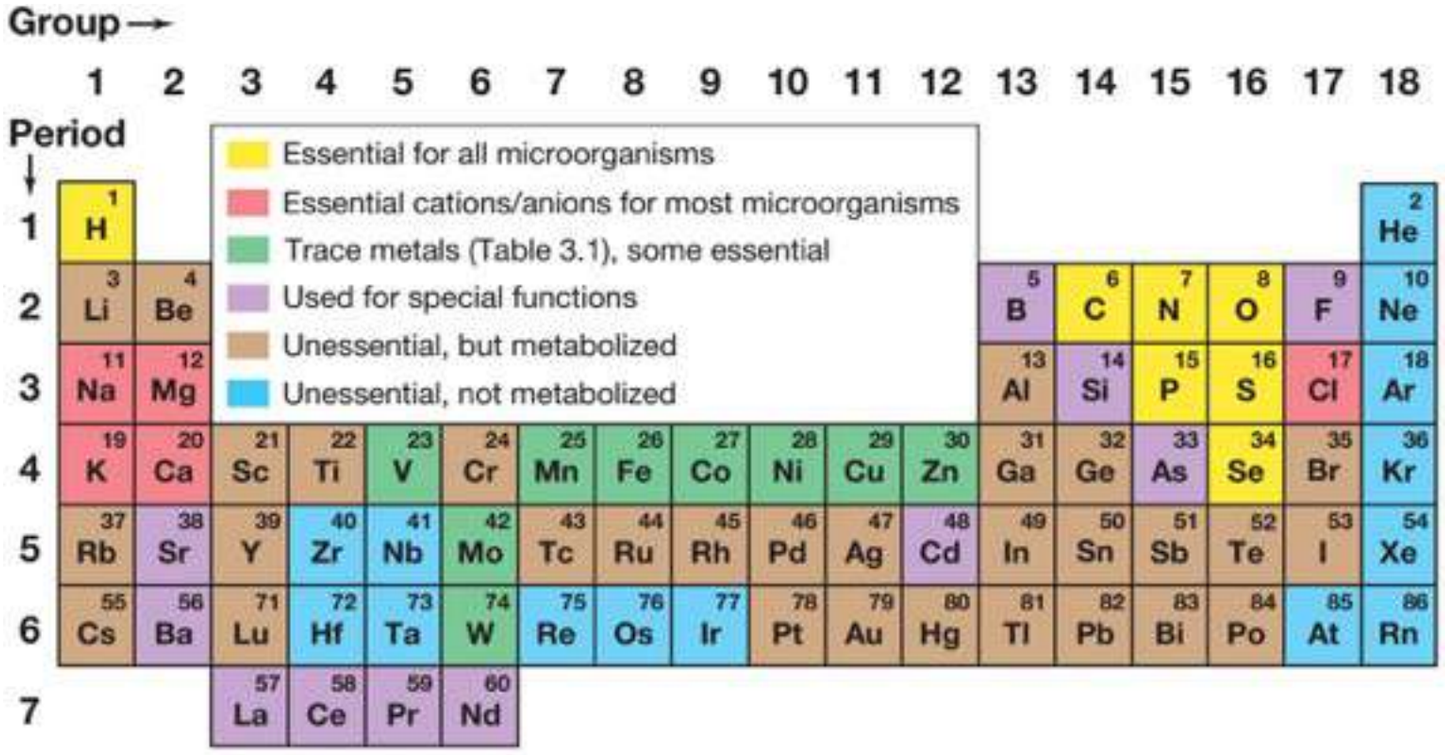
Aston,1924

- Universe, Earth, Life share important elements
- Majority of Universe is H and He and some others
- The essence of Life is the <u>other elements</u>



The Elemental Composition of *E. coli*

Element	% dry	Substrate Source	Cellular Components
C	55	DOC, CO_2	Main constituent of cellular material
O	20	O_2, DOM, CO_2	Constituent of cell material and cell water; O_2 primary electron acceptor in aerobic respiration
N	10	NH_3, NO_3^-, NO_2^-, DON, N_2	Constituent of amino acids, nucleic acids, nucleotides, and coenzymes
H	8	DOM, H_2	Main constituent of organic compounds and cell water
P	3	PO_4^{3-}, DOP	Constituent of nucleic acids, nucleotides, phospholipids, LPS, teichoic acids in gram positives
S	1	SO_4, H_2S, HS, DOM	Constituent of cysteine, methionine, glutathione, several coenzymes
K	1	Potassium salts	Main cellular inorganic cation and cofactor for certain enzymes
Mg	0.5	Magnesium salts	Inorganic cellular cation, cofactor for certain enzymatic reactions
Ca	0.5	Calcium salts	Inorganic cellular cation, cofactor for certain enzymes
Fe	0.002	Iron salts, DOM	Component of cytochromes and Fe-proteins; cofactor for many enzymes

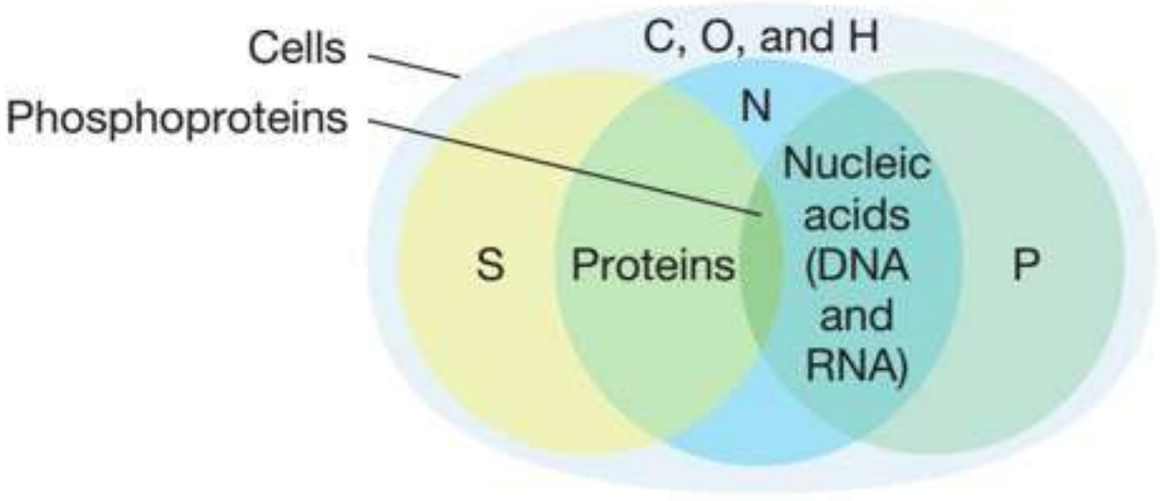


Demain, A. L. and Solomon, N. A. (1981), Manual of Industrial Microbiology & Biotechnology, American Society for Microbiology, Washington, DC, p. 108

Madigan et al. 2018

From macromolecules to cell

Elemental composition of informational macromolecules



(b)

Macromolecular composition of a cell

Macromolecule	Percent of dry weight
Protein	55
Lipid	9.1
Polysaccharide	5.0
Lipopolysaccharide	3.4
DNA	3.1
RNA	20.5

(c)

- About 75% of microbial cell wet weight (a single cell of *Escherichia coli* weighs just 10^{-12} g) is water
- The remainder ~ 25% is primarily macromolecules–proteins, nucleic acids, lipids, and polysaccharides
- The building blocks of these macromolecules are the amino acids, nucleotides, fatty acids, and sugars, respectively

Madigan et al. 2018

Core Concept

02: Unique goal of microbial life: survival, maintenance, generation of ATP, growth of new cells

LIVING vs NON living

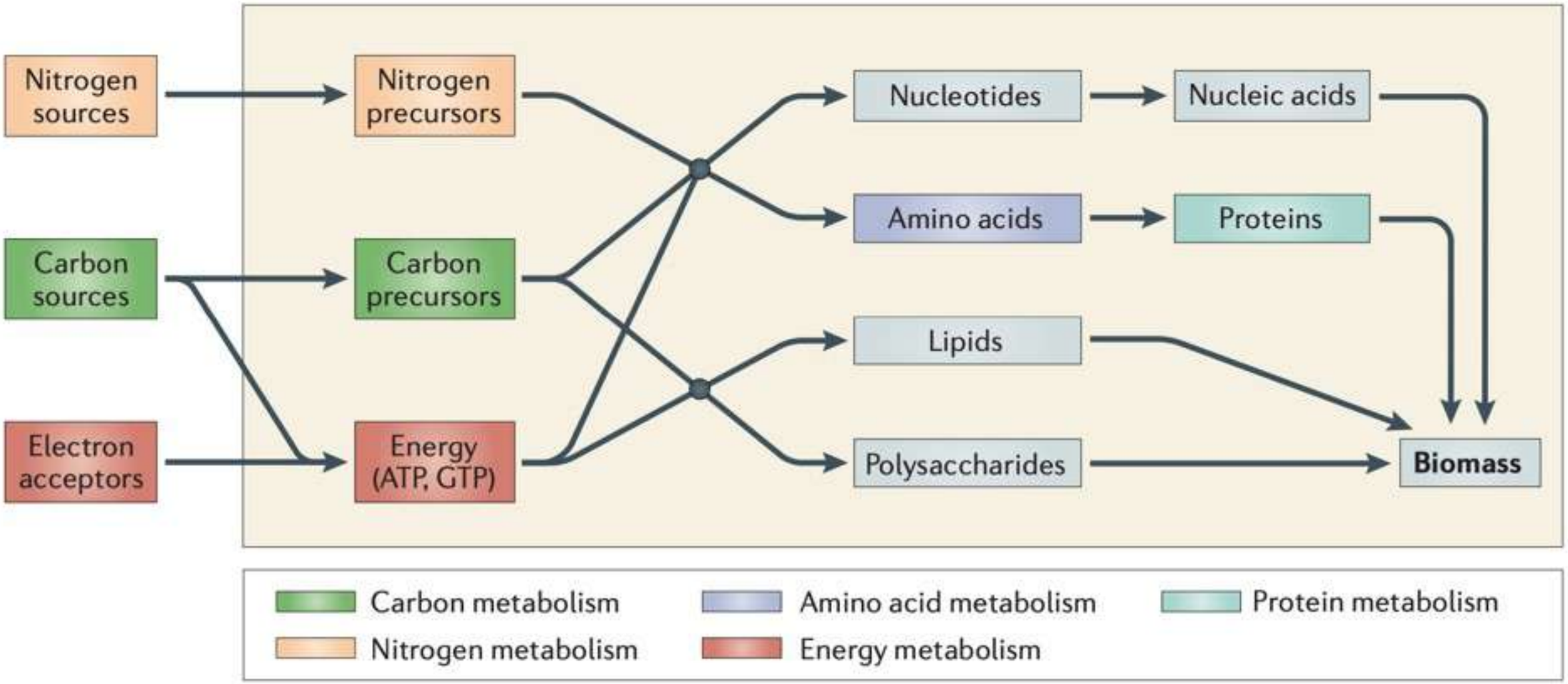
1. A membrane subsystem for compartmentalizing the functional network components
2. An autocatalytic metabolic subsystem that functions out-of-equilibrium by capturing energy and material resources

What is the metabolisms?
Which are the chemical reactions that are essential to the cell?

