The Cost of Cellular Features

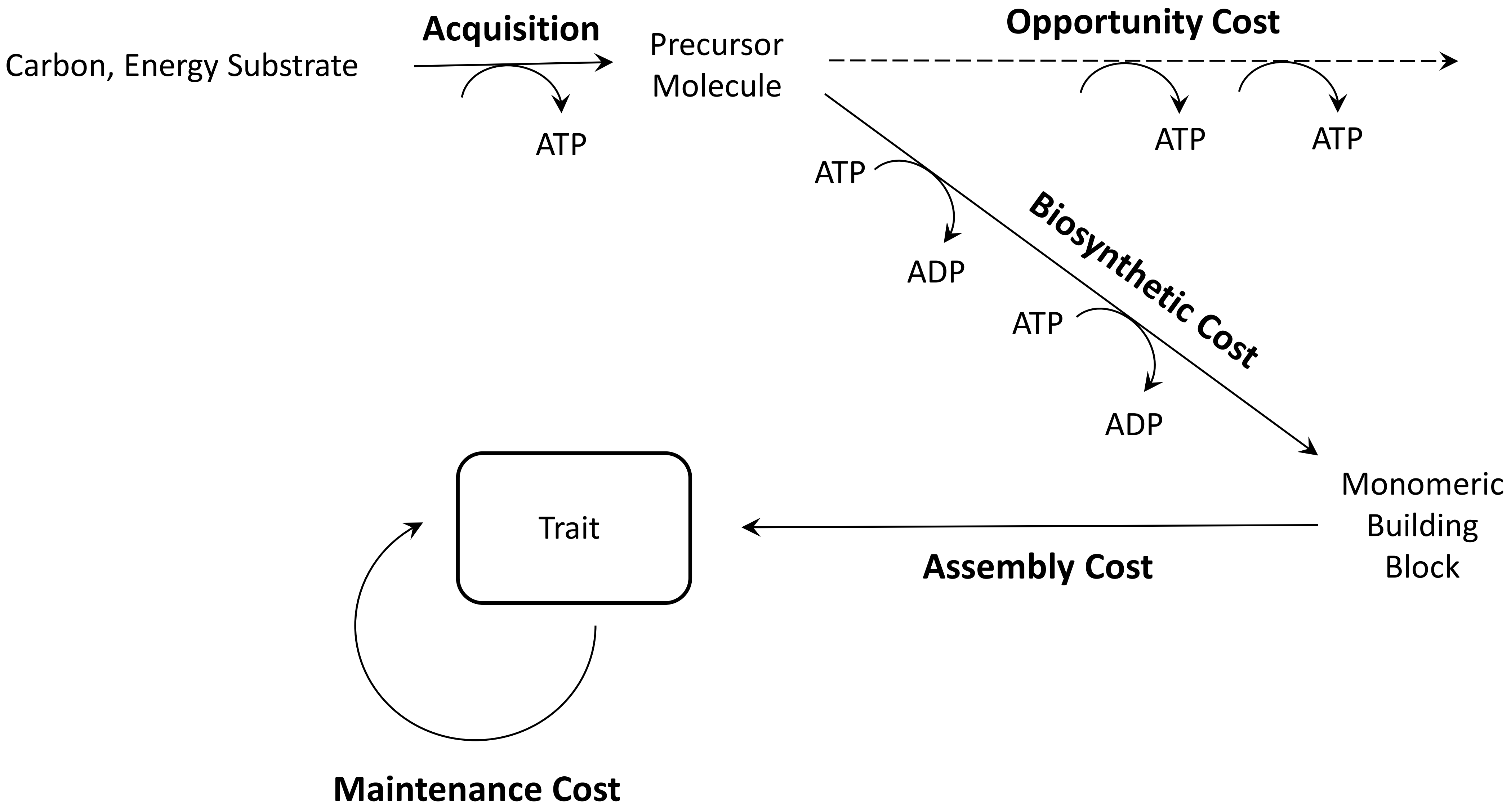
- How do cells apportion their energy budgets into alternative functions, and to what extent does such fractionation vary among phylogenetic lineages?

- What is the appropriate currency for cost measures?
  - ATP hydrolyses constitute the universal currency of bioenergetics across the Tree of Life.
  - Elemental composition contributes to construction costs, but certain elements are relevant to only certain traits, and do not relate in obvious ways to maintenance and/or operational costs.

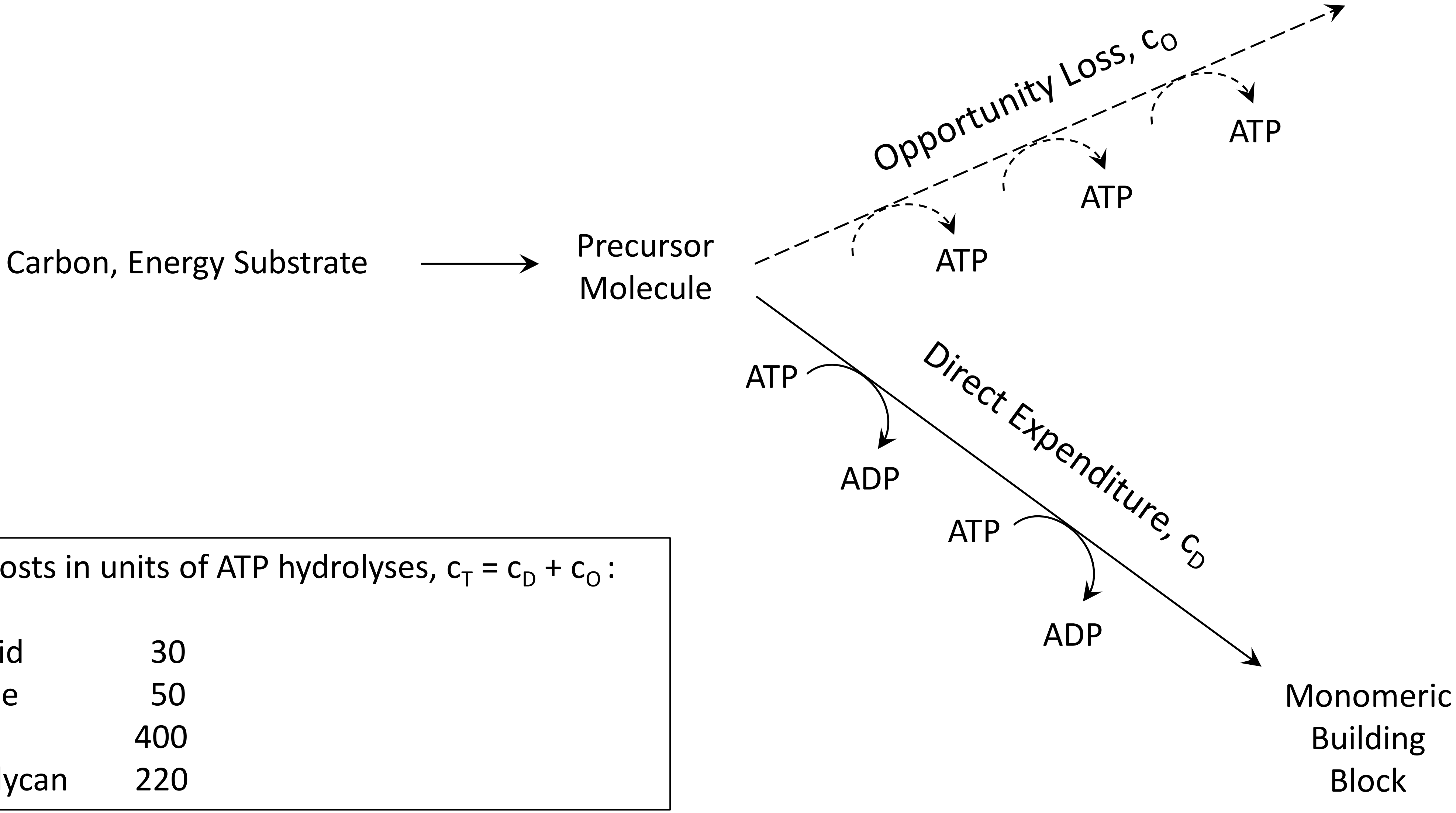
- How can bioenergetic costs be related to the concept of fitness and evolutionary theory?

- Example applications:
  - The cost of maintaining and operating a gene: DNA, RNA, and protein.
  - The costs of membranes in eukaryotes.
  - Total energy budget of a cell: ciliated protozoans.

The Four Primary Cost Components



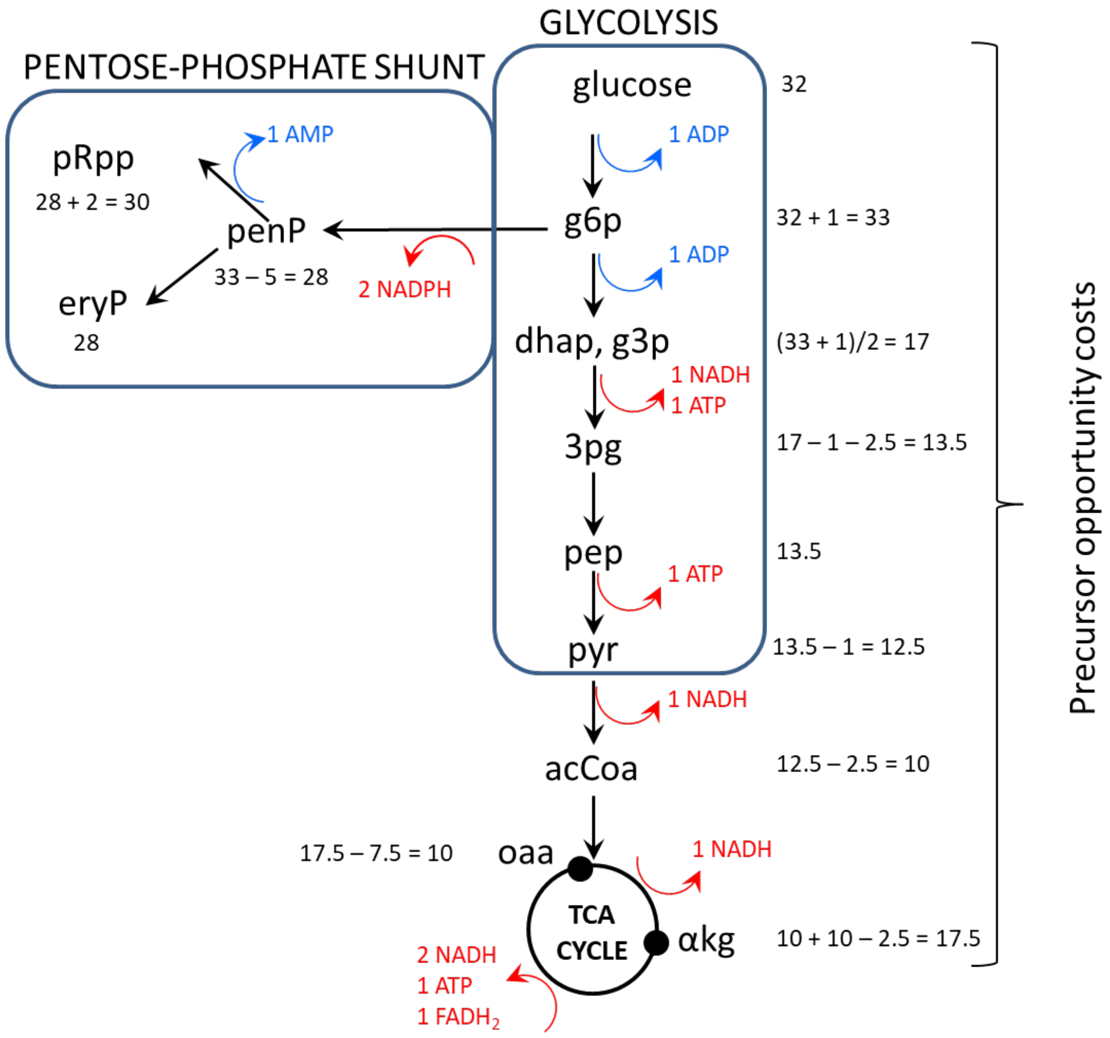
The Cost of Biosynthesis of Elementary Building Blocks



