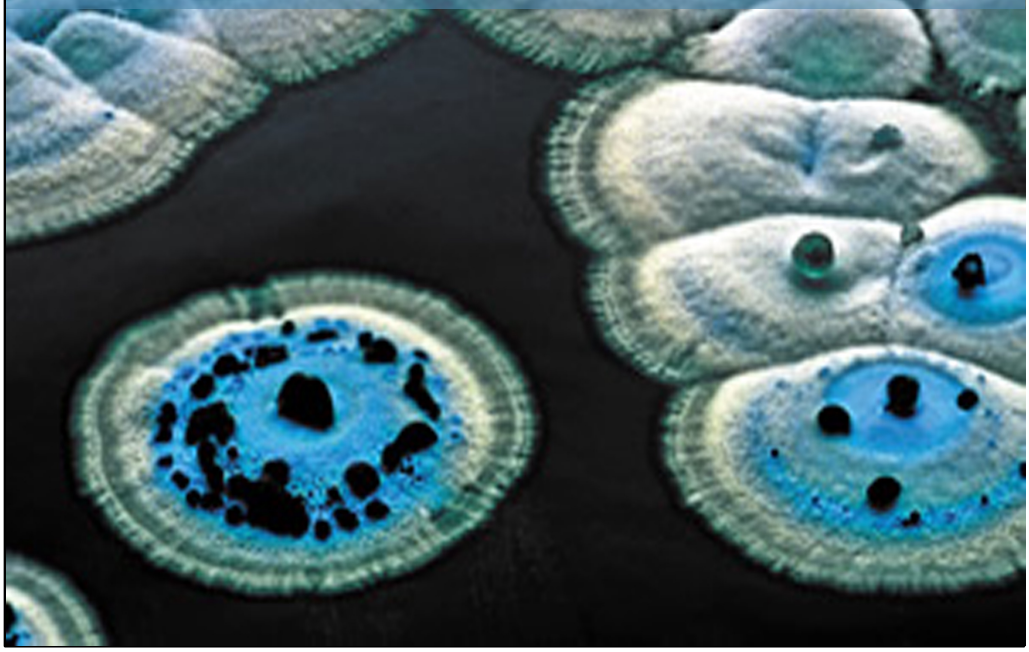
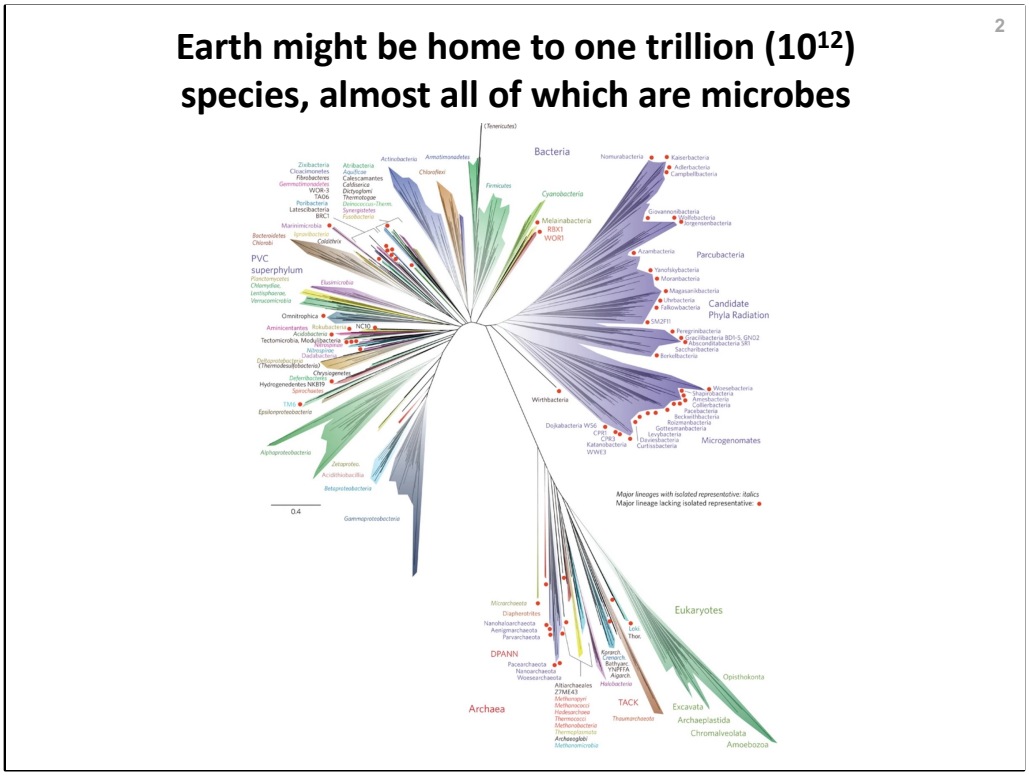
Microbiology (BIO-372)





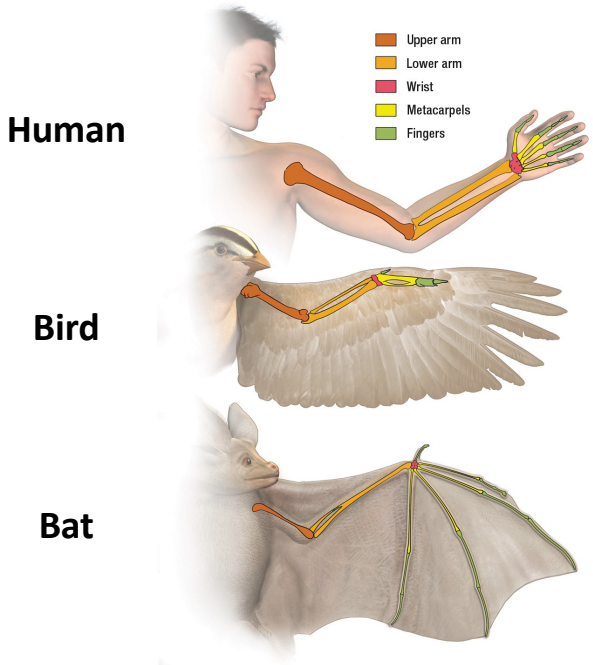
Source: Hug LA et al. (2016) A new view of the tree of life. 1: 16048 PMID: 27572647.

The tree includes 92 named **Bacterial** phyla, 26 **Archaeal** phyla, and all 5 of the **Eukaryotic** supergroups. Major