Coordinated Metabolism

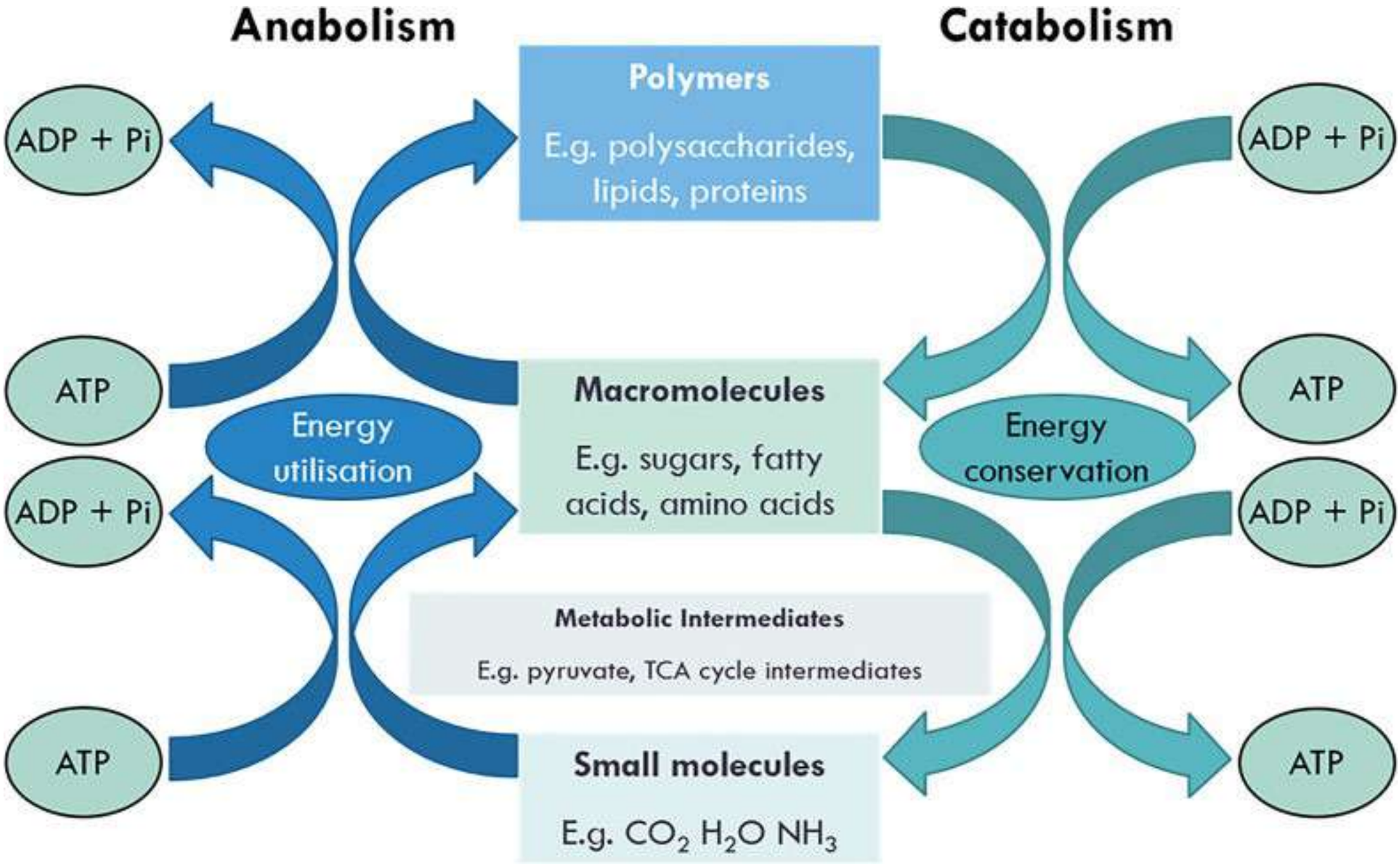
Beyond fuelling cellular activities with building blocks and energy, metabolism also integrates environmental conditions into intracellular signals

Metabolisms underlying regulatory network is complex and multifaceted



Chubkov et al., 2014

Anabolism and Catabolism



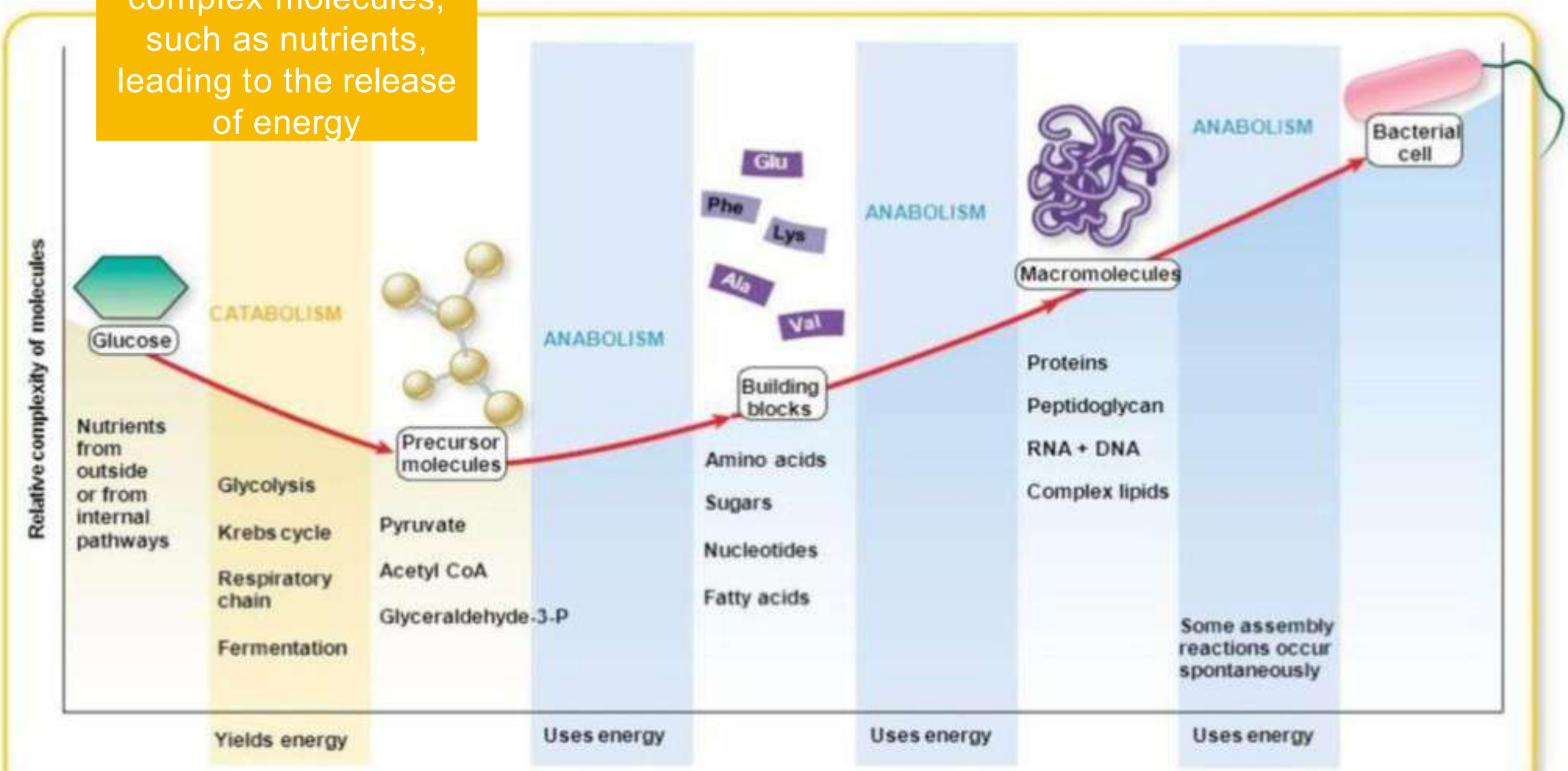
(Judge and Dodd, 2020)

https://app.jove.com/science-education/v/12114/concepts/overview-of-metabolism

Metabolism

Catabolism: The degradation of complex molecules, such as nutrients, leading to the release of energy

Anabolism: The energy-dependent formation of building blocks and macromolecules in a cell



Real Growth in a limited environment

Rapid exponential growth is not real in the environment

In **48 h** assuming that ***E.coli*** that **doubles every 20 min, given its weight 10^{-12} g, there will be $2.2x10^{31}$ g**

In the environment:

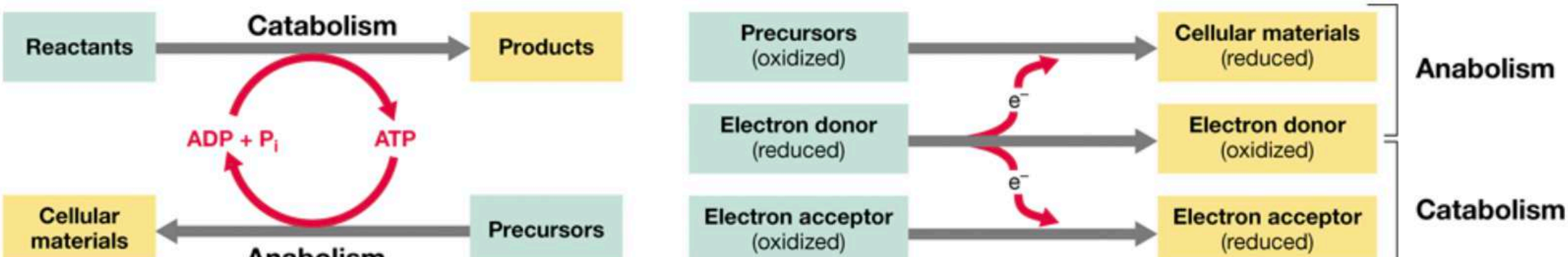
- **Sporadic rapid growth**
- **Slow growth**
- **Sporadic slow growth**
- **Dormancy**

Estimates of microbial growth rate, dormancy, ...

Habitat	Organism	Doubling time (DT) or survival time (ST)	References
Growth rate			
Laboratory medium	*E. coli*	20 min DT	Koch, 1971
Human intestine	*E. coli*	12 h DT	Koch, 1971
Mouse	*Salmonella typhimurium*	10–24 h DT	Brock, 1971
Rumen	Heterotrophic bacteria	~12 h DT	Brock, 1971
Pond	Heterotrophic bacteria	2–10 h DT	Brock, 1971
Lake water	Heterotrophic bacteria	10–280 h DT	Jannasch, 1969
Ocean	Heterotrophic bacteria	20–200 h DT	Vaulot et al., 1995
Ocean	Autotroph, *Prochlerococcus*	~24 h DT	Gray and Williams, 1971
Soil	Heterotrophs: α Proteobacteria, rhizobia	100 days DT	
Shallow groundwater	Heterotrophs: *Acidovorax, Commamonas*	15 days DT	Mailloux and Fuller, 2003
Marine surface sediments	Sulfate reducers	1 year DT	Hoehler and Jorgensen, 2013
Shallow subsurface	*Geobacter*	46 h DT	Holmes et al., 2013
Deep subsurface	Heterotrophs	100 years DT	Phelps et al., 1994; Fredrickson and Onstott, 2001
Deep marine sediments	Sulfate reducers, heterotrophs	200–3000 year DT	Hoehler and Jorgensen, 2013

Madsen, 2016

Figure 3.1 Metabolic coupling with respect to energy conservation and electron flow.



(a) Catabolism employs exergonic reactions to drive the synthesis of ATP. Anabolism employs endergonic reactions, which consume ATP, to drive the biosynthesis of cellular material. Some energy would be lost as heat and cannot be conserved in the formation of ATP (not shown). *(b)* Cells require reducing power, in the form of a reduced electron donor, as a source of electrons (e^-) needed to carry out anabolic and catabolic reactions. Inputs to metabolism are labeled in {green} and outputs of metabolism are labeled in {yellow}.

Madigan et al. 2020

Catabolic and Anabolic pathways

Catabolic pathways are exergonic processes in which cells generate free energy by transforming reactants into products. Free energy is energy available to do work. The free energy produced in catabolism is conserved by synthesizing energy-rich molecules such as ATP. The formation of ATP requires at least $\Delta G^{0\prime} = -31.8\,\text{kJ/mol}$. Hence, the aerobic respiration of a mole of glucose could produce up to 91 moles of ATP under standard conditions, though under natural cellular conditions this reaction actually produces closer to 38 moles of ATP. This difference in ATP yield occurs because reactions in the cell do not occur under standard conditions. free energy available under natural conditions differs from the free energy calculated at standard conditions. In addition, chemical reactions release some portion of energy in the form of heat, which cells cannot conserve in the formation of ATP. The heat lost during metabolic reactions is what makes your body warm and what makes a decomposing compost pile become steaming hot.