Average total costs in units of ATP hydrolyses, $c_T = c_D + c_O$:

amino acid	30
nucleotide	50
lipid	400
peptidoglycan	220

Energetic Content of Metabolic Precursors

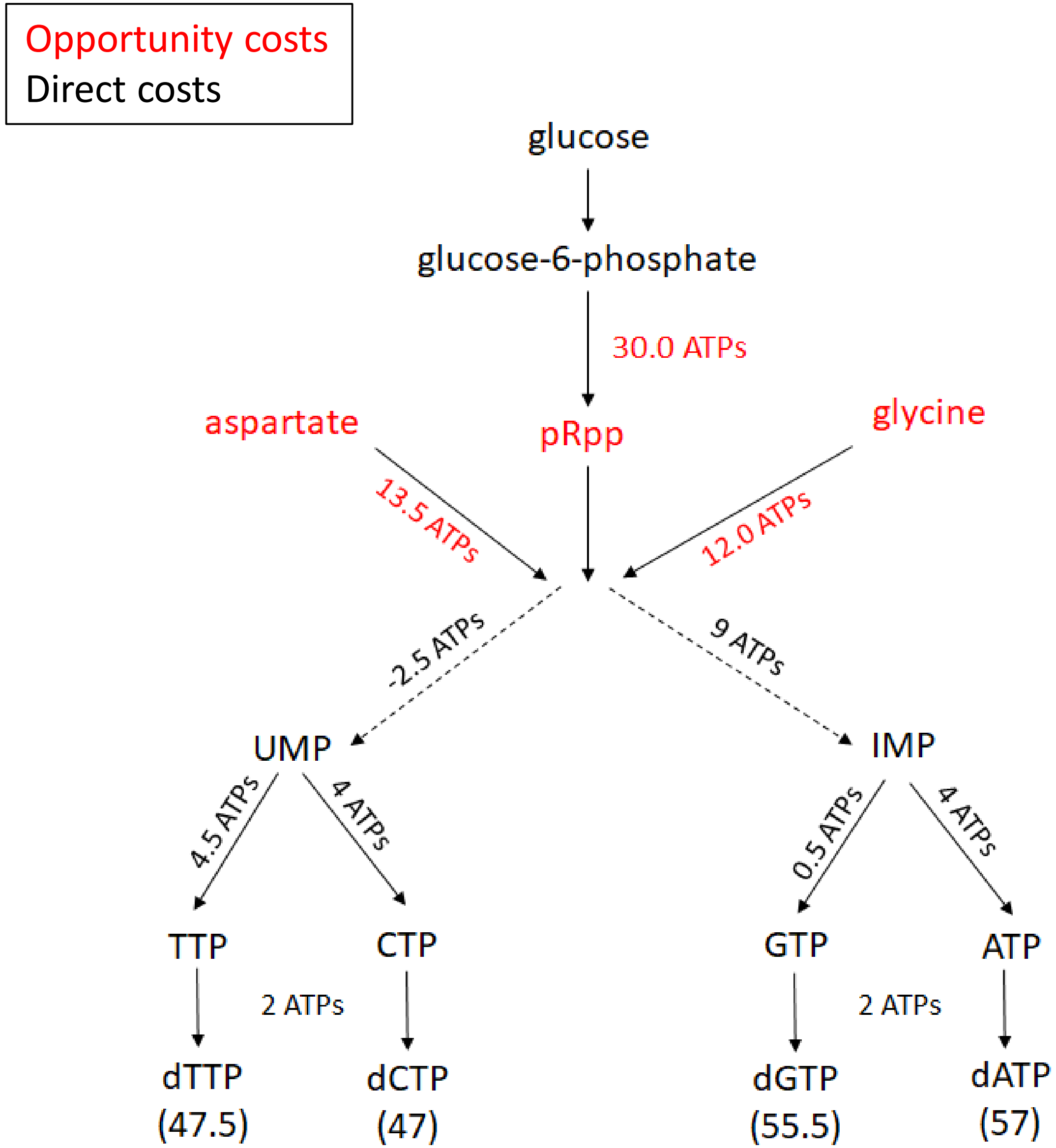


1 NADH ≈ 2.5 ATPs; drives the pumping of 10 protons

1 FADH$_2$ ≈ 1.5 ATPs; drives the pumping of 6 protons

Precursor	Abbrev.	ATP	NADH	FADH2	Total
Ribose 5-phosphate	penP	5	8	2	28.0
5-Phosphoribosyl pyrophosphate	pRpp	7	8	2	30.0
Erythrose 4-phosphate	eryP	5	8	2	28.0
Dihydroxyacetone phosphate	dhap	3	5	1	17.0
Glyceraldehyde-3-phosphate	g3p	3	5	1	17.0
3-Phosphoglycerate	3pg	2	4	1	13.5
Phosphoenolpyruvate	pep	2	4	1	13.5
Pyruvate	pyr	1	4	1	12.5
Acetyl-CoA	acCoA	1	3	1	10.0
Oxaloacetate	oaa	1	3	1	10.0
α-ketoglutarate	αkg	2	5	2	17.5

Energetic Costs of Nucleotides



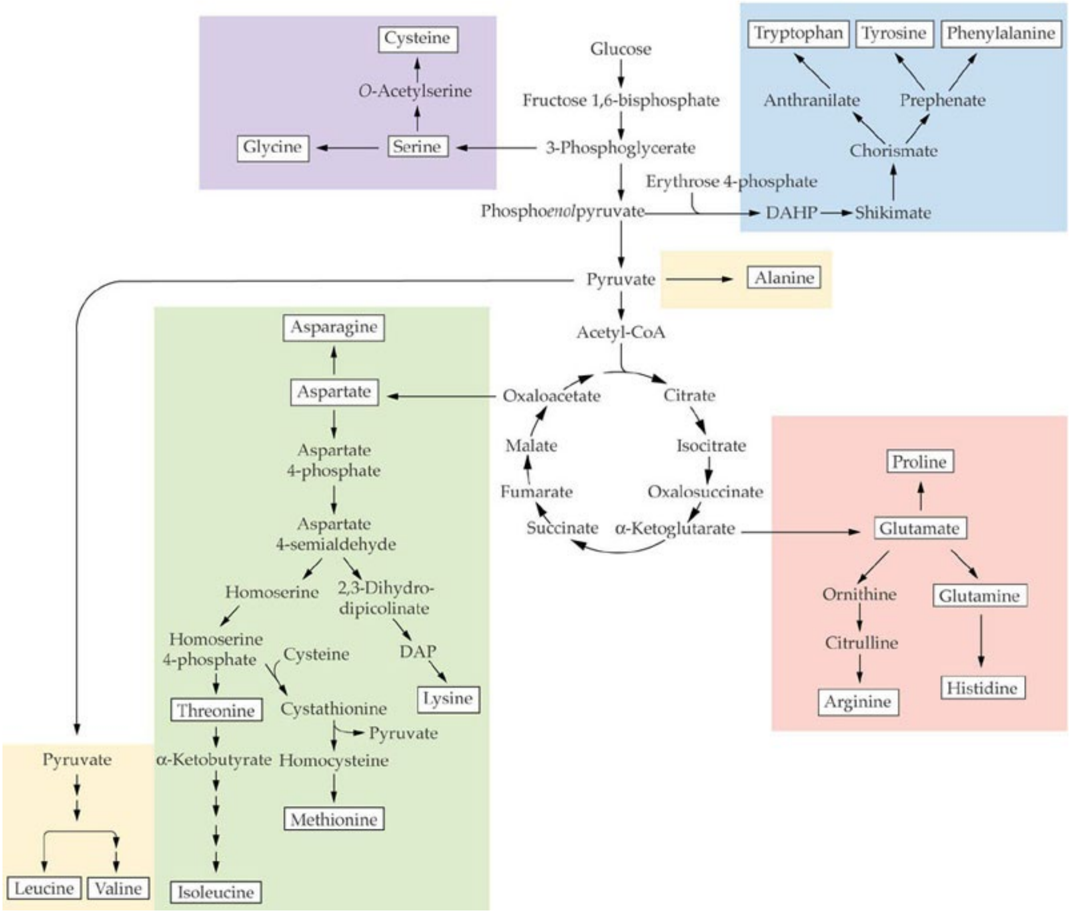
Nucleotide	Opportunity	Direct	Total
Adenine (ATP)	42.0	13.0	55.0
Guanine (GTP)	42.0	11.5	53.5
Cytosine (CTP)	43.5	1.5	45.0
Uracil (UTP)	43.5	−0.5	43.0
Thymine (TTP)	43.5	2.0	45.5

Average ribonucleotide: 43 6

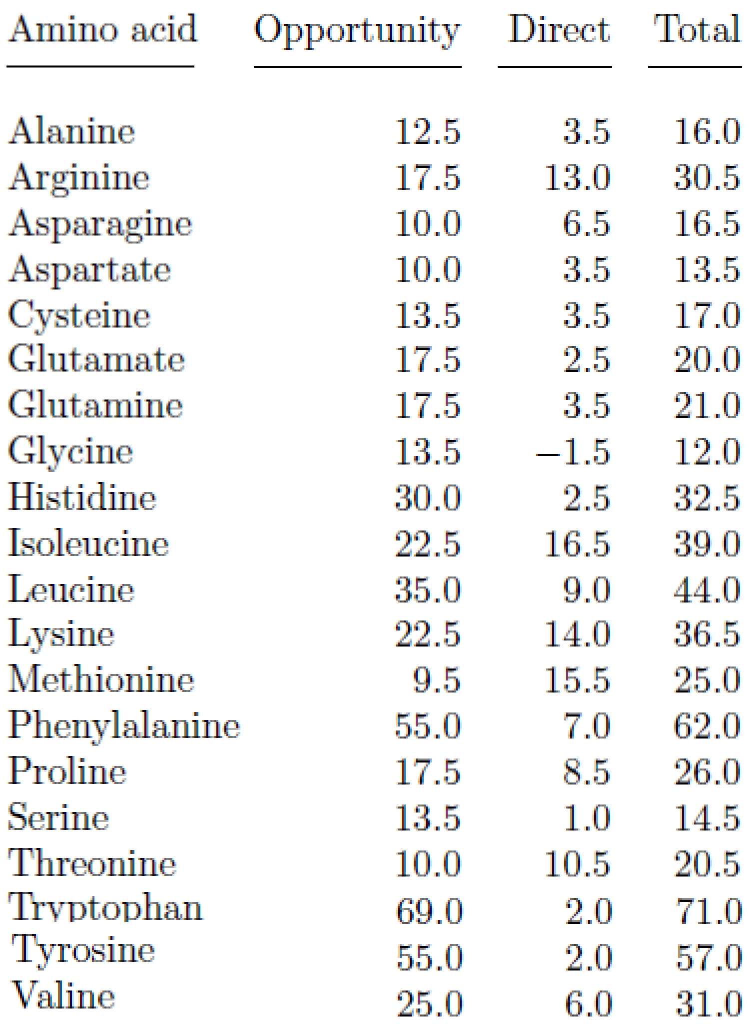
Add 8 to direct costs for deoxyribonucleotide.

