Resource Acquisition and Homeostasis

- Adaptive fine-tuning of elemental composition.
- Nutrient uptake kinetics and mechanisms.
- Photosynthesis: the transformation of light to chemical energy.
- Osmoregulation: fighting the water-intake problem.
- Circadian rhythms: 24-hour cycles in cell physiology.

Can Selection Favor the Use of Particular Nucleotides or Amino Acids Based on Their Elemental Compositions?

- Organisms living in environments depleted in a key element are expected to evolve towards reduced reliance on the same element.

- Enzymes involved in metabolism of a scarce element are expected to have nucleotides and/or amino acids depleted for the same element.

(Baudouin-Cornu et al.\ 2001; Alves and Savageau 2005; Acquisti et al.\ 2009; Grzymski and Dussaq 2012; Fasani and Savageau 2014).

- Enzymes involved in the assimilation of sulfur and carbon in *E. coli* and yeast contain amino acids that are, respectively, depleted with sulfur and carbon (e.g., methionine and cysteine in the case of sulfur) (Baudouin-Cornu et al. 2001).

- Fewer methionine / cysteine residues in cyanobacterial proteins expressed during times of sulfur depletion (Mazel and Marliere 1989).

- No such depletion for sulfur metabolizing enzymes in mammals.
    - Is this is because mammals are less deprived of sulfur (Baudouin-Cornu et al. 2001)?

DNA-level selection:

- A:T → 10 C and 7 N atoms.
- G:C → 9 C and 8 N atoms.
    - *E. coli* – sized bacterium contains ~ 10^{10} C and 10^9 N atoms.
    - Exchange of one pair for another alters the elemental budget by fractions 10^{-10} C and 10^{-9} N.
    - Using the benchmark of $2N_e s > 0$, to avoid selective neutrality, selective discrimination requires $N_e > 0.5 \times 10^9$ to 10^{10}.

Amino-acid-level selection:

- Maximum C-content difference / AA is 9 (tryptophan vs. glycine).
- Maximum N-content difference / AA is 3 (arginine vs. several others).
    - Maximum fractional impact of exchange of a single amino acid is ~ 10^{-9}.
    - With a mean copy number of proteins / cell in *E. coli*, this can place the total fractional impact in range of 10^{-6} to 10^{-5}.

Nutrient Uptake

- Kinetics.
- Channels and transporters.
- Physiological acclimation.
- Advantages of motility.

Michaelis-Menten Enzyme Kinetics

$$\mathrm{E} + \mathrm{S} \underset{k_d}{\overset{k_a}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_{cat}} \mathrm{E} + \mathrm{P}$$

Three kinetic parameters.

$$k_{\mathrm{a}}[\mathrm{E}][\mathrm{S}] = (k_{\mathrm{d}} + k_{\mathrm{cat}})[\mathrm{ES}], \quad [\mathrm{ES}] = \frac{[\mathrm{E}][\mathrm{S}]}{K_{\mathrm{S}}}$$

Concentration of the postulated intermediate at equilibrium.