Anabolic pathways are endergonic processes in which the synthesis of cellular material from simple precursors requires an input of energy. The energy required to fuel anabolic reactions, and to biosynthesize cellular materials, comes from the hydrolysis of ATP. In this way, catabolic and anabolic reactions are fundamentally linked.

Madigan et al. 2020

Principle of Bioenergetics

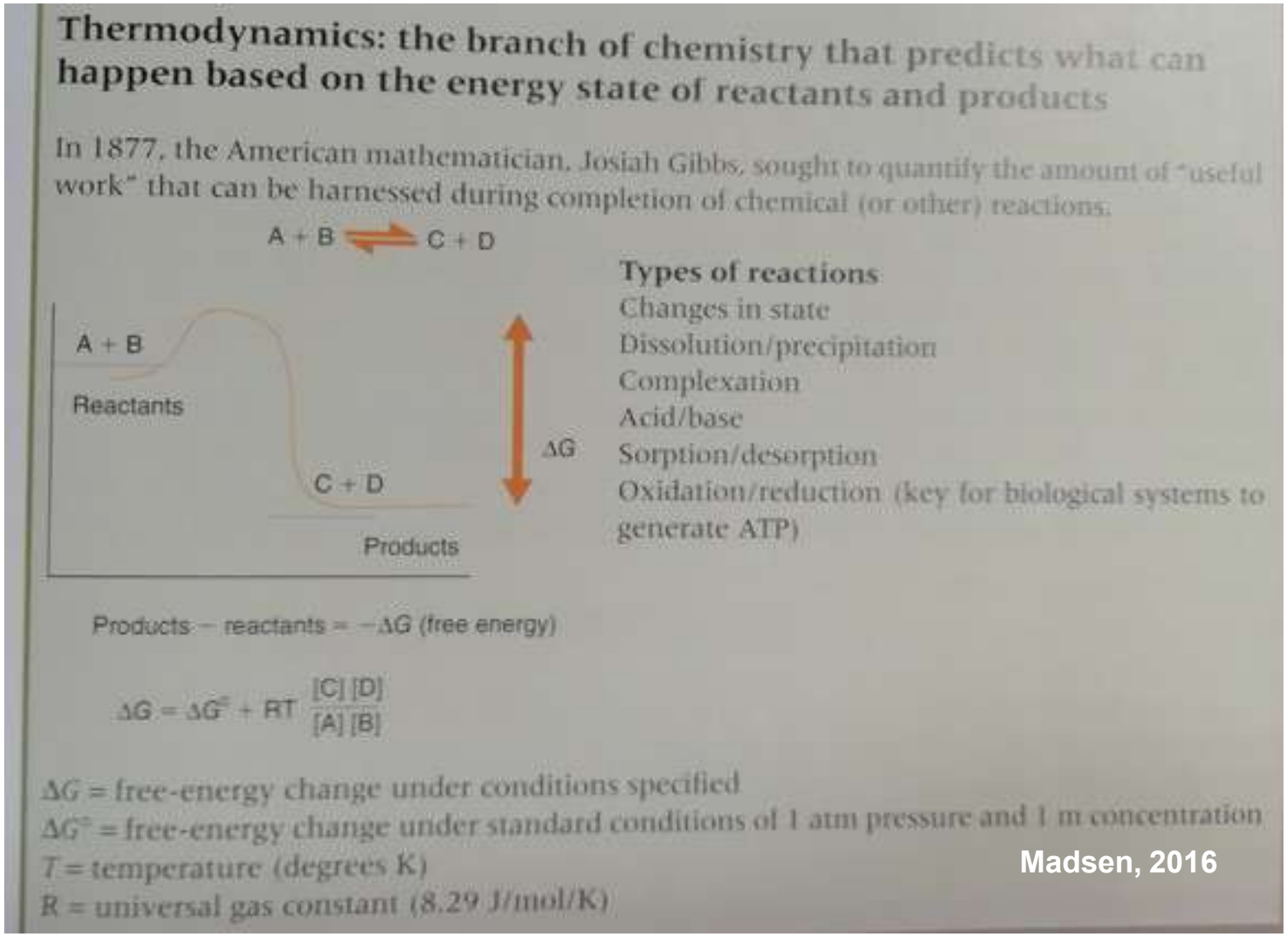
Energy flows (radiation—>chemical—> heat)
Matter cycles (uptake—>growth—> death—>recycle)

Energy is defined as the **ability to do work**, kilojoules (kJ), a unit of heat energy

All chemical reactions in a cell are accompanied by **changes in energy**, energy being either required or released as a reaction proceeds

$\Delta G^{0'} < 0$, reaction will proceed with release of free energy- exergonic

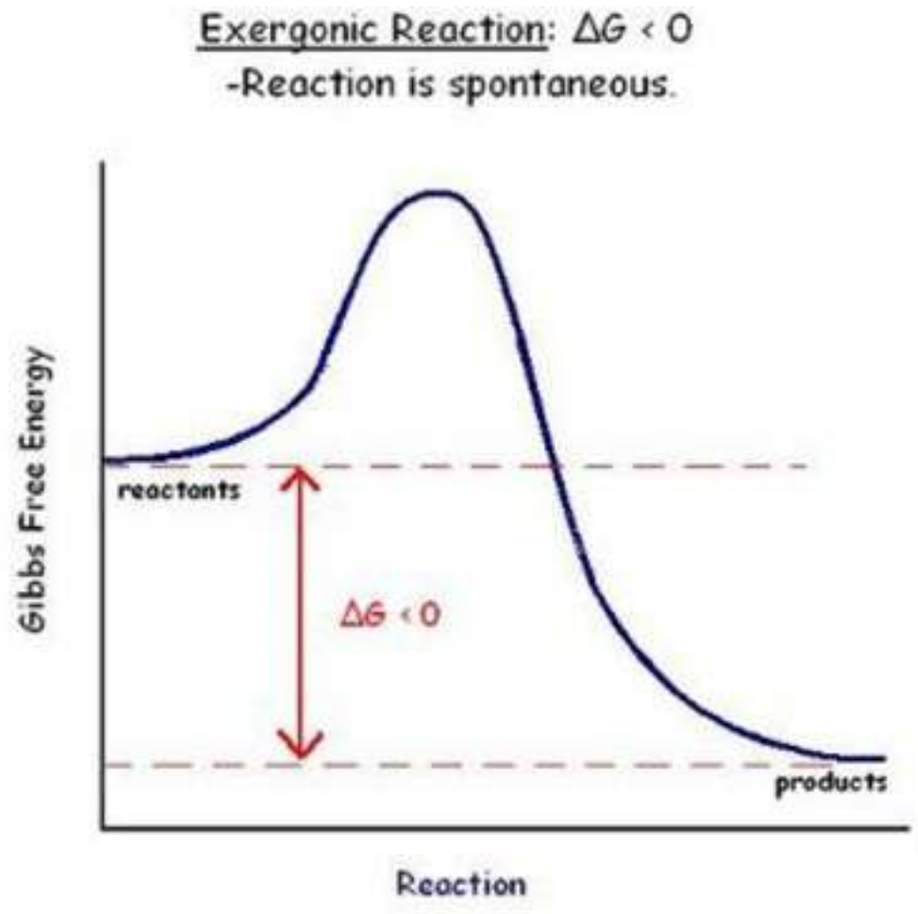
$\Delta G^{0'} > 0$, reaction requires energy in order to proceed- endergonic



A $\Delta G^{0'} > 0$ reaction under standard conditions can become exergonic **$\Delta G^{0'} > 0$** under the actual conditions present in the microbial habitat

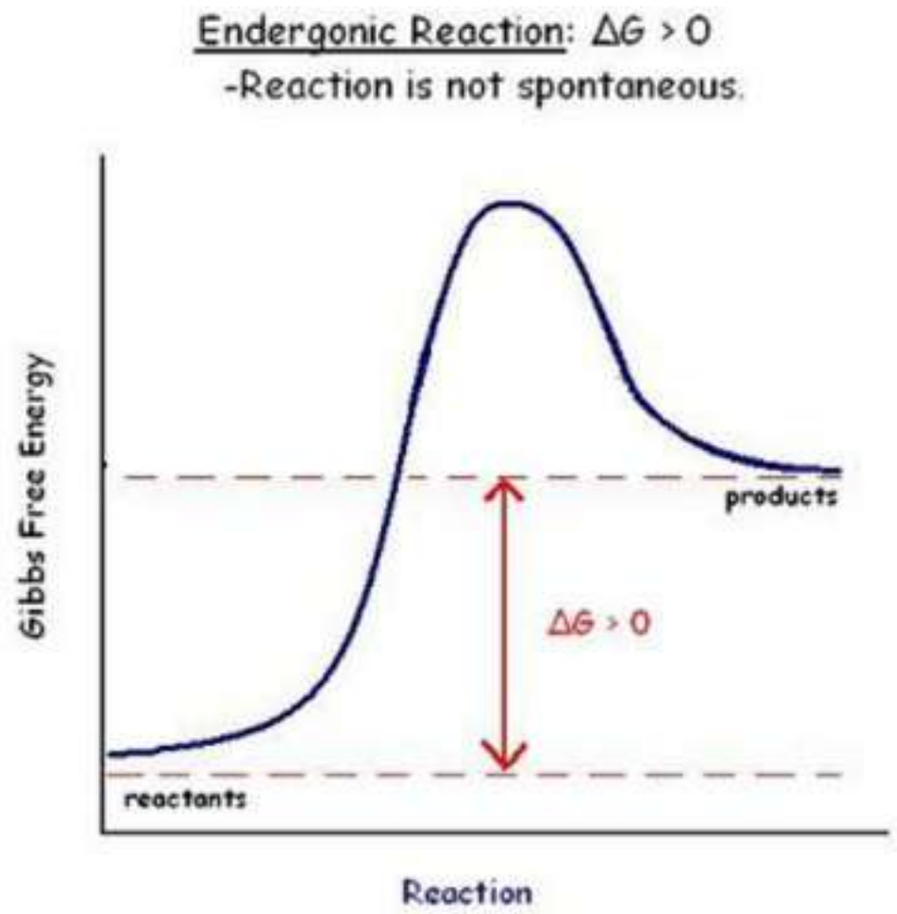
Spontaneous

$\Delta G^{0'} < 0$, reaction will proceed with release of free energy- EXErgonic