- A:T bond ≈ 100.5 ATPs
- G:C bond ≈ 98.5 ATPs

Energetic Costs of Amino Acids



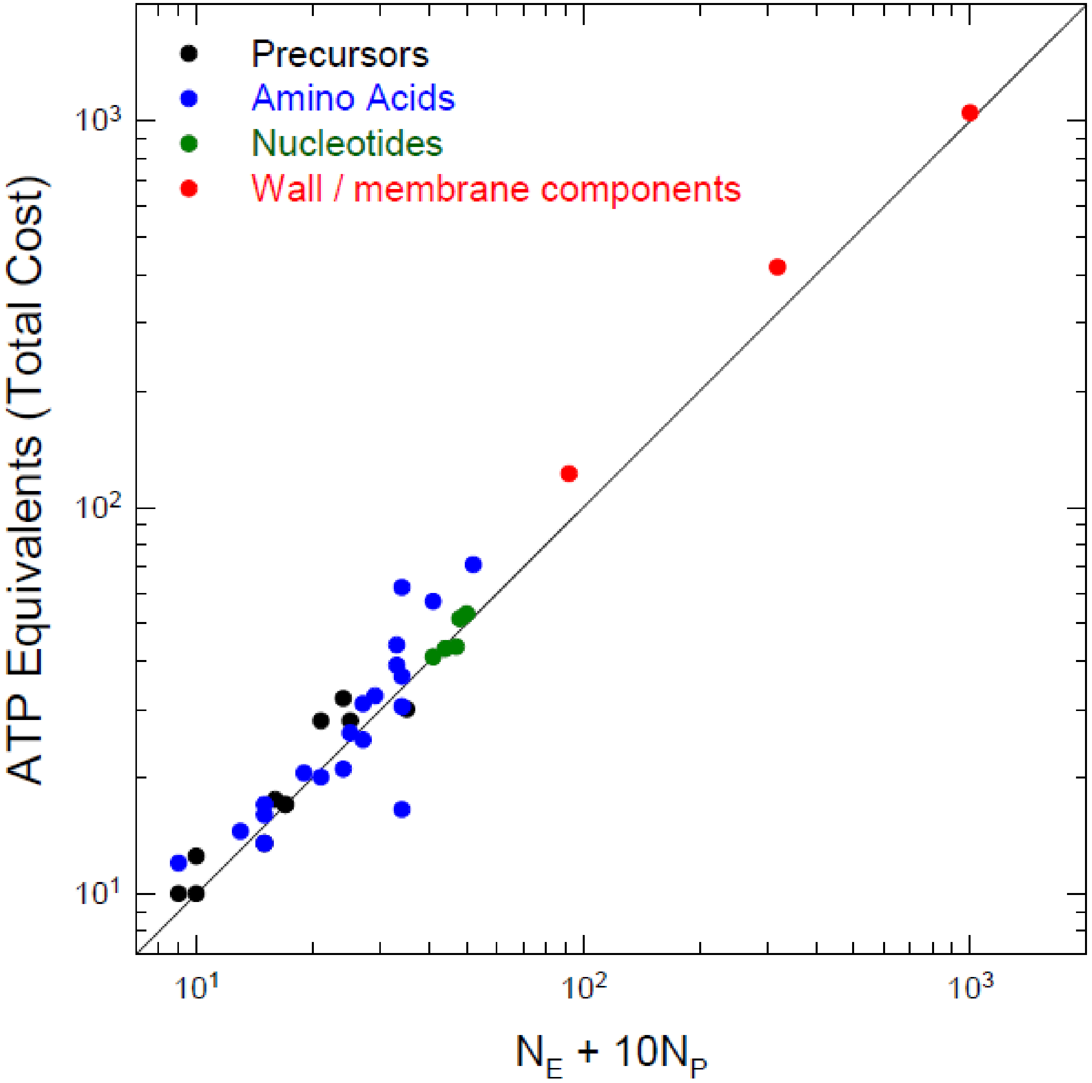
Near Universal Biosynthetic Pathways for Amino Acids



Amino acid	Opportunity	Direct	Total
Alanine	12.5	3.5	16.0
Arginine	17.5	13.0	30.5
Asparagine	10.0	6.5	16.5
Aspartate	10.0	3.5	13.5
Cysteine	13.5	3.5	17.0
Glutamate	17.5	2.5	20.0
Glutamine	17.5	3.5	21.0
Glycine	13.5	−1.5	12.0
Histidine	30.0	2.5	32.5
Isoleucine	22.5	16.5	39.0
Leucine	35.0	9.0	44.0
Lysine	22.5	14.0	36.5
Methionine	9.5	15.5	25.0
Phenylalanine	55.0	7.0	62.0
Proline	17.5	8.5	26.0
Serine	13.5	1.0	14.5
Threonine	10.0	10.5	20.5
Tryptophan	69.0	2.0	71.0
Tyrosine	55.0	2.0	57.0
Valine	25.0	6.0	31.0

Average: 24 6

An Empirical Shortcut to Cost Estimates



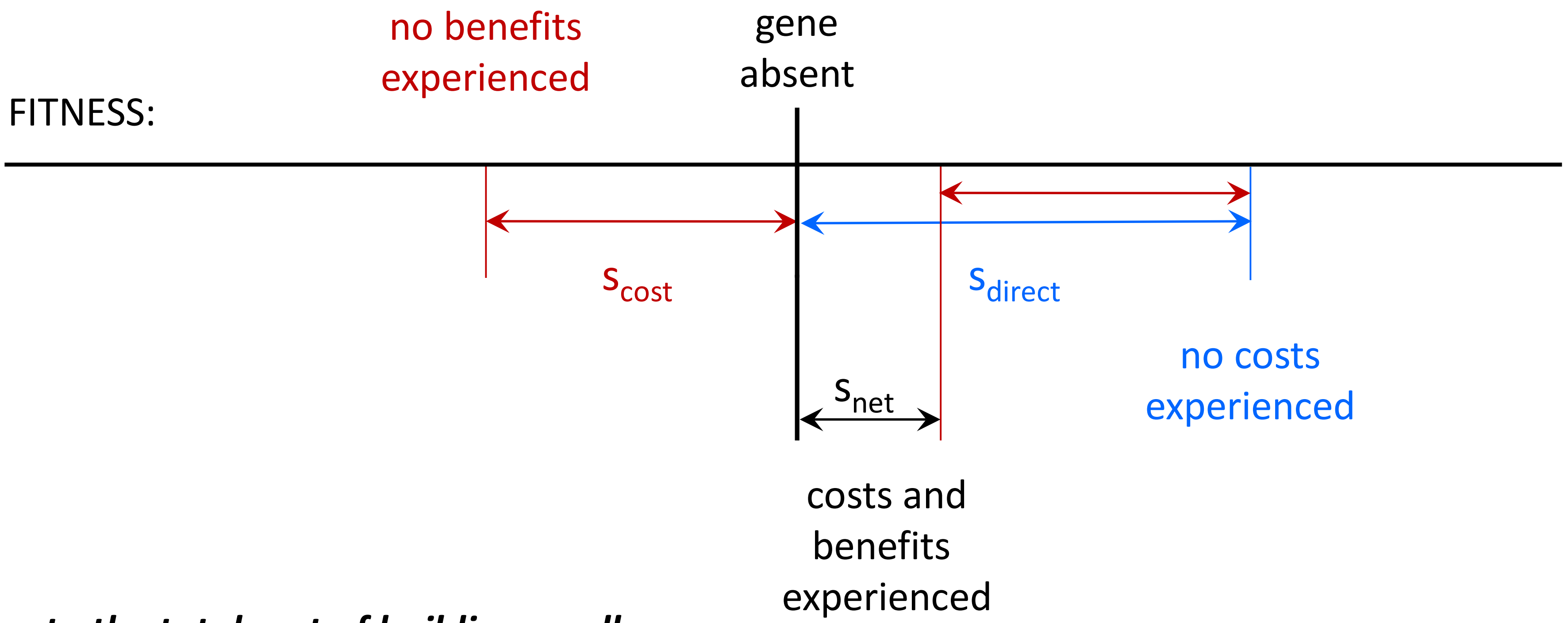
$$\underbrace{N_{\mathrm{ATP}} = 4N_C + N_H - 2N_O}_{} + 10N_P$$

Kharasch and Sher (1925)
“degree of reduction”

Energetic and Evolutionary Consequences of a Genomic / Cellular Modification

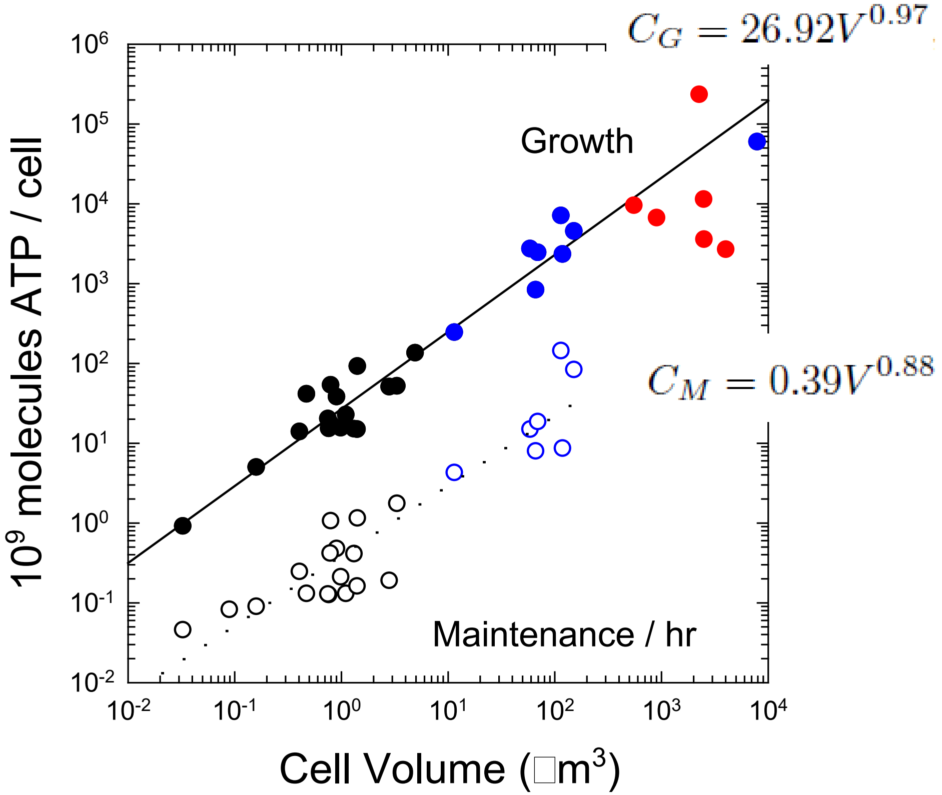
Total baseline energetic cost: $s_{cost} = s_{DNA} + s_{RNA} + s_{PRO}$

Net selective advantage of expressed features: $s_{net} = s_{direct} - s_{cost}$



All scaled relative to the total cost of building a cell.

Lifetime Energy Requirements of Cells



Bacteria
Unicellular eukaryotes
Multicellular eukaryotes

- Scaling is nearly isometric with cell volume.

- It takes ~27 x 10^9 ATP hydrolyses to build 1 μm^3 of cell volume (an *E. coli* cell).

- Total ATP consumption / cell division: $C_T = C_G + TC_M$, where T = cell division time (hours).

Lynch and Marinov (2015)

Conversion of a Bioenergetic Cost to a Baseline Selective Disadvantage

- Selective disadvantage = reduction in population-level growth rate = s_c

  Fitness prior to trait modification = 1
  Fitness after investment in the trait = $1 - s_c$

- Ancestral cell-division time proportional to C.