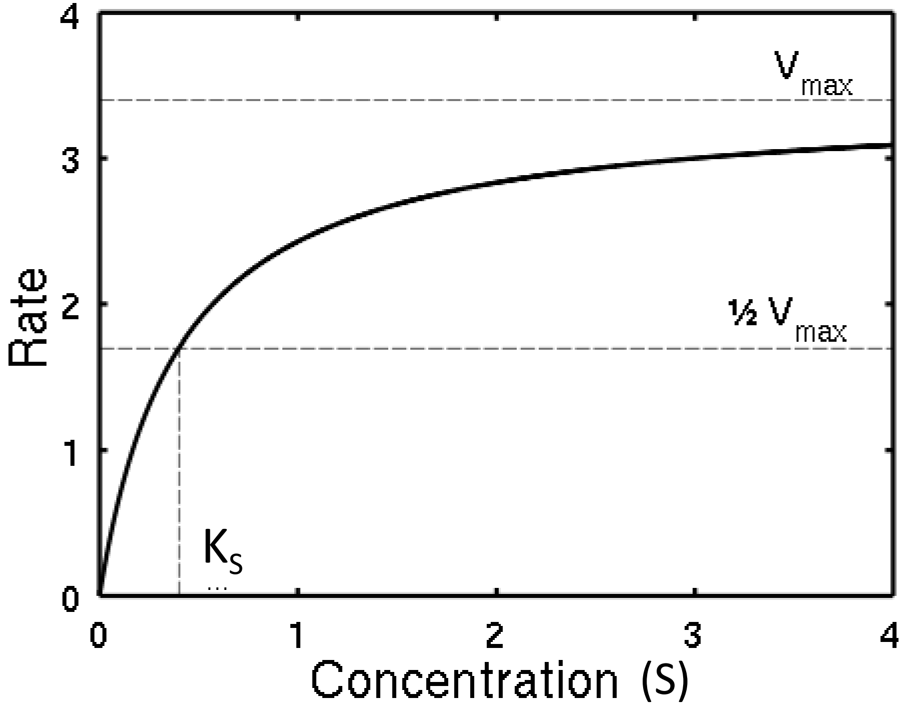
$(K_{\mathrm{S}} = (k_{\mathrm{d}} + k_{\mathrm{cat}})/k_{\mathrm{a}})$

$$V = k_{\mathrm{cat}}[\mathrm{ES}] = \frac{k_{\mathrm{cat}}[\mathrm{E}][\mathrm{S}]}{K_{\mathrm{S}}} = \frac{k_{\mathrm{cat}}[\mathrm{E_T}][\mathrm{S}]}{K_{\mathrm{S}} + [\mathrm{S}]}$$

Reaction rate in terms of total enzyme and substrate concentration.

German biochemist Leonor Michaelis and Canadian physician Maud Menten

Measuring Enzyme Efficiency



k_{cat} = turnover rate

k_{cat} / k_S has units of $M^{-1} \cdot sec^{-1}$

(uptake affinity or catalytic activity)

$$K_S = (k_d + k_{cat})/k_a$$

$$\text{Reaction rate} = \frac{V_{max} \cdot S}{k_S + S} = \frac{k_{cat} \cdot E \cdot S}{k_S + S} \longrightarrow$$

Low S: $(k_{cat} / k_S) \cdot E \cdot S$

High S: $k_{cat} \cdot E$

Nutrient-Uptake Parameters in Phytoplankton Species

Scaling of Nutrient-Uptake Parameters (per cell) with Cell Volume (V^x)

X	Nitrogen	Phosphorus
V_{max}	0.82	0.94
V_{max} / k_{cat}	0.75	0.85

- Uptake rates per unit biomass decline with increasing cell volume.

(Edwards et al. 2012)

Channels and Transporters

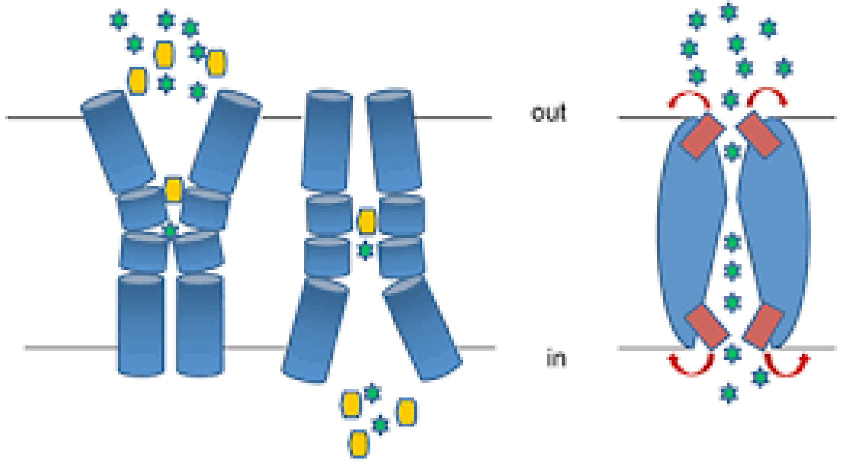
- 20 to 35% of the genes in prokaryotes and eukaryotes encode for membrane proteins.