Not Spontaneous

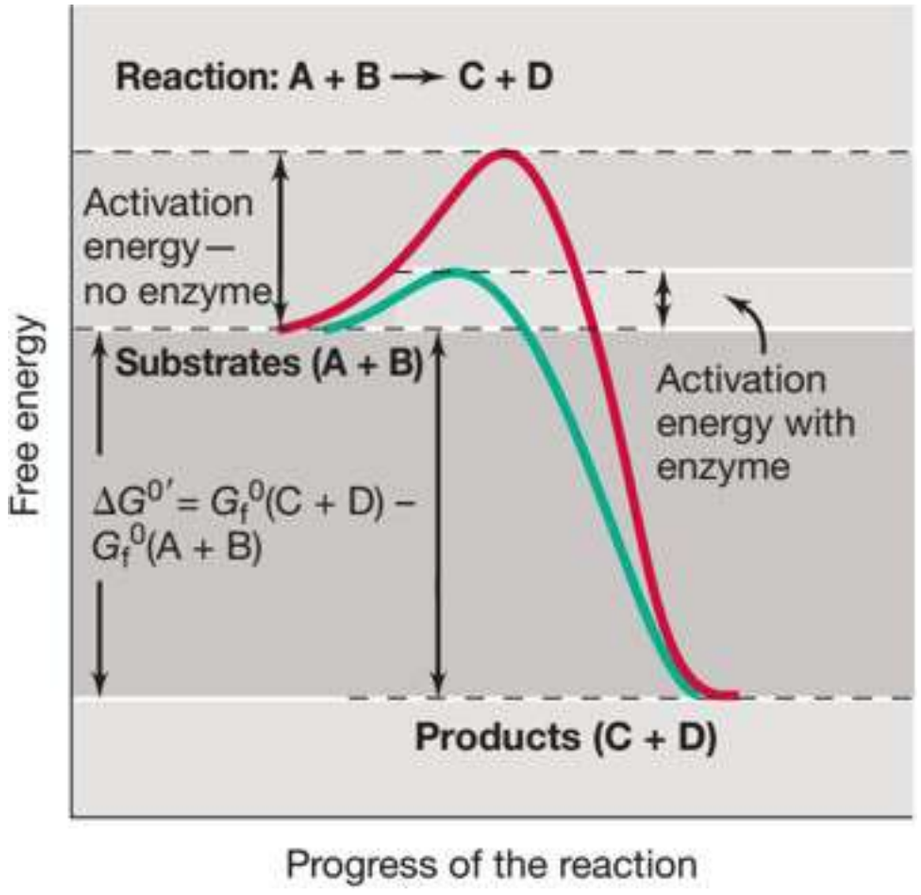
$\Delta G^{0'} > 0$, reaction requires energy in order to proceed- ENDergonic



1. Libretexts. “11.5: Spontaneous Reactions and Free Energy.” Chemistry LibreTexts, Libretexts, 13 July 2018
2. Science, Ck12. “Spontaneous and Nonspontaneous Reactions.” CK-12 Foundation, CK-12 Foundation, 28 Mar. 2017

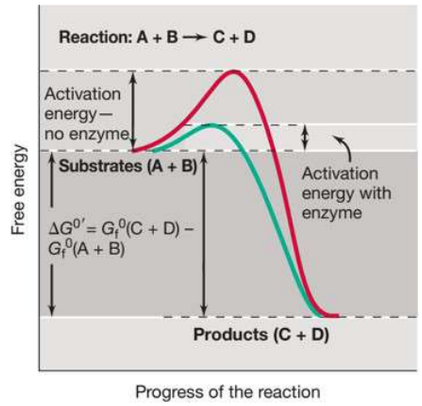
Enzyme, I

- Free-energy calculations reveal only whether energy is released or required in a given reaction
- $\Delta G^{0'}$ says nothing about the rate of the reaction
- **Activation energy** can be viewed as the **minimum energy required** for a chemical reaction to begin
- **Catalysts** function **by lowering the activation energy** of a reaction thereby increasing the reaction rate



Madigan et al. 2018

Enzyme, II



- **Catalysts** have no effect on the energetics or the equilibrium of a reaction but **affect the rate at which a reaction proceeds**
- **Most cellular reactions will not proceed at significant rates without catalysis**
- The **major** catalysts in cells are enzymes, **proteins** (or in a few cases, **RNAs**) that are **highly specific**
- This **specificity** is a function of the precise **3D structure** of the enzyme.
- In an enzyme-catalyzed reaction, the enzyme combines with the reactant, called a substrate, forming an enzyme-substrate complex. Then, as the reaction proceeds, the product is released and the enzyme is returned to its original state, ready to catalyze a new round of the reaction
- **Prosthetic** groups bind **tightly** to their enzymes, usually covalently and permanently (e.g. heme group present in cytochromes such as cytochrome c)
- **Coenzymes**, with a few exceptions, **are loosely and often transiently** bound to enzymes
- Single coenzyme molecule may associate with a number of different enzymes (e.g. vitamins)

Madigan et al. 2018

Speed and specificity of a reaction

*If the enzyme has more than o**ne possible substrate, the k_{cat}/K_m** values determine the **specificity** of the enzyme for each*

*The **higher** this value the **more specific** the enzyme is for that substrate*

This is because a high value of k_{cat} and a low value of K_m are expected for the best substrates

*If k_{cat}/K_m – which is the apparent second-order rate constant for the **enzyme-catalyzed reaction** – **approaches the diffusion limit (~ 10^8–10^9 M^{-1} s^{-1})**, the enzyme cannot catalyze the reaction any better and is said to have reached '**catalytic perfection**'*
—-> second-order rate constants that approach their rates of encounter (~10^9 s^{-1} M^{-1}) with the substrate in solution

Triosphosphate isomerase, superoxide dismutase and carbonic anhydrase are examples of perfect enzymes

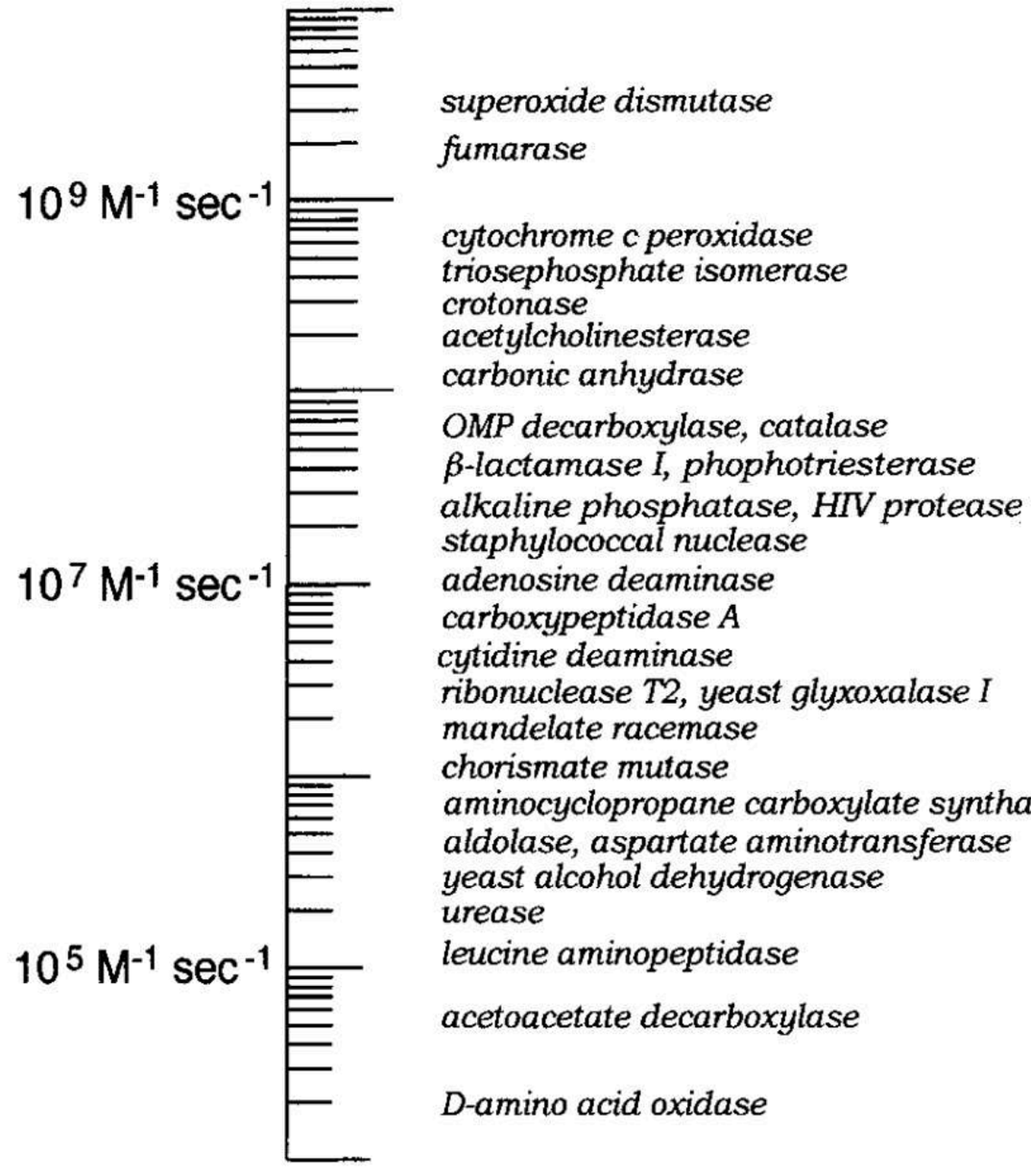


FIGURE 1. Representative values of k_{cat}/K_m at 25 °C compiled from the literature.

WOLFENDEN and SNIDER, 2001

Evolution in action !

The **half-life** of a reaction: **amount of time needed for a reactant concentration to decrease by half compared to its initial concentration**

Enzymes allow organisms to **channel the flow of matter to their own advantage**, allowing some reactions to proceed rapidly compared with other reactions that offer no selective advantage

After a substrate is bound at an enzyme's active site, its half-life is usually a small fraction of 1 s

Rapid turnover is necessary if any enzyme is to produce a significant rate of reaction at the limited concentration (<10^{-5} M) at which enzymes are present within the cell

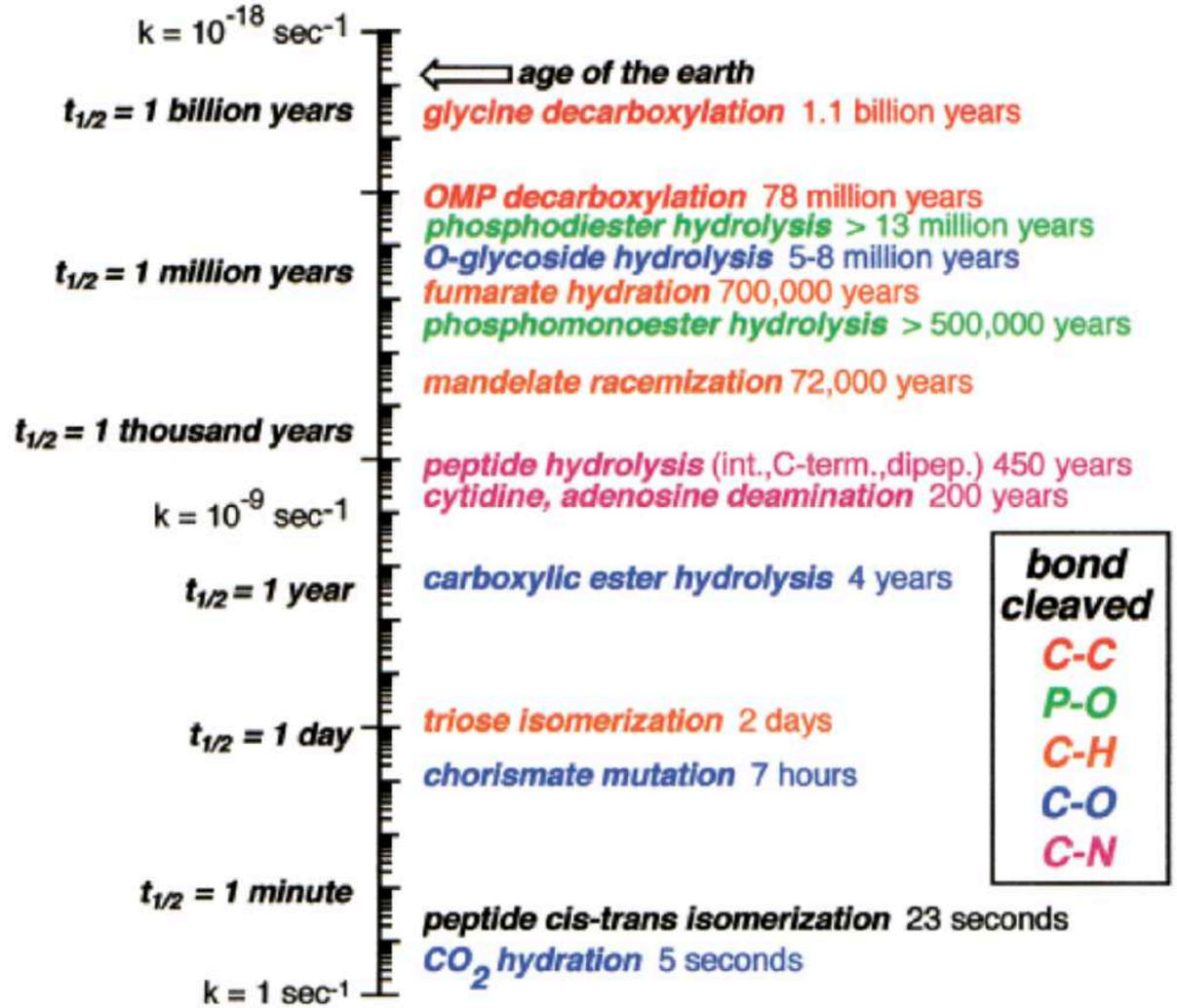


FIGURE 4. Natural half-times of some biological reactions in neutral solution at 25 °C.