- Division time after trait modification proportional to C + c.

s_c = energetic fitness cost of the trait.

C = total energy budget of ancestral cell.

c = added energy cost of the trait.

- Assuming $c \ll C$,

  $s_c = \ln(2) \cdot (c / C)$ for binary fission.

Ilker and Hinczewski (2019, Phys. Rev. Letts.)

Can Selection Promote Particular Amino Acids on the Basis of Their Biosynthetic Costs Alone?

- Maximum cost differential = 59 ATPs / amino acid (glycine → tryptophan).
- Lifetime cost of an entire cell ≈ (3×10^{10} ATPs) x cell volume (um^3).

- *E. coli* cell volume ≈ 1 um^3
- Highly expressed gene, 10^4 proteins per cell.
- Relative cost ≈ (59×10^4) / (3×10^{10} ATPs) = 2×10^{-5}
- Visibility to selection requires $2N_es > 1$, so N_e need only exceed 4×10^4
- Lowly expressed gene with 10 proteins / cell → critical $N_e = 4 \times 10^7$

- For yeast ~100 um^3, critical N_e for lowly and highly expressed genes ≈ (4×10^8) and (4×10^4).
- For animal cell ~1000 um^3, critical N_e for lowly and highly expressed genes ≈ (4×10^9) and (4×10^4).

Three Levels for the Cost of a Protein-Coding Gene

Chromosome: synthesis of nucleotides, chain elongation, and downstream transactions.

Transcription: synthesis of mRNAs for steady-state number of transcripts and accounting for turnover.

Protein: synthesis for steady-state number and turnover; downstream modifications.

- All measured relative to the total energy budget of the cell in units of ATP hydrolyses.

Evolutionary Consequences:

Total baseline cost: $s_c = s_{DNA} + s_{RNA} + s_{PRO}$

Net selective advantage: $s_n = s_p - s_c$

Effective Neutrality:

- If $|s| < 1/N_e$ (N_e = the effective population size), selection is unable to eradicate or promote a gene modification.

Costs at the Chromosome Level

- Primary cost is nucleotide synthesis: ~50 ATPs per nucleotide x 2 strands x length of gene in bp.
- In eukaryotes, there is an additional cost of nucleosomes (eight proteins + linker): ~160 ATP per bp in gene length.
- Additional small costs: opening of origins of replication, double-helix unwinding, replacement of RNA primers, ligation of Okazaki fragments.

Bacteria: $C_{\mathrm{DNA},b} \simeq 101 L_g$

Haploid eukaryote: $C_{\mathrm{DNA},h} \simeq 263 L_g$

Diploid eukaryote: $C_{\mathrm{DNA},d} \simeq 526 L_g$

Costs at the Transcriptional Level

- Ribonucleotide synthesis: ~48 ATPs per nucleotide x steady-state number of mRNAs/gene x length of transcript in bases.

- mRNA turnover: ~2 ATPs per nucleotide / base of replacement transcripts.

  Transcription Rate = decay rate (δ) x steady-state number.

- 100 bp for poly(A) tails in eukaryotes.

- Additional costs, not well understood, but small enough to be ignored: splicing, histone remodeling.

- One-time cost for steady-state replacement; recurrent costs for maintenance:
  - Bacteria: $C_{\mathrm{RNA},b} \simeq 2N_r L_g(23 + \delta_r t)$
  - Eukaryotes: $C_{\mathrm{RNA},e} \simeq N_r(46 \cdot L_{r,mat} + 2.17 \cdot \delta_r t L_{r,pre})$

Costs at the Protein Level

- Amino-acid synthesis: c_{AA} ATPs per residue x steady-state number of proteins/gene x length of protein.

- Chain elongation: ~4 ATPs per residue x total proteins produced/cell lifetime x length of protein.

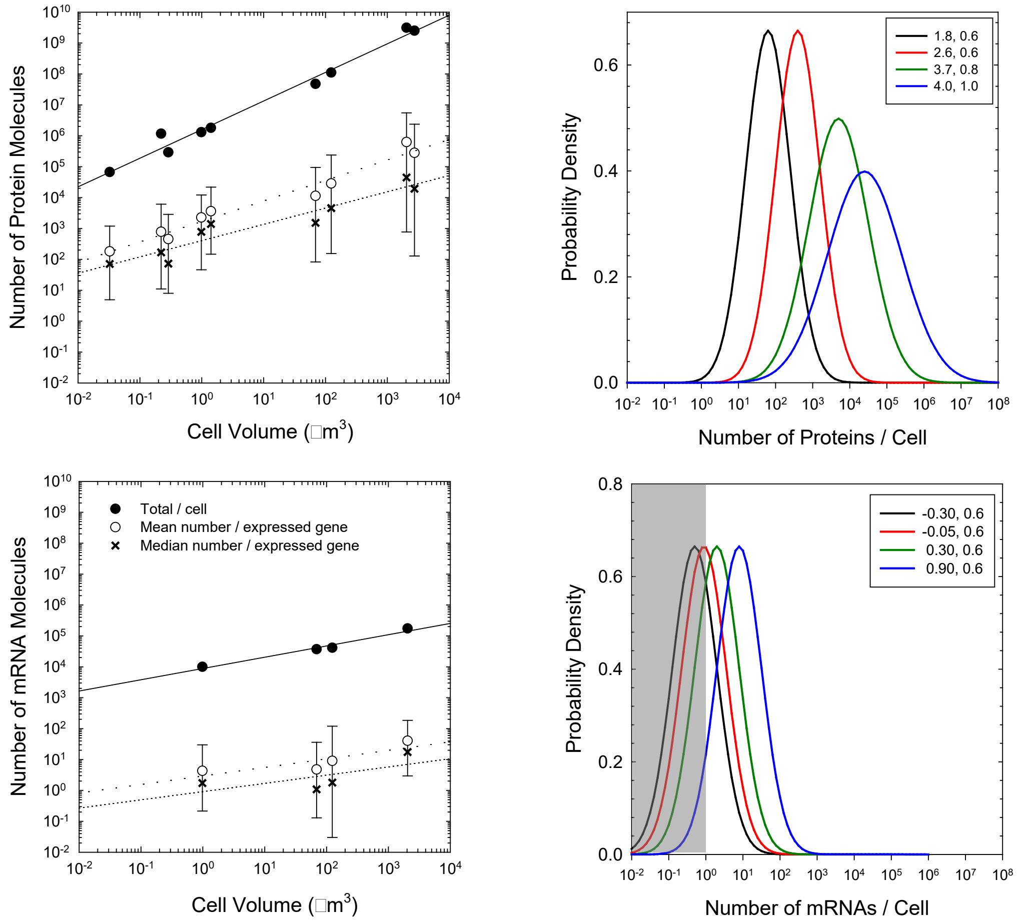
- Small cost of degradation associated with protein turnover.

- Additional costs, not well understood, but small enough to be ignored: translation initiation and termination, post-translational modifications, and protein folding.

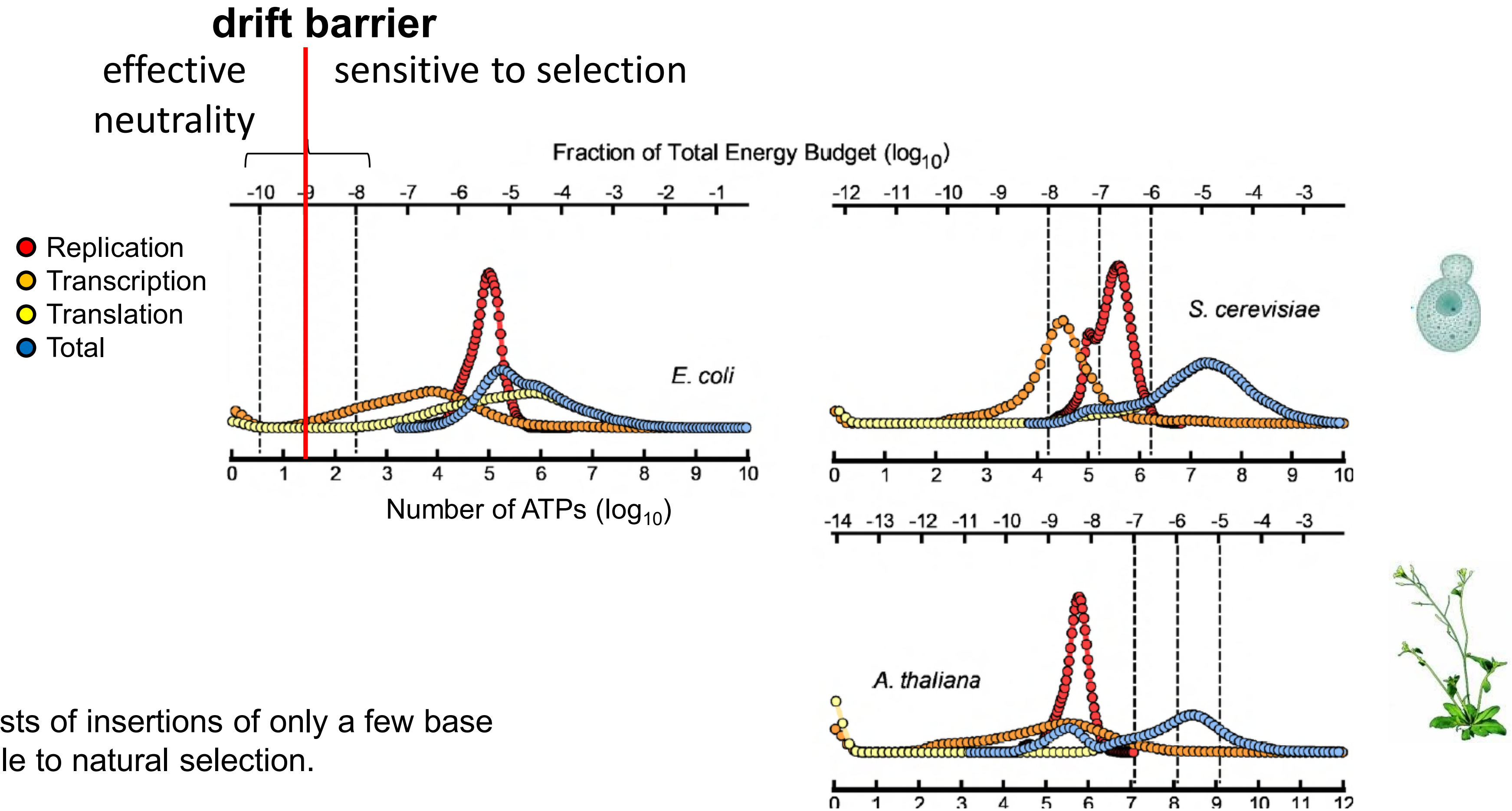
- One-time cost for steady-state replacement; recurrent costs for maintenance:

$$c_{\mathrm{PRO}} \simeq N_p L_p (34 + 7\delta_p T)$$

Scaling of Steady-state Numbers of mRNAs and Proteins With Cell Volume



Frequency Distribution of the Costs for All Genes



- **Bacteria** – costs of insertions of only a few base pairs are visible to natural selection.

- **Multicellular eukaryotes** – absolute costs are ~10 to 100x those in bacteria, but the relative costs are smaller, and often too small to be perceived by selection.