- Phylogenetic distributions indicate that many of these have pre-LUCA origins.

    - Many lineage-specific losses and gains, e.g., no K-channel genes in fission yeast, 1 in budding yeast, 300 in *Paramecium*.

- The energy for transport of single solute particles is thought to $\approx$ 2 ATP hydrolyses.

Transporters | Channels



Transporters

- Transport requires structural change of membrane protein, elicited by cargo binding.

- Slow rates of transport, requiring energy input

Channels

- No structural change.

- Do not bind solute directly.

- Rates can approach the diffusion rate, but only transport down a concentration gradient.

Some Rough Computations: Glucose Uptake in *E. coli*.

- Assuming a 40 min cell-division time, and ~2 x 10^9 glucose molecules to build a new cell implies:

  - 1) a total cellular uptake rate of ~833,000 glucose molecules/sec, which at a transport rate of 100 molecules / sec requires ~8,000 glucose transporters / cell;

  - 2) ~4% of membrane areas must be allocated to glucose transporters;

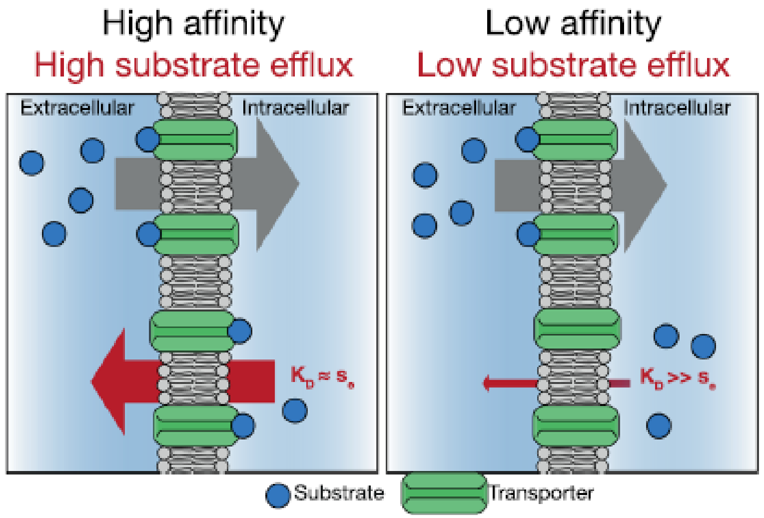
  - 3) assuming a cost of 2 ATPs / imported glucose molecule, the cost of glucose uptake / cell division ≈ 4 x 10^9 ATP hydrolyses, which ≈ 15% of the total cost of building an *E. coli* cell.

- The cost of import / transporter ≈ 500,000 ATP hydrolyses / cell division.

  - Greatly exceeds the cost of building the transporters, as even a 1000 residue protein costs ~30,000 ATP hydrolyses.

Phillips and Milo (2009)

Physiological Acclimation



- Model predicts that at higher nutrient concentrations, lower-affinity transporters have higher rates of influx because they experience less conflict with internal cell concentrations

- When cells are acclimated to lower nutrient concentrations, substrate affinity increases.
- Often associated with a shift from use of low- to high-affinity transporters in low nutrient conditions (Eide 2012).
- Can be explained if transporters behave in a bidirectional manner.