WOLFENDEN and SNIDER, 2001

Table 1. Cleavage of Polymers at 25 and 100 °C

	bond $t_{1/2}$			$t_{1/2}$ per cleavage event	
reaction	25 °C	100 °C	no. of bonds per polymer	25 °C	100 °C
protein hydrolysis	400 years	5.5 weeks	123 (RNase A)	4 years	7 hours
polysaccharide hydrolysis	4.7×10^6 years	160 years	10^5 residues (glycogen)	50 years	12 hours
RNA hydrolysis	4 years	9 days	70 residues (tRNA)	20 days	3 hours
DNA hydrolysis	140 000 years	22 years	10^9 residues (human DNA)	1 month	2 hours

Energy conservation from chemical and light reactions in to energy-rich compounds

Reducing power, soluble e^- carriers

Coupling energy producing with energy consuming reactions

Multi step reactions

Basic Cellular Metabolism

1. Harvesting energy source to store it in ATP
2. Generation of reducing power
3. Carbon and other nutrient source (H_2O) for assembling cellular building blocks

Carbon source	Energy source		
	Chemical, organic	Chemical, inorganic	Light
Fixed organic	**Chemosynthetic organoheterotroph** (Example: humans, fungi, *Pseudomonas*)	**Chemosynthetic lithoheterotroph** (Example: *Beggiatoa* sp.)	**Photosynthetic heterotroph** (Example: purple and green bacteria; *Rhodospirillum*)
Gaseous CO_2		**Chemosynthetic lithoautotroph** (Example: ammonia-, hydrogen-, and sulfur-oxidizing bacteria; *Nitrosomonas, Aquifex*)	**Photosynthetic autotroph** (Example: plants, algae, *Prochlorococcus*)

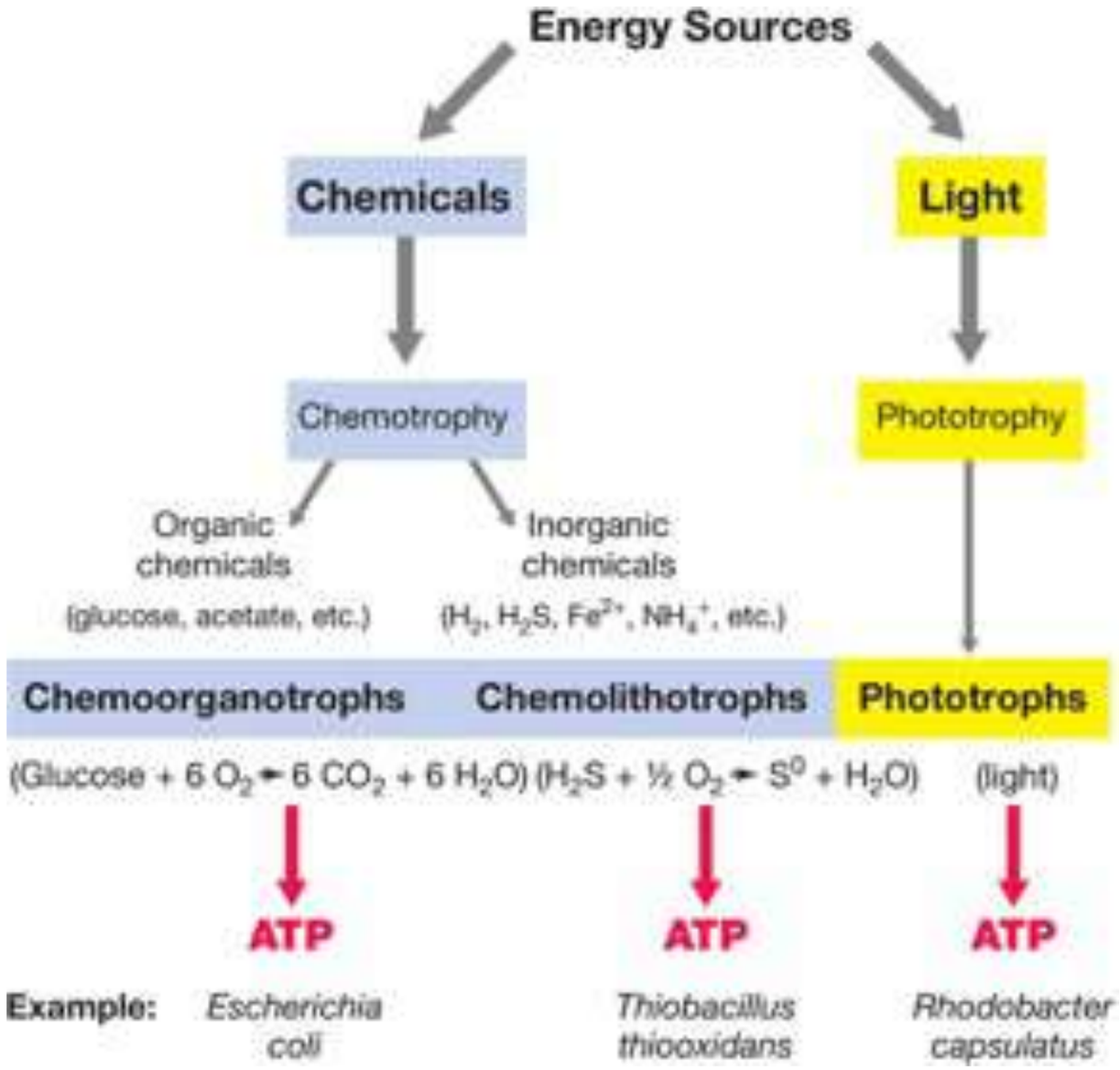
Terminology:

- Autotroph: carbon from CO_2 fixation
- Heterotroph: carbon assimilated from (fixed) organic compounds
- Photosynthetic: energy from light
- Chemosynthetic: energy from oxidizing reduced chemicals
- Chemolitho: energy from oxidizing inorganic reduced chemicals
- Chemoorgano: energy from oxidizing organic reduced chemicals.

Madsen, 2016

Basic Cellular Metabolism

—> For maintenance of existing cells and growth of new cells
—> Successful exploitation well-defined energy and carbon source
—> Physical, biological and chemical properties vary in space and time



Madigan et al. 2020

Metabolism & Growth in a limited environment

Habitat characteristics and nutrient limitations faced by three physiological classes of microorganisms

Habitat type	Photoautotroph	Chemolithotroph	Chemoorganoheterotroph
Ocean water	Daily light cycle, light penetration depth; scarce iron	Flux of reduced inorganic compounds, especially NH_3, H_2S, H_2, or CH_4 from nutrient turnover and hydrothermal vents	Carbon flux from phototrophs, dead biomass, and influent waters
Lake water	Daily light cycle, light penetration depth; scarce phosphorus	Flux of reduced inorganic materials, especially NH_3, H_2, and CH_4 from nutrient turnover	Carbon flux from phototrophs, dead biomass and influent waters
Sediment (freshwater and oceanic)	Daily light cycle, light penetration depth	Flux of reduced inorganic materials, especially NH_3 and H_2 from nutrient turnover or H_2, H_2S, or CH_4 from hydrothermal vents	Flux of organic carbon from phototrophs and dead biomass; flux of final electron acceptors to carbon-rich anaerobic strata
Soil	Daily light cycle, light penetration depth	Flux of reduced gaseous substrates, especially methane from nutrient turnover by anaerobes	Slow turnover of soil humus, dead biomass, plant root exudates; leaf fall from vegetation
Subsurface sediment	No light	Flux of reduced inorganic materials, especially H_2 and CH_4 from geothermal origin	Carbon flux from nutrient turnover

Madsen, 2016

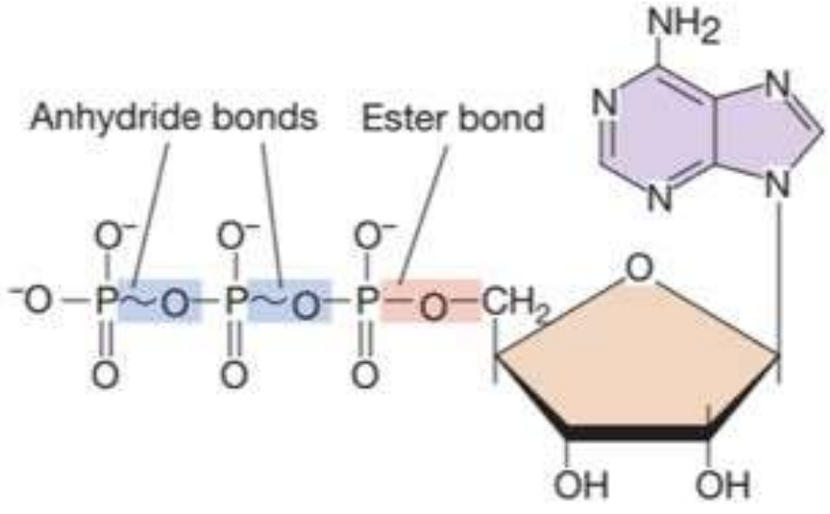
Energy conservation

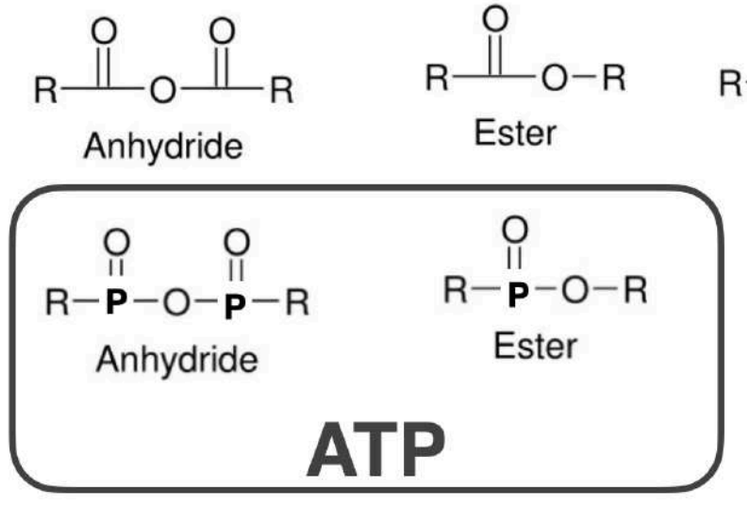
According to the first law of thermodynamics, energy is neither created nor destroyed. Hence, in order to grow, cells must *conserve energy* by converting energy available from their surroundings into a form that can do work. Cells accomplish this by generating energy-rich compounds such as *adenosine triphosphate* (ATP)—a molecule capable of storing energy and releasing it to fuel cellular processes