General Conclusions

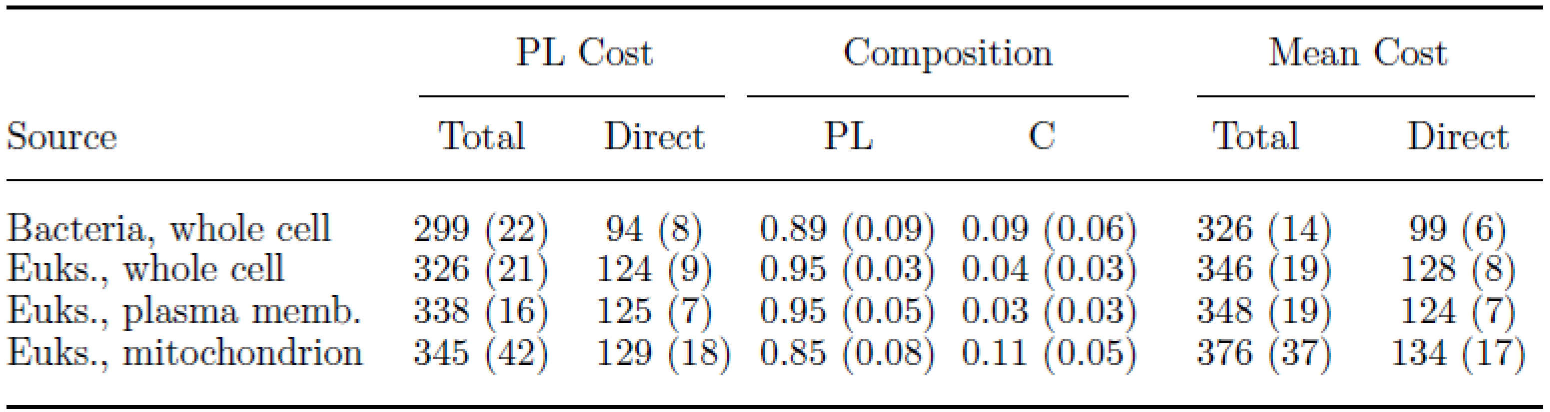
- The energetic cost of just a few nucleotides is sufficient to be perceived by natural selection in bacteria, but insertions of >10 kb are often effectively neutral in large eukaryotes with small N_e.

- Costs at the RNA level often exceed those at the DNA level, but are often still too small to be perceived by selection in large eukaryotes.

- Costs at the protein level are often substantial enough to be perceived even in low N_e species.

- Increased cell size does not impose a burden on the colonization of genes, but has the opposite effect.

Costs of Lipid Molecules



	PL Cost		Composition		Mean Cost	
Source	Total	Direct	PL	C	Total	Direct
Bacteria, whole cell	299 (22)	94 (8)	0.89 (0.09)	0.09 (0.06)	326 (14)	99 (6)
Euks., whole cell	326 (21)	124 (9)	0.95 (0.03)	0.04 (0.03)	346 (19)	128 (8)
Euks., plasma memb.	338 (16)	125 (7)	0.95 (0.05)	0.03 (0.03)	348 (19)	124 (7)
Euks., mitochondrion	345 (42)	129 (18)	0.85 (0.08)	0.11 (0.05)	376 (37)	134 (17)

- Cost per molecule is substantially greater than that for amino acids and nucleotides.

- Despite the large differences in molecular composition, average costs are similar across membrane types and species.

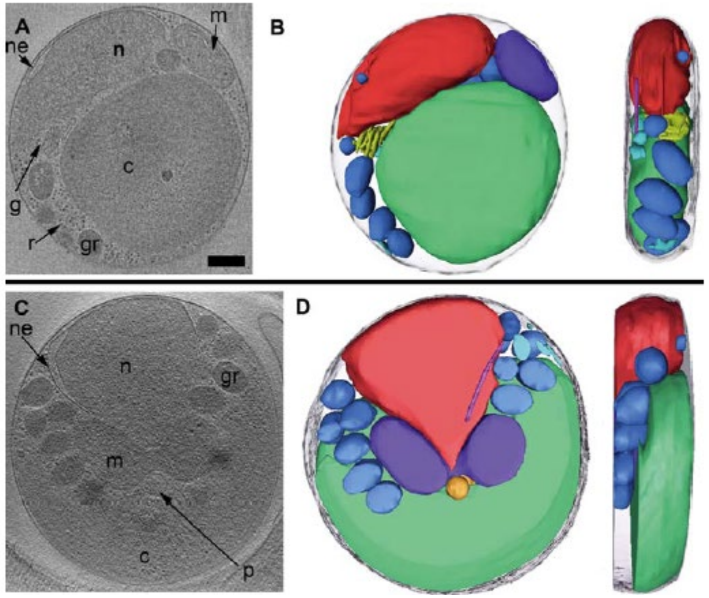
Total Costs of Membranes

- Total number of lipid molecules x average cost per molecule

  = [Twice the total membrane surface area / (head space / lipid molecule)] x cost per molecule.

$$C_L \simeq 2A \cdot \bar{c}_L / (0.65 \times 10^{-6})$$

- Use scanning electron micrograph (SEM) stacks to obtain total membrane surface areas.

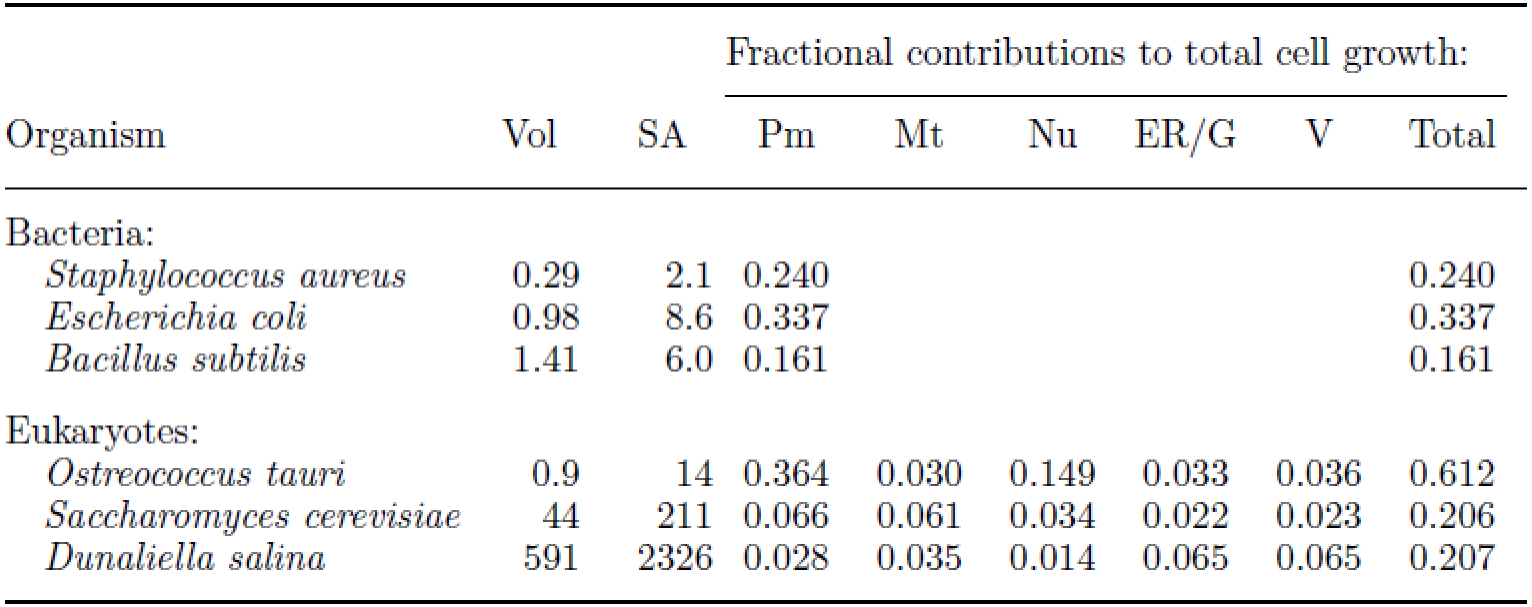


Nuclei
Chloroplast
Mitochondria
Golgi
Endoplasmic reticulum
Peroxisomes
Granules

Green alga, *Ostreococcus tauri*

Henderson et al., 2007, PLoS ONE

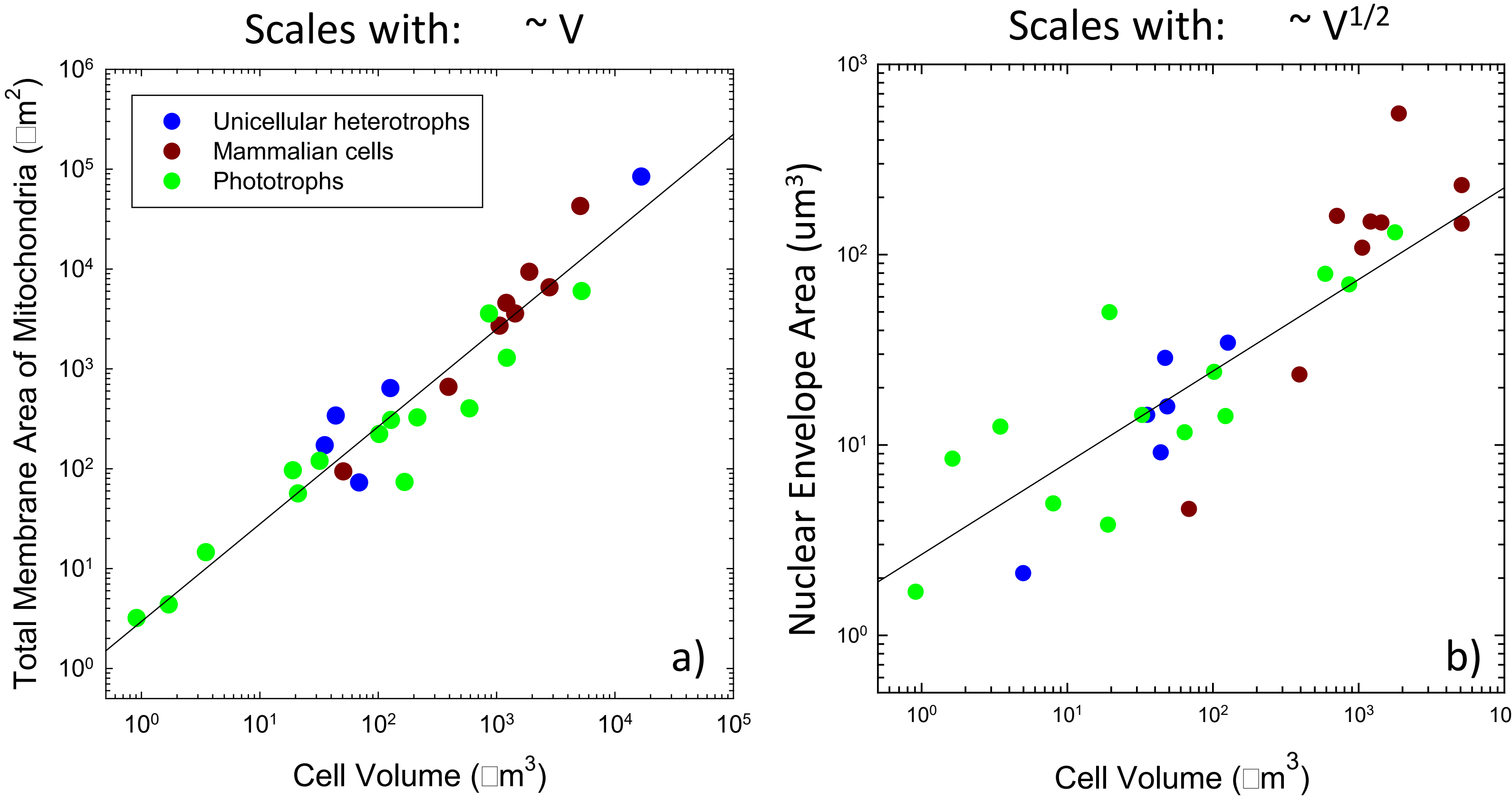
Membrane-Cost Partitioning



			Fractional contributions to total cell growth:					
Organism	Vol	SA	Pm	Mt	Nu	ER/G	V	Total
Bacteria:								
Staphylococcus aureus	0.29	2.1	0.240					0.240
Escherichia coli	0.98	8.6	0.337					0.337
Bacillus subtilis	1.41	6.0	0.161					0.161
Eukaryotes:								
Ostreococcus tauri	0.9	14	0.364	0.030	0.149	0.033	0.036	0.612
Saccharomyces cerevisiae	44	211	0.066	0.061	0.034	0.022	0.023	0.206
Dunaliella salina	591	2326	0.028	0.035	0.014	0.065	0.065	0.207

- 20 to 60% of the total energy budgets of cells is associated with membranes.
- In eukaryotes, >50% of total membrane costs are associated with organelles, more so for larger-celled species.