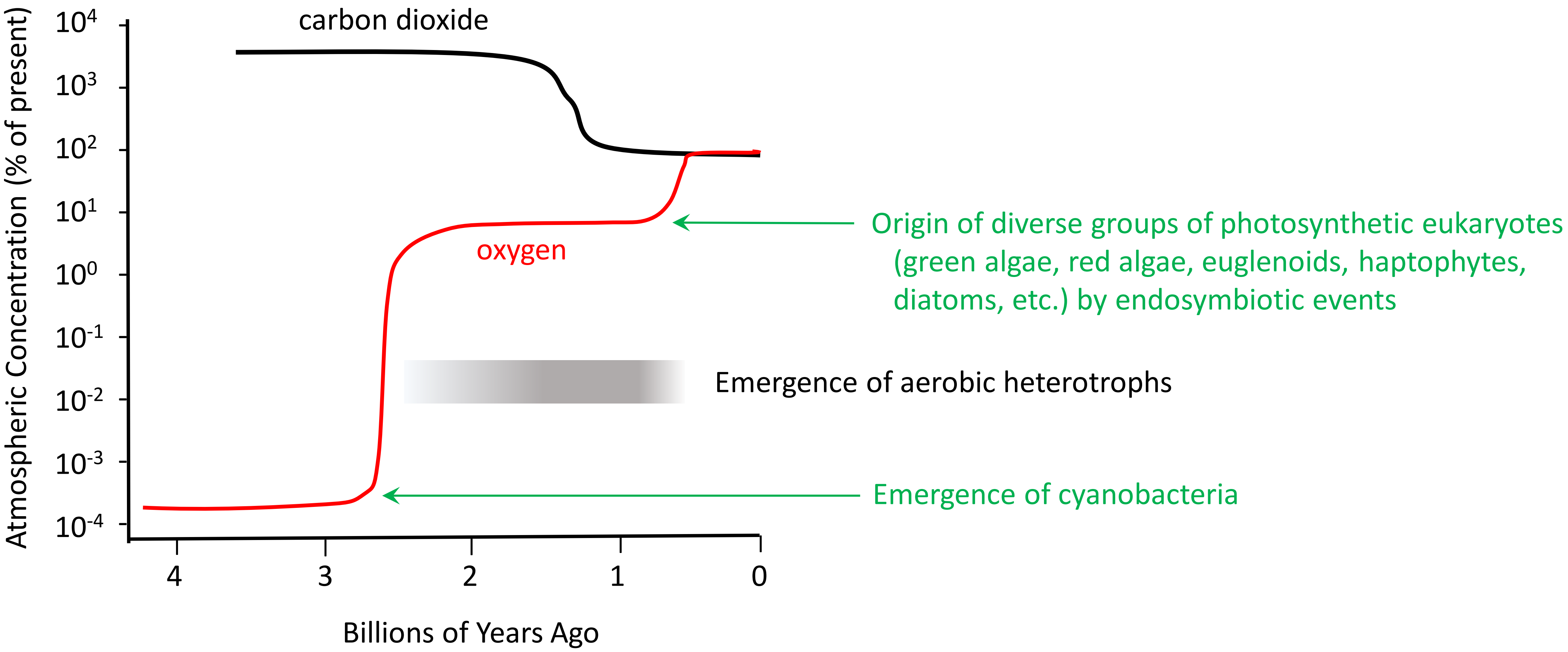
Photosynthesis: the transformation of solar to chemical energy.

- Autotrophy: five other carbon-fixation mechanisms, not requiring light, are exploited by various anaerobic prokaryotes, but ~99% of total global primary production is generated by RuBisCO, the key carbon-fixation enzyme in phototrophs.

- Photosynthesis occurs in 7 bacterial phyla, but only in the cyanobacteria and photosynthetic eukaryotes does it release O_2.
  - Anoxygenic photosynthesizers use ferrous iron, molecular hydrogen, and sulfur as electron donors.
  - Oxygenic photosynthesizers use water as an electron donor -- a key moment in evolutionary history, providing a means for exploiting a permanent and reliable supply of light energy to produce the ATP and NADPH necessary for the downstream synthesis of organic matter.

- Because the early-Earth atmosphere was anoxic but had CO_2 with concentrations 100x higher than in today's atmosphere, it is thought that anoxygenic photosynthesis was the first established form of phototrophy.
  - Given its phylogenetic distribution across bacteria, it may have been present in the ancestral bacterium (Woese 1987), with many bacterial lineages subsequently experiencing loss and transition to heterotrophic life styles.