All living cells share certain fundamental metabolic requirements. All cells require *water* in which to perform metabolic reactions, as well as sources of *carbon* and other *nutrients* with which to synthesize cellular materials. All cells also require **free energy**—*the energy available to do work*—and **reducing power**—a source of electrons (e^-) that can be used to both generate free energy and perform certain biosynthetic reactions

Madigan et al. 2020

Energy conservation

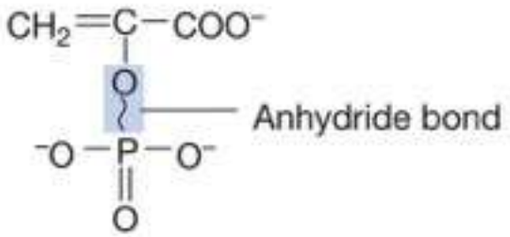




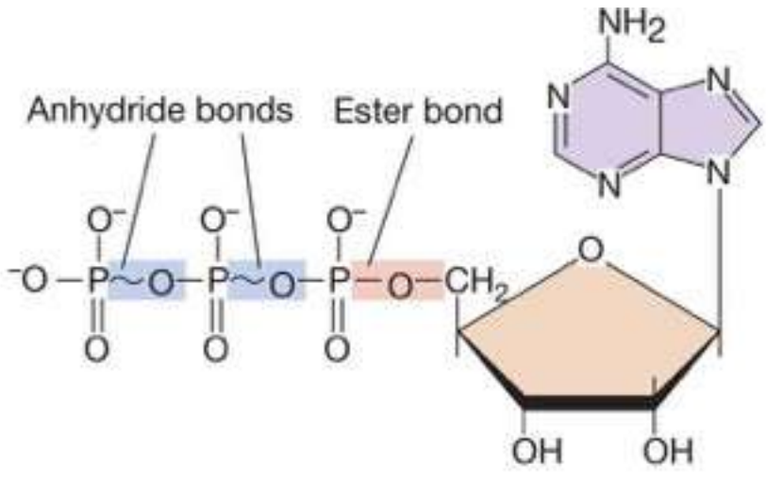
- ATP generation, adenosine triphosphate
- **ATP the energy currency of the cell** –> cell motility, biosynthetic reactions, replication, cell growth and heredity, its generation relays:
    - 1. **Substrate-level phosphorylation**
    - 2. **Membrane-bound e- transport chain** –> create **H+ motive force** that drives ATP synthetase embedded in cytoplasmic membrane
- Earth including biota are a very heterogeneous and complex environment –> thermodynamics (prediction of reactions that are energetically favorable)

Madigan et al. 2018

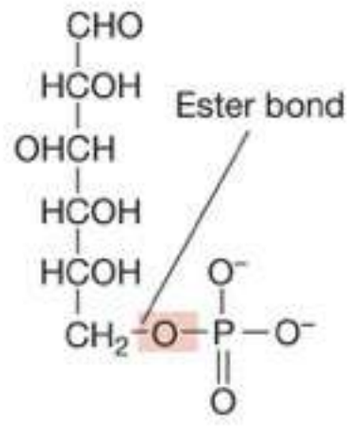
Energy-rich compounds



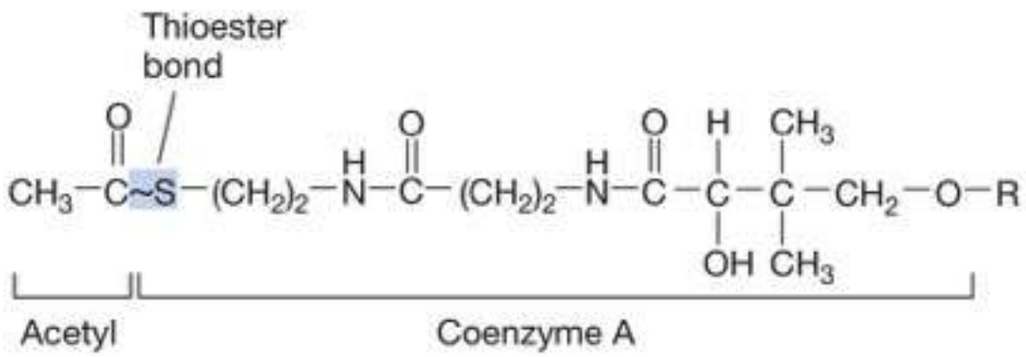
Phosphoenolpyruvate



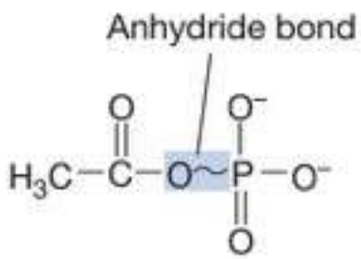
Adenosine triphosphate (ATP)



Glucose 6-phosphate



Acetyl-CoA



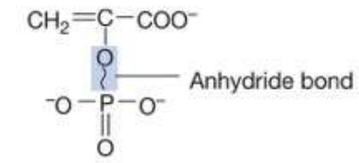
Acetyl phosphate

Compound	$G^{0'}$ kJ/mol
$\Delta G^{0'}$ > 30kJ	
Phosphoenolpyruvate	−51.6
1,3-Bisphosphoglycerate	−52.0
Acetyl phosphate	−44.8
ATP	−31.8
ADP	−31.8
Acetyl-CoA	−35.7
$\Delta G^{0'}$ < 30kJ	
AMP	−14.2
Glucose 6-phosphate	−13.8

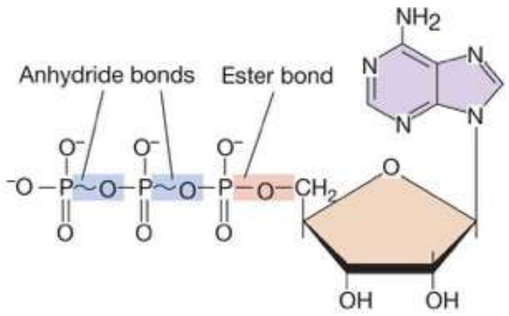
Madigan et al. 2018

Energy-rich compounds

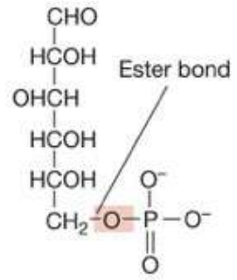
Madigan et al. 2018



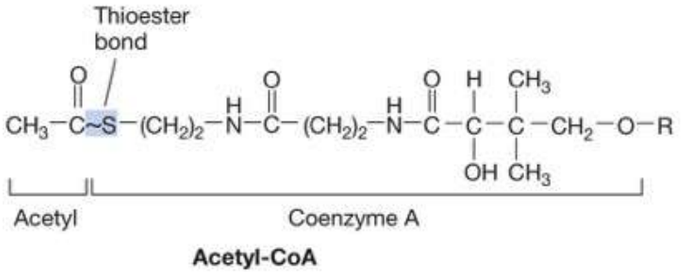
Phosphoenolpyruvate



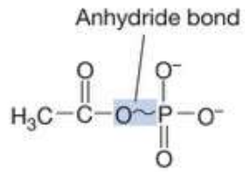
Adenosine triphosphate (ATP)



Glucose 6-phosphate



Acetyl-CoA



Acetyl phosphate

Compound	$G^{0'}$ kJ/mol
$\Delta G^{0'}$ > 30kJ	
Phosphoenolpyruvate	−51.6
1,3-Bisphosphoglycerate	−52.0
Acetyl phosphate	−44.8
ATP	−31.8
ADP	−31.8
Acetyl-CoA	−35.7
$\Delta G^{0'}$ < 30kJ	
AMP	−14.2
Glucose 6-phosphate	−13.8

- The energy released from **redox** reactions **fuels energy-requiring cell functions**
- Free energy released in the coupled **exergonic redox** reaction **must first be trapped** by the cell and conserved
- Energy conservation in cells is accomplished through the formation of a set of compounds containing **energy-rich phosphate or sulfur bonds**
- The biosynthesis of these compounds functions as the **free-energy trap**, and their hydrolysis releases this energy **to drive endergonic reactions** ($\Delta G^{0'} > 0$)

Mechanisms of Energy Conservation

Cells conserve energy by generating ATP through one of three fundamental mechanisms. The first mechanism is **substrate-level phosphorylation**. In substrate-level phosphorylation the energy-rich bond of a substrate is hydrolyzed to directly drive the formation of ATP. For example, hydrolysis of the phosphate bond in phosphoenolpyruvate is sufficiently exergonic to drive ATP formation . We will see that substrate-level phosphorylation is the dominant mechanism of energy conservation in fermentative organisms .

The second mechanism of energy conservation is **oxidative phosphorylation**. In oxidative phosphorylation the movement of electrons from an electron donor to an electron acceptor generates a *proton motive force*. The **proton motive force** is an electrochemical gradient formed by energy-conserving reactions that transport protons outside the cytoplasmic membrane. This electrochemical gradient creates a force that is ultimately used to synthesize ATP . Oxidative phosphorylation is the defining feature of respiration reactions and it is performed by diverse chemotrophic organisms

The third mechanism of energy conservation is **photophosphorylation**. In photophosphorylation light energy is used to form the proton motive force that powers ATP synthesis and is the dominant mechanism of energy conservation in phototrophic organisms. Both photophosphorylation and oxidative phosphorylation ultimately rely on electron transfer reactions to drive the formation of the proton motive force

Madigan et al. 2020

Reducing power

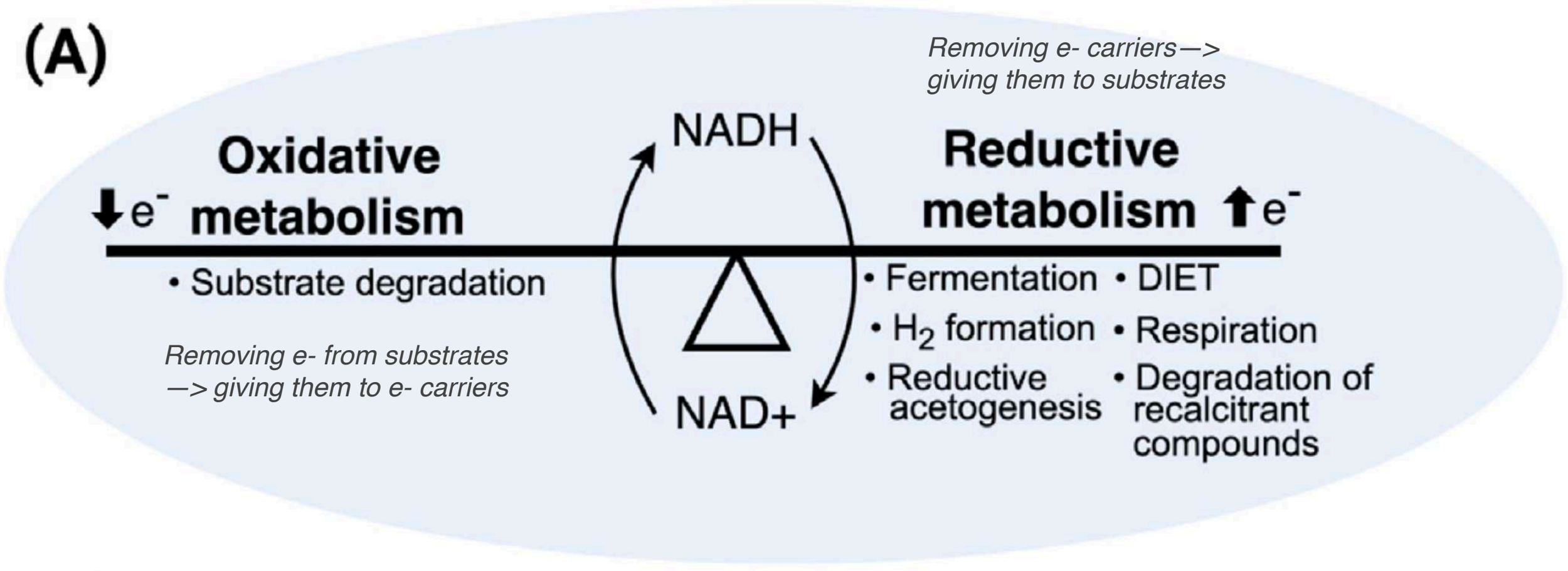
Electron Carriers and NAD^+/NADH Cycling

Cells require a net movement of electrons from an electron donor to an electron acceptor, but such reactions are rarely performed in a single step. More often, the movement of electrons from electron donor to electron acceptor proceeds through a series of consecutive reactions at different locations within the cell. Hence, the cell needs soluble electron carriers such as **nicotinamide adenine dinucleotide** (NAD^+/NADH) to carry electrons from one place to another within the cell . NAD^+/NADH is a redox couple with a reduction potential of $-0.32\,V$, which makes NADH a good electron donor and NAD^+ a weak electron acceptor . The reduction of NAD^+ to NADH requires $2\,e^-$ and $1\,H^+$, but the oxidation of electron donors typically results in the production of $2\,e^-$ and $2\,H^+$. Therefore, the reduction of NAD^+ typically results in the production of $NADH + H^+$ with the extra proton released into solution .