Some General Scaling Relationships for Membrane Areas in Eukaryotes

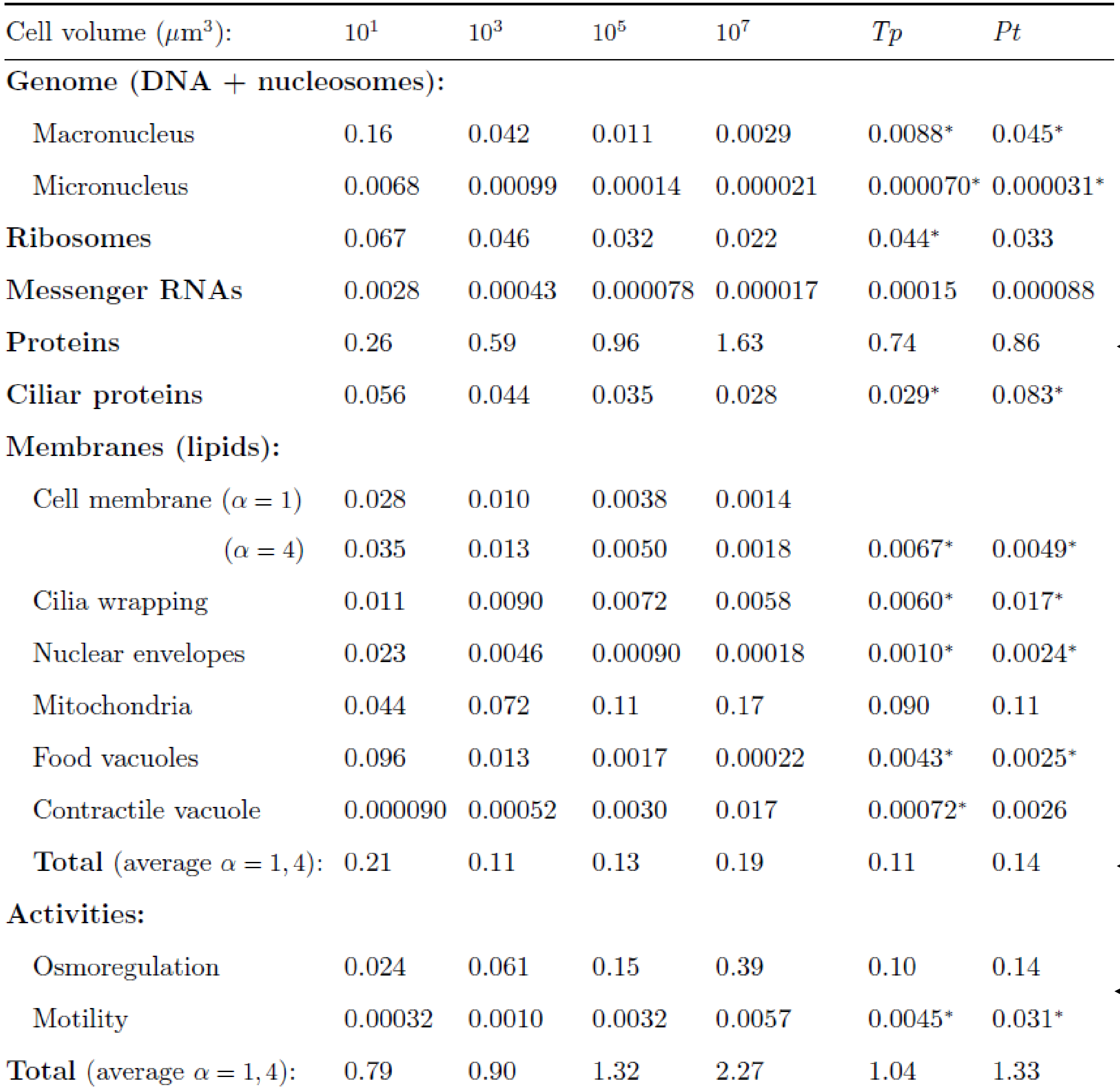


Scales with: ~ V

Scales with: ~ $V^{1/2}$

- Relative to total cellular ATP requirements, cost of mitochondrial membranes ≈ 5% of cell’s energy budget

Approximate Total Cell Budgets in Ciliates (*Tp = Tetrahymena; Pt = Paramecium*)



Cell volume (μm^3):	10^1	10^3	10^5	10^7	*Tp*	*Pt*
Genome (DNA + nucleosomes):						
Macronucleus	0.16	0.042	0.011	0.0029	0.0088*	0.045*
Micronucleus	0.0068	0.00099	0.00014	0.000021	0.000070*	0.000031*
Ribosomes	0.067	0.046	0.032	0.022	0.044*	0.033
Messenger RNAs	0.0028	0.00043	0.000078	0.000017	0.00015	0.000088
Proteins	0.26	0.59	0.96	1.63	0.74	0.86
Ciliar proteins	0.056	0.044	0.035	0.028	0.029*	0.083*
Membranes (lipids):						
Cell membrane ($\alpha = 1$)	0.028	0.010	0.0038	0.0014		
($\alpha = 4$)	0.035	0.013	0.0050	0.0018	0.0067*	0.0049*
Cilia wrapping	0.011	0.0090	0.0072	0.0058	0.0060*	0.017*
Nuclear envelopes	0.023	0.0046	0.00090	0.00018	0.0010*	0.0024*
Mitochondria	0.044	0.072	0.11	0.17	0.090	0.11
Food vacuoles	0.096	0.013	0.0017	0.00022	0.0043*	0.0025*
Contractile vacuole	0.000090	0.00052	0.0030	0.017	0.00072*	0.0026
Total (average $\alpha = 1, 4$):	0.21	0.11	0.13	0.19	0.11	0.14
Activities:						
Osmoregulation	0.024	0.061	0.15	0.39	0.10	0.14
Motility	0.00032	0.0010	0.0032	0.0057	0.0045*	0.031*
Total (average $\alpha = 1, 4$):	0.79	0.90	1.32	2.27	1.04	1.33

← Bulk of investment is associated with proteins, more so in larger cells.

← Costs of membranes are relatively independent of cell volume, and most due to mitochondria.

← Costs of osmoregulation and motility increase with cell volume.

Status of the Mitochondrial Theory for the Origin of Cellular Complexity

- An energetic boost associated with the emergence of the mitochondrion was not a precondition for the expansion of genome or cellular complexity in eukaryotes.
    - There is continuity in scaling of cellular energetic features between bacteria and eukaryotes.
    - Two of the central costs of a gene, the steady-state numbers of mRNA and protein molecules, scale sublinearly with cell volume.
    - Within bacteria alone, although larger cells have higher energetic requirements per cell lifetime, species with larger cell sizes have reduced cell-division times, implying a higher efficiency of energy conversion, despite having larger genome sizes.

A Singular Event: the Origin of the Mitochondrion

Did this give rise to a Lane/Martin bioenergetic revolution
that led to the evolution of:

- Novel protein folds
- Expansion in gene number and genome size
- Introns
- Internal complexity of cells
- Multicellularity
- Development
- Sex
- Etc.

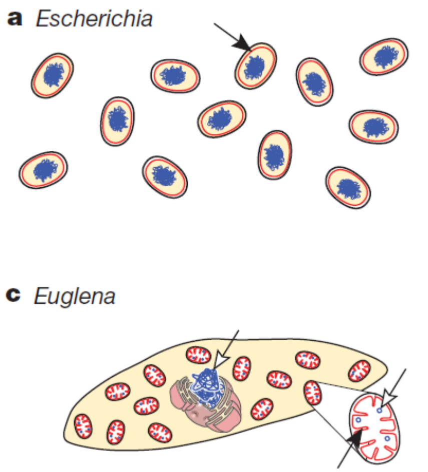
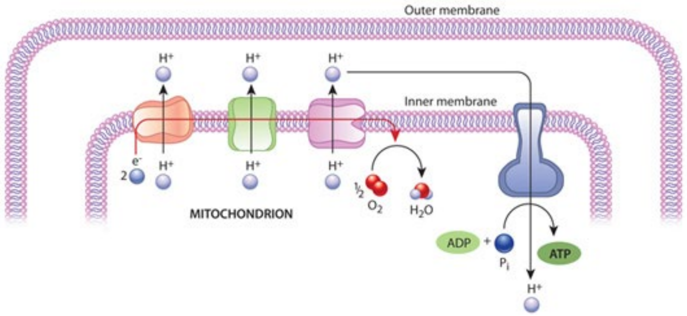


Figure 2 | The cellular power struggle.

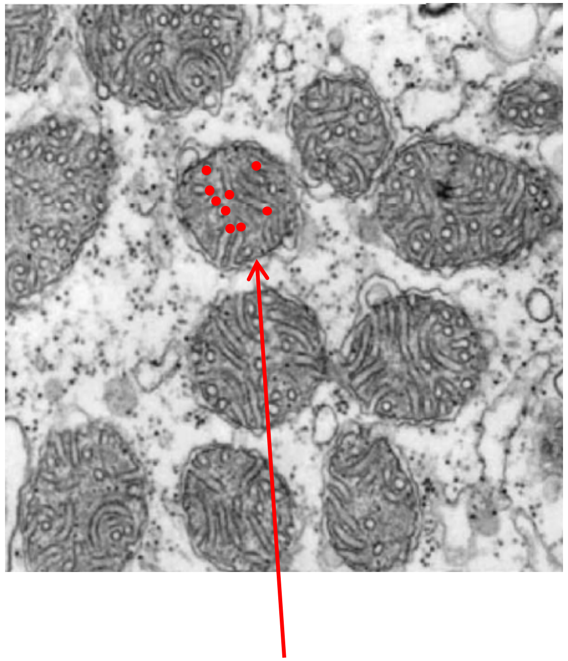


Membrane scaling and prokaryote-eukaryote divide:

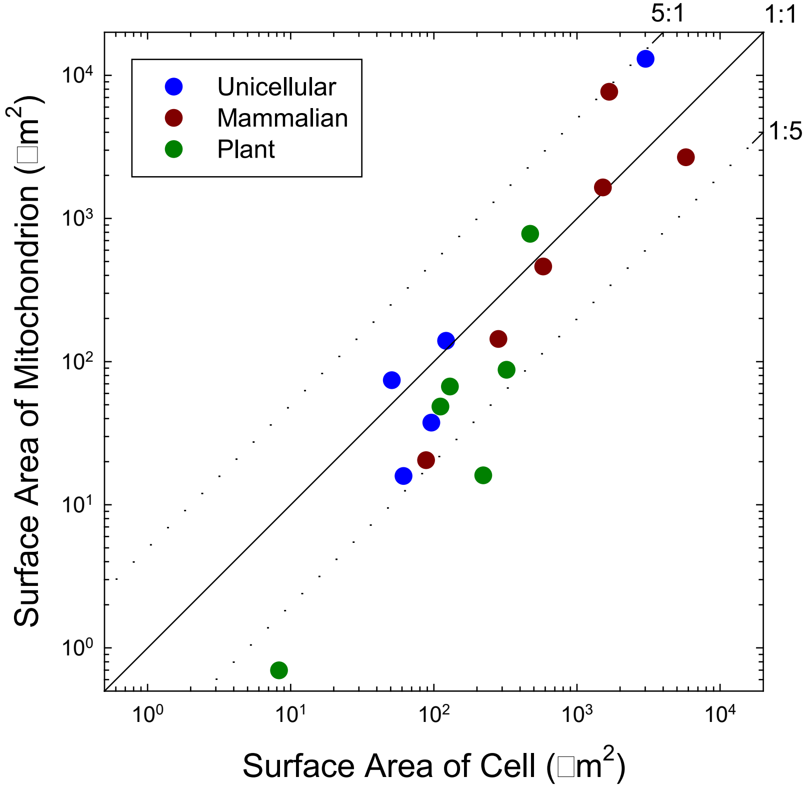
- 10 to 20% of a eukaryotic cell’s total energy budget is associated with membranes, which is comparable to the ~20% composition in bacterial species.
- The cost of synthesizing mitochondrial membranes is ~5% of a eukaryotic cell’s energy budget.
- The total membrane area of mitochondria is not much different than that of the cell surface area.
- The number of ATP synthase complexes and ribosomes in eukaryotic cells is approximately the same as expected for a bacterial cell of comparable volume.