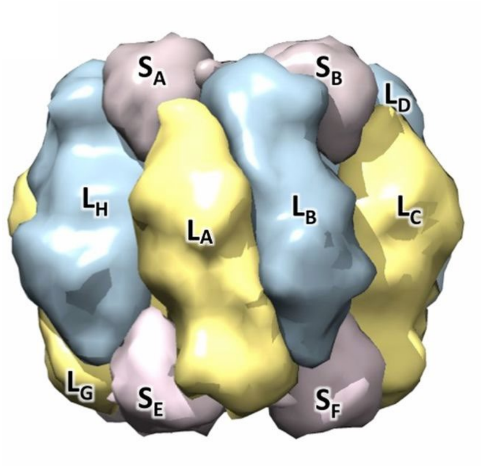
The Great Oxygenation Events: ~10^6-fold increase in atmospheric O_2 concentration



Historical record of the Earth's atmospheric CO_2 and O_2 concentrations. Vertical axis is on a logarithmic scale.

RuBisCo (Ribulose-1,5-bisphosphate carboxylase / oxygenase): the world's most abundant enzyme.

- Catalyzes the joining of ribulose-1,5-bisphosphate (a 5-carbon molecule) and CO_2 into a 6-carbon product that is subsequently split into two 3-carbon molecules (glyceraldehyde-3-phosphates), which are then deployed in downstream biosynthetic pathways and in the recycling of RuBP for another round of carbon fixation.



- ~10^{13} kg on Earth (Bar-On and Milo 2019).

- Substantial structural diversity:
  - Form I (cyanobacteria, most eukaryotes) – hexadecamer, 8 large subunits, two rings of 4 small subunits.
  - Form II: no small subunits – anaerobic photosynthetic proteobacteria, and dinoflagellates.
  - Homohexamer and homodimer in two other photosynthetic bacteria.
  - Homooctamer and homodecamer in two archea.

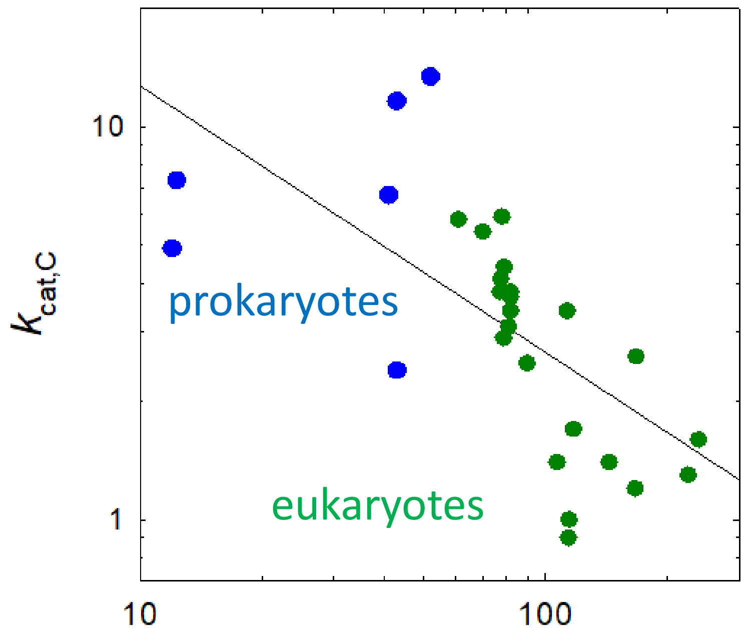
The World's Most Abundant Enzyme is Also One of the Least Efficient

- k_{cat}, a measure of the maximum processing rate / molecule, and k_{cat} / K_s, a measure of uptake affinity, are orders of magnitude below the rates based on diffusion limitation.

- RuBisCo is extremely error-prone, competitively binding O_2 vs. CO_2, with error rates ≈ 15 to 35% at atmospheric concentrations.

- Is the lower specificity of bacterial RuBisCo a "frozen accident" of the bacterial enzymes having evolved during times of low O_2 / high CO_2?