Catabolism, and indeed life itself, depends on the directed flow of electrons from an *electron donor* to an *electron acceptor* during redox reactions . Redox reactions are also required in many biosynthetic reactions that occur during anabolism
Redox reactions can be understood in terms of *reduction potential* which measures the affinity of a substance for electrons.

Madigan et al. 2020

Redox Tower for electron carriers molecules

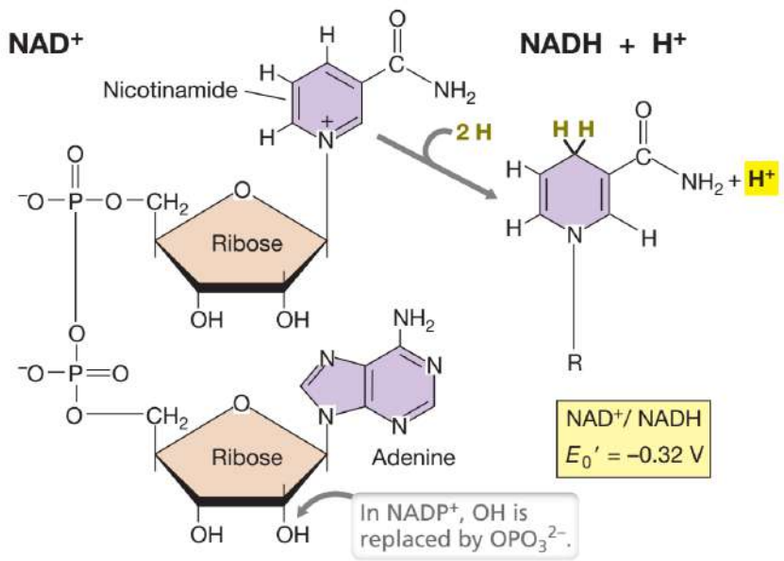


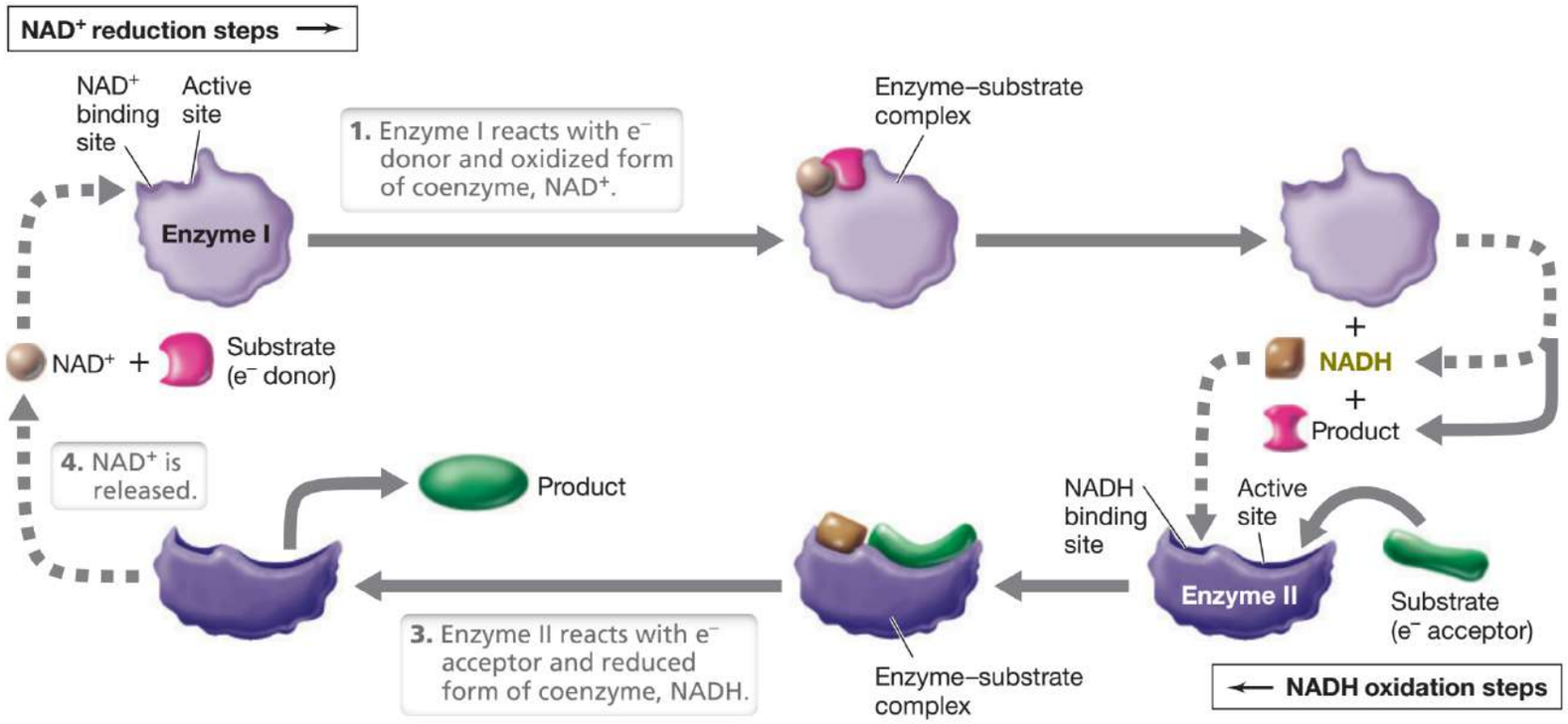
NAD+ and NADH balance in microbial communities: microbial processes constantly balance NAD+ (oxidized) and NADH (reduced) levels

Substrate breakdown (oxidation) generates NADH, while various pathways (respiration, fermentation) reoxidize NADH

Nicotinamide adenine dinucleotide, II

- NADH is a good electron donor
- Nicotinamide adenine dinucleotide phosphate ($NADP^+$) is made from NAD^+ by adding a phosphate molecule
- $NADP^+$ /NADPH participates in anabolic redox reactions (biosynthesis of cellular)
- NAD^+/NADH participates in catabolic redox reactions





Madigan et al. 2018

Electron Donors and Electron Acceptors

- Cells conserve energy released **from exergonic reactions by coupling** the reaction to the **biosynthesis** of **energy-rich compounds**, such as ATP

- Reactions that **release** sufficient **energy to form ATP require oxidation–reduction** biochemistry

- An **oxidation** is the **removal of an electron** (or electrons) from a substance, and a **reduction is the addition of an electron** (or electrons) to a substance: OILRIG

- In redox reactions, we refer to the **substance oxidized** as the **electron donor**, and the **substance reduced** as the **electron acceptor**

- By convention, in writing a redox couple, the **oxidized** form of the couple is always placed on the **left** (before the forward slash) followed by the **reduced** form **after** the forward slash

Electron Donors and Electron Acceptors

- **Substances differ** in their tendency to donate or accept electrons

- This **tendency is expressed as their reduction potential (E$^{0'}$**, standard conditions), a value measured in volts (V) compared with that of a reference substance, H_2

- When two redox couples react, the **reduced substance** of the couple ($E^{0'}$ is < 0, negative) **donates electrons** to the **oxidized substance** ($E^{0'}$ >0, positive)

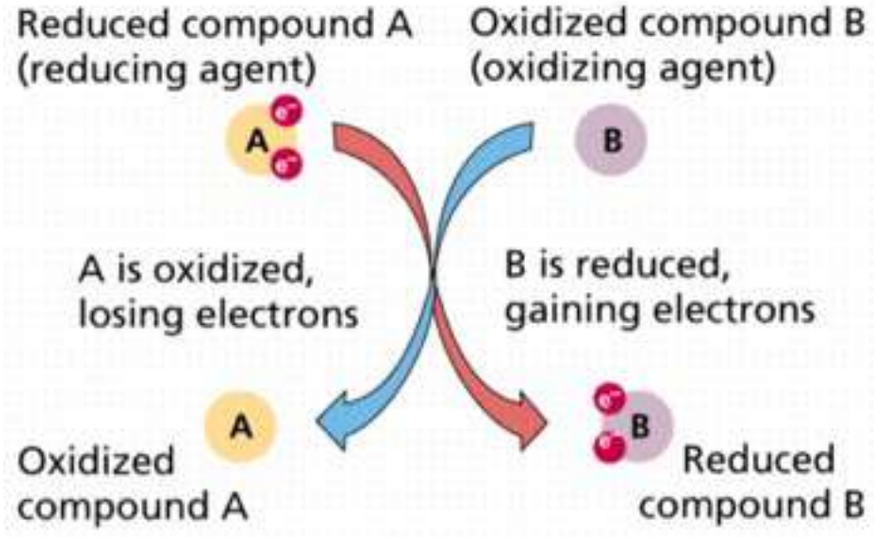
- The half reaction with the more negative $E^{0'}$ proceeds as an oxidation and is therefore written in the opposite direction

- **Redox reactions: reactions consisting of the movement of electrons between substrates constituting reduction (addition of electrons) and oxidation (removal of electrons) reactions**

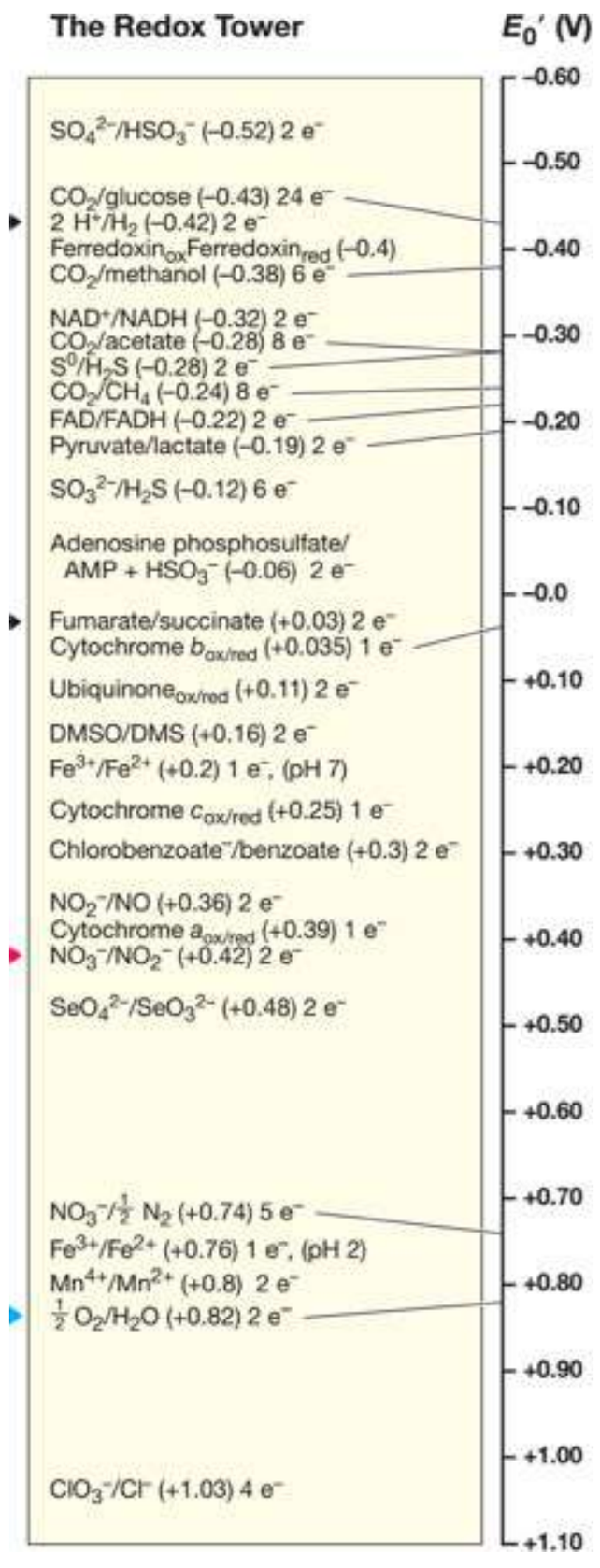
Redox Tower

- Redox couples are arranged **from the strongest donors at the top ($E^{0'}<0$)** to the **strongest acceptors at the bottom ($E^{0'}>0$)**

- **The larger the difference in reduction potential** between electron donor and electron acceptor, **the more free energy is released** ($\Delta G^{0'}$ can be computed via Nerst equation from reduction potential)



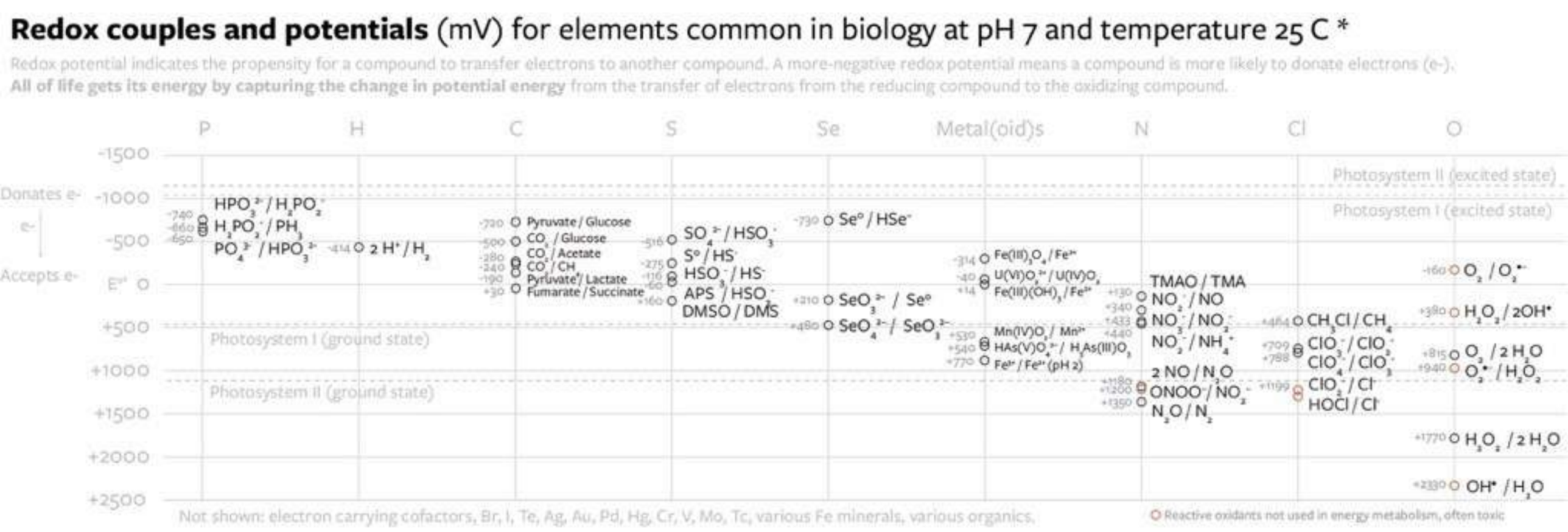
Reduced



Oxidized

Madigan et al. 2018

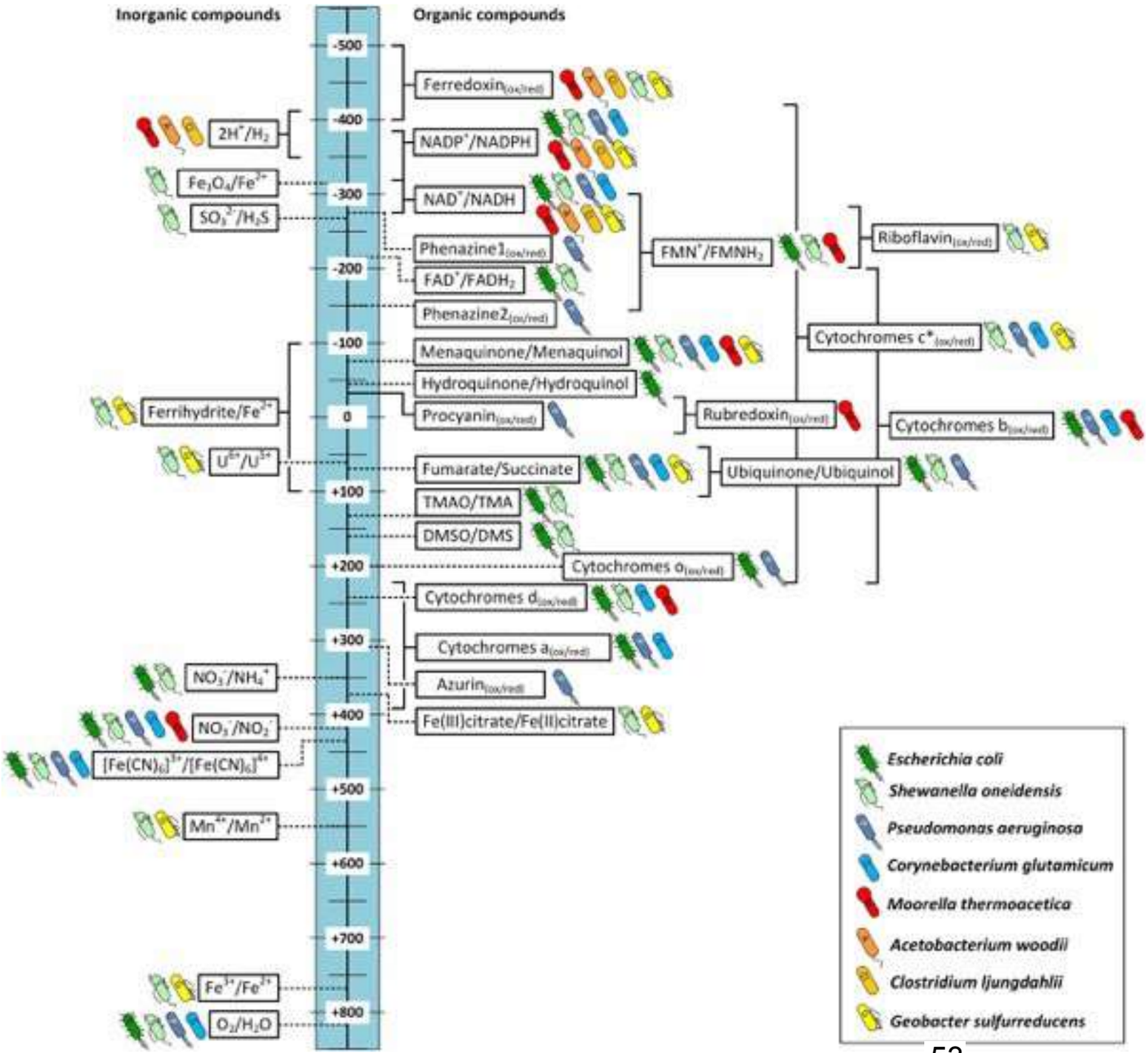
Redox couples in the environment



Across periodic table

From Tyler Barnum

High diversity of key molecules in the Electron Transport Chain



Standard redox potential (E0′ [mV, 25°C, pH = 7]) are indicated by dashed (- - -) lines

If physiological or environmental conditions are known to shift the potential from the E0′, redox windows are indicated (solid lines)

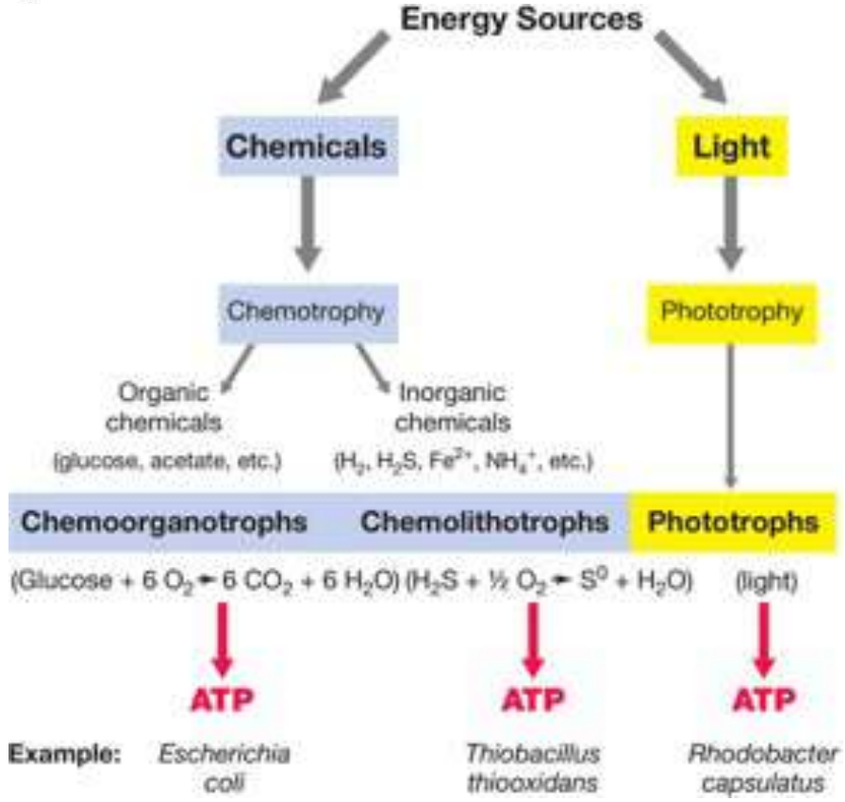
- **Blue: aerobes**
- **Green: facultative anaerobes**
- **Red–yellow: obligate anaerobes**

c-type cytochromes can cover a broad range of redox potentials as indicated.

Not all bacteria mentioned will cover the whole range.

Kracke et al., 2015

All types of metabolism can be classified based on their source of energy
Phototrophs obtain energy for metabolism from light. Plants are one type of phototroph, but we will learn that many different types of phototrophic metabolism exist in the microbial world . **Chemotrophs** obtain energy for metabolism from chemical reactions . The aerobic respiration of glucose is an example of chemotrophic metabolism in that free energy comes from the chemical oxidation of glucose to CO_2 Chemotrophic reactions are classified as *aerobic* if they require O_2 as an electron acceptor, but they are classified as anaerobic if their electron acceptor is anything other than O_2 . Chemotrophs can conserve energy from either *respiration* reactions or *fermentation* reactions .
For example, in the presence of O_2, yeast can perform aerobic respiration of glucose to CO_2, but when O_2 is limiting they alter their metabolism to perform anaerobic fermentation of glucose to ethanol and CO_2.



Madigan et al. 2020