Surface Area of Mitochondria vs. Plasma Membrane

Paramecium mitochondria



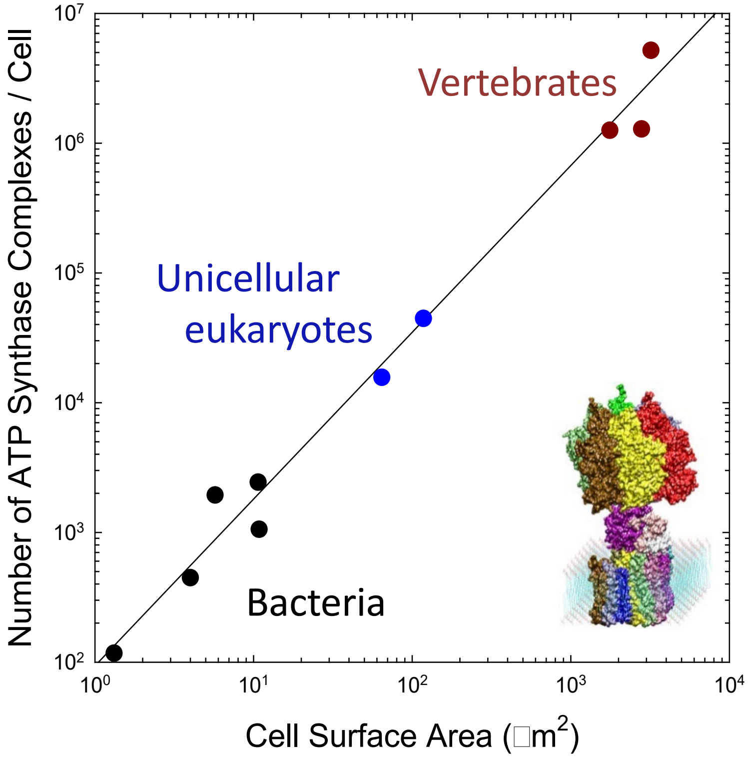
ATP synthase is restricted to the tips of cristae



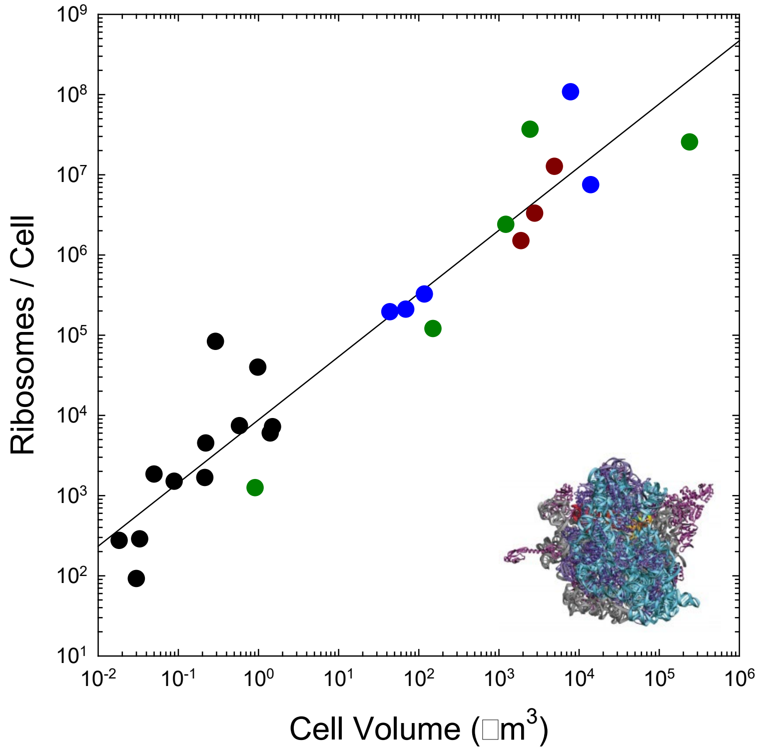
Size-dependent Scaling: Numbers of ATP Synthase Complexes and Ribosomes / Cell

- Continuity of scaling across bacteria and eukaryotes.

$N_{\text{ATP synthase}} = 94S^{1.3}$



$N_{\text{ribosomes}} = 8800V^{0.8}$



Conversion of a Bioenergetic Cost to a Baseline Selective Disadvantage

- Selective disadvantage = difference in rates of increase between two genotypes: $s = r - r'$

- Rate of increase = ln(2) / (cell-division time): $s = \ln(2)\left(\frac{1}{\tau} - \frac{1}{\tau'}\right) \simeq \frac{\ln(2) \cdot \Delta_\tau}{\tau}$.

Noting that $\Delta_\tau \simeq \tau c_T / C_T$ leads to $s \simeq \frac{\ln(2) \cdot c_T}{C_T}$

where (c_T / C_T) is the proportional change in division time.

Across the Tree of Life, the Cost of a Gene Declines with Increasing Organismal Size