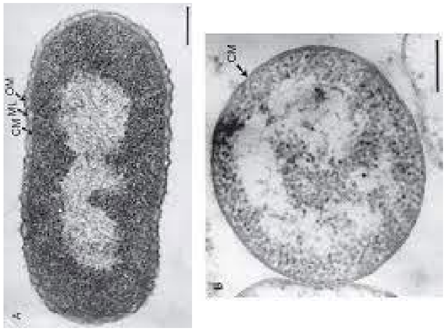
- Is the negative correlation between catalytic rate and specificity an inevitable consequence of a structural tradeoff?



CO_2 / O_2 Specificity
(ratio of uptake affinities)

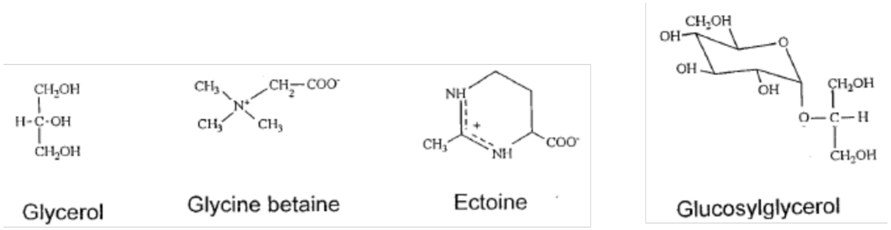
- Many species have a CO_2-concentrating mechanism (CCM) to store CO_2 intracellularly, e.g., C4 and CAM plants, and carboxysomes in cyanobacteria.
  - Bivariate drift barrier? Inverse relationship between the CCM factor and the specificity factor.

Osmoregulation



- Hyperosmotic (saline) environments promote cell dehydration and excess molecular crowding.
- Hypoosmotic environments induce cell swelling and potential membrane rupture.
  - Example: “L forms” (wall-less) bacteria, produced with antibiotics that inhibit peptidoglycan formation, must be grown in an osmoprotective environment (containing nonmetabolizable sucrose).

- Mechanisms of solving the osmoregulation problem:
  - Aquaporins for passive movement of water molecules, excluding other ions.
  - Gated ion transporters.
  - Synthesis of “compatible” solutes in saline environments.
  - Cell walls to counter turgor pressure.
  - Contractile vacuoles to expel excess water.



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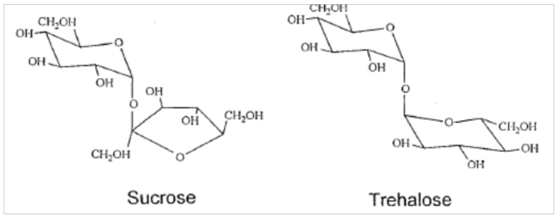

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costs of compatible solutes (in ATPs)

- Use of synthesized compatible solutes (a one-time investment) vs. active pumping of ions (1 to 2 ATPs per ion transported, plus the cost of constructing the transporters.
    - Up to 80% of the construction costs of halophilic cells can be associated with compatible-solute production.

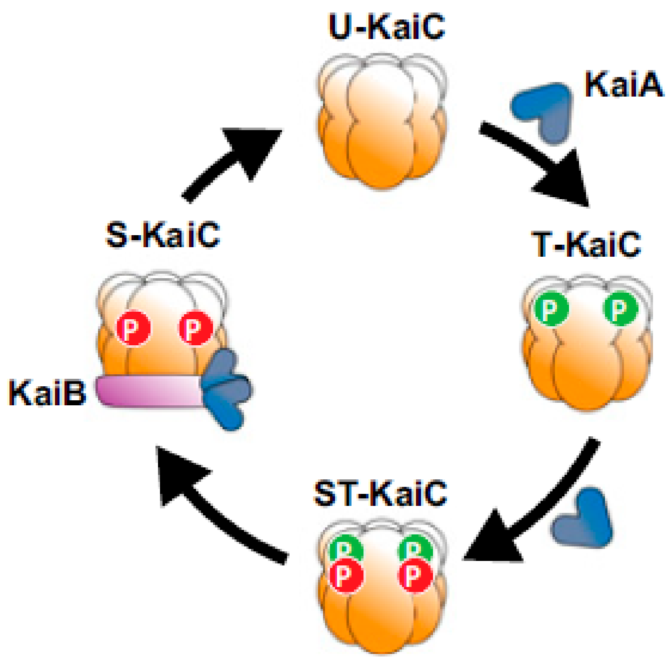
- Use of cell walls (a one-time investment) vs. contractile vacuoles.
    - Ciliates: the equivalent of 90 to 150 cell volumes is expelled per day (2 to 40% of total cell budget, increasing with cell volume).
    - The green alga *Chlamydomonas* expels the equivalent of 270 cell volumes / day.
    - *Mesostigma*, another green alga, expels the equivalent of 1200 cell volumes / day.