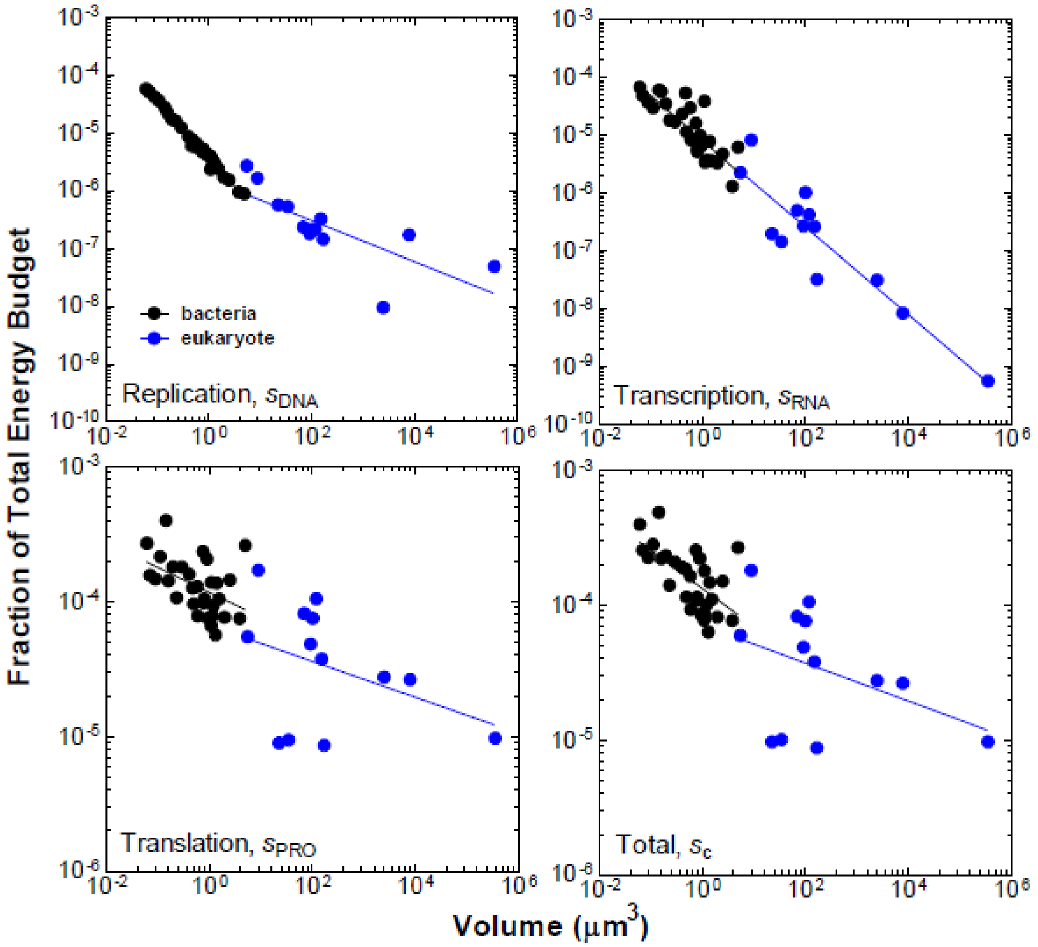
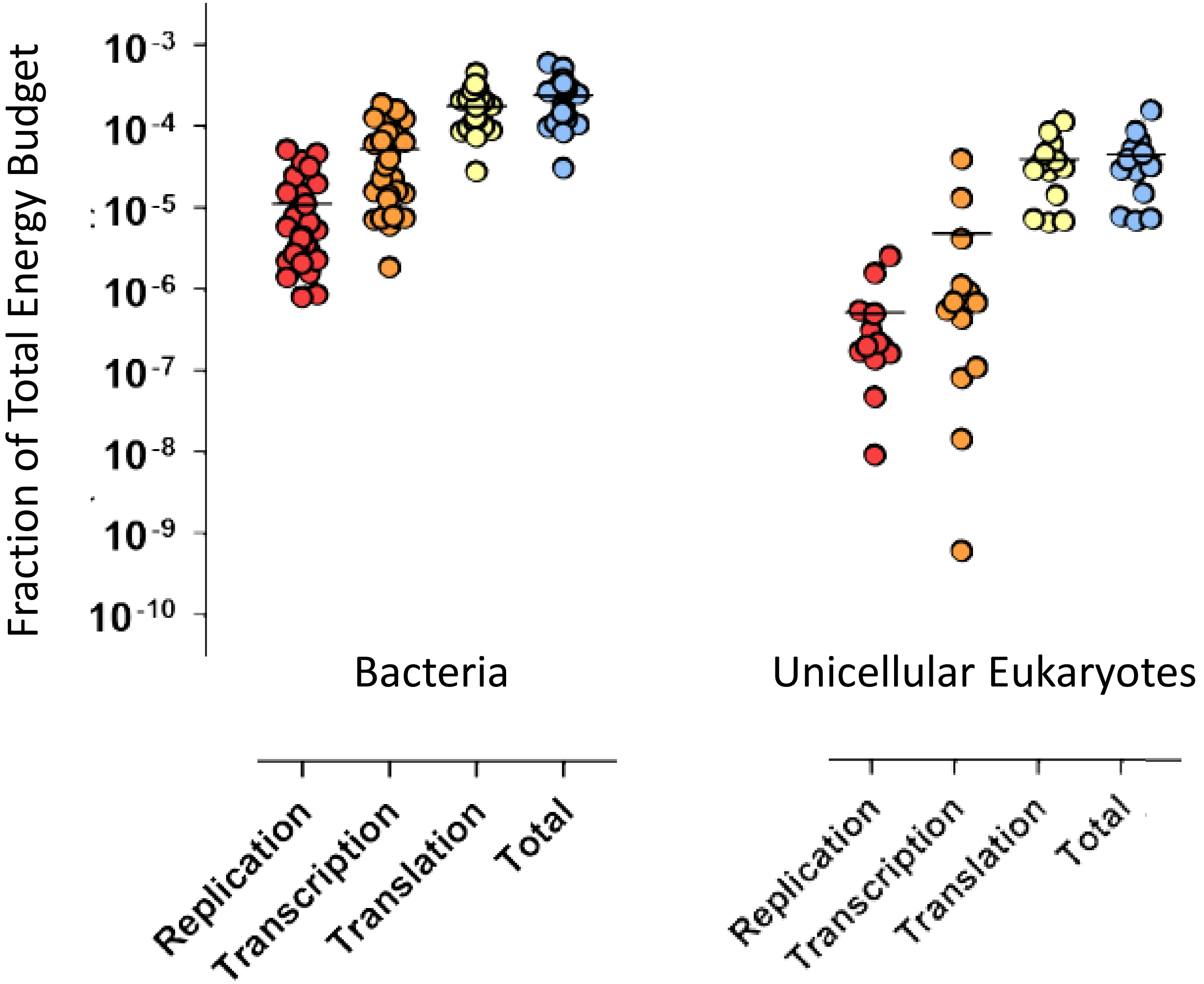
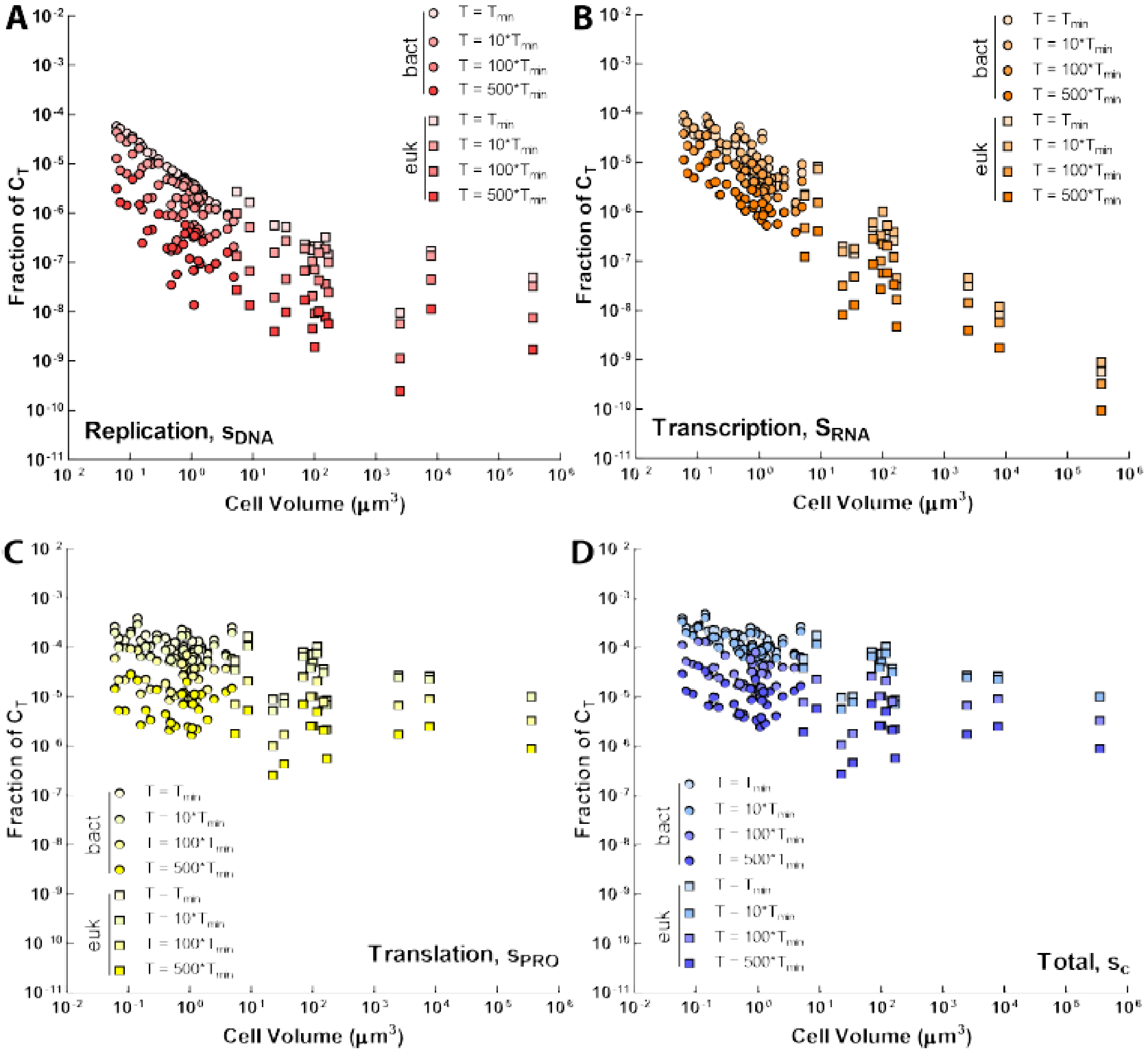
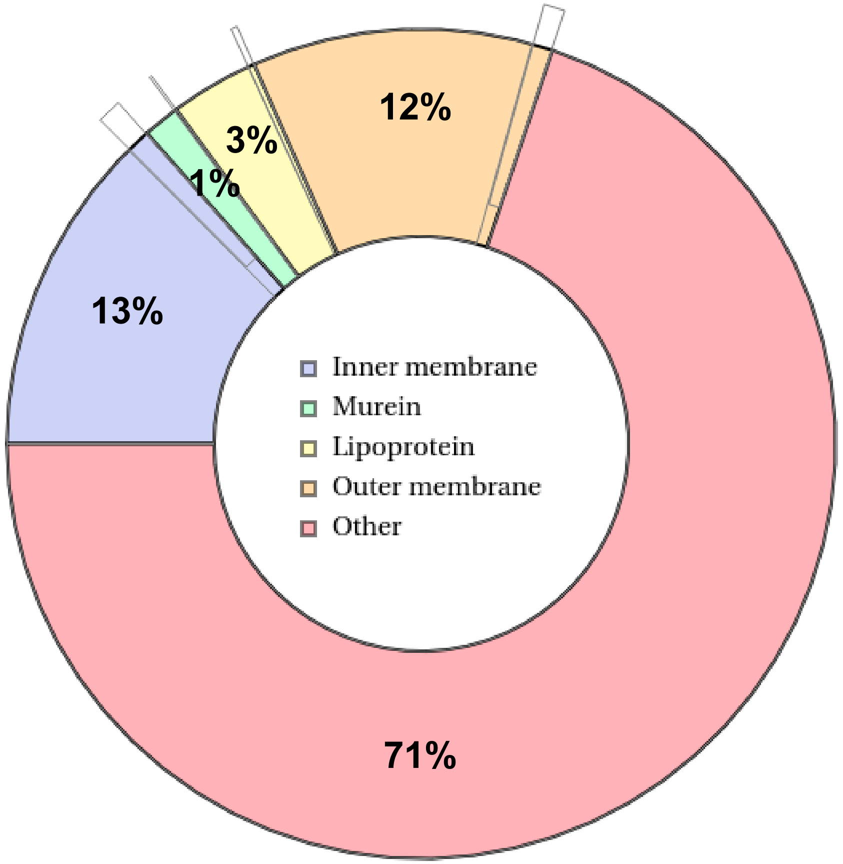


Fig. 4. Fractional costs of average genes in bacteria and unicellular eukaryotes (relative to total cellular energy budgets), subdivided into components at the level of replication, transcription, and translation.

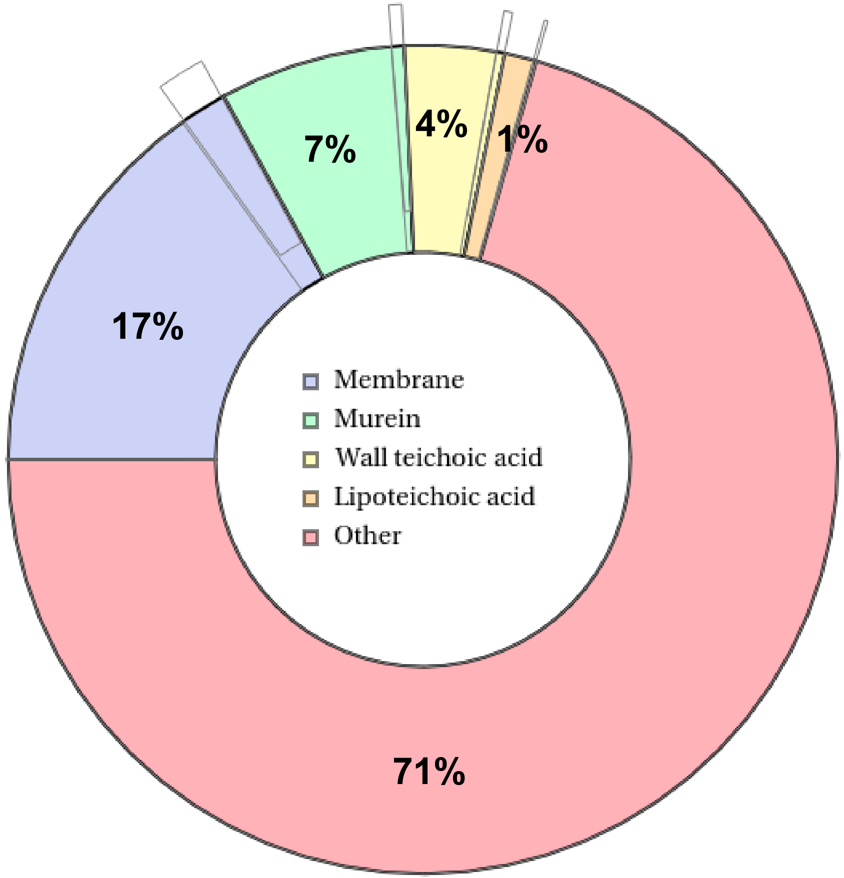

Fraction of Total Energy Budget
10^-3
10^-4
10^-5
10^-6
10^-7
10^-8
10^-9
10^-10
Bacteria
Unicellular Eukaryotes
Replication
Transcription
Translation
Total
Replication
Transcription
Translation
Total



Supplementary Figure 6: Scaling between energy costs, cell volume and division time. A) Replication; B) Transcription; C) Translation; D) Total. The figure shows the same species shown in Figure 4 in the main text. The model in which $\delta(t_n|t_n \geq 1) = \delta_{t_{min}}/t_n$ was used (Equation 9).



Escherichia coli (Gram negative)



Bacillus subtilis (Gram positive)

- Cost of cell wall is less than that of the cell membrane, but still 5 to 10% of total budget.
- In both cases, the total cost of cell exterior is ~30% of the cell’s energy budget.

The Price of Mitochondrial Membranes

Total cost of membranes = (no. of lipid molecules / surface area)
x (cost / lipid molecule) x surface area

$$C_L \simeq (3.08 \times 10^6) \cdot \bar{c}_L \cdot A,$$

Relative to total cellular ATP requirements,
cost of mitochondrial membranes

= $0.05\ V^{0.04} \approx 5\%$ of cell’s energy budget