Circadian Rhythms

- Internal molecular clocks generate robust ~24-hour rhythmicity that can be accurately entrained by an external factor (such as a light : dark cycle) and remain free-running for extended periods in a constant environment.
    - A lack of sensitivity to absolute temperature is generally taken to be a hallmark of a true circadian rhythm.

- Do such clocks exist in unicellular organisms with cell-division times less than 24 hours?
    - Unknown in heterotrophic bacteria.

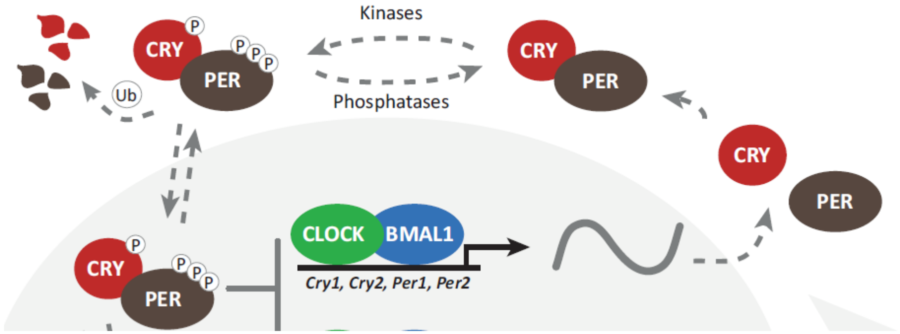
Three-Component Oscillator in the Cyanobacterium *Synechococcus*



- Central hub, KaiC, is phosphorylated in the presence of KaiA, but once this occurs, KaiB forms a complex that inhibits KaiA, thereby promoting dephosphorylation of KaiC, starting the cycle anew. Cycle elicits a signal-transduction cascade of gene-expression changes leading to growth-inhibition in the absence of light.

- Core oscillator can be made to operate *in vitro* in a solution containing just the three proteins and ATP.

Phong et al. (2013)



- Simplified view of the vertebrate molecular clock.

- The heterodimeric transcription factor Clock/Bmal1 activates transcription of the Per and Cry genes. The proteins of the latter two genes then heterodimerize, return to the nucleus, and suppress their own expression.

- This leads to stable 24-hour cycles.

Partch et al. (2014)

Cost of Running the *Synechococcus* Clock

- Primary cost is in building rather than running the clock:
  - Each monomer of the hexameric KaiC contains 518 AAs, so construction cost / molecule ≈ 6 x 518 x 30 = 93,000 ATPs.
  - 24-hour cycle involves the hydrolysis of just 60 ATPs per hexamer.

- The ~4,000 KaiA, 11,000 KaiB, and 8,000 KaiC proteins/cell imply a total biosynthetic cost $\approx$ 1.5% of the total life-time cell energy budget.

- The tiny eukaryotic green alga *Ostreococcus* (40x larger cell volume than *Synechococcus*):
  - Clock runs by a simple transcriptional-loop mechanism, with one protein (CCA1) repressing the transcription of another (TOC1), and the latter activating transcription of the former (Bouget et al. 2014).
  - Only ~100 copies of each protein per cell.
  - Total cost of the clock ≈ 0.01% of total cell energy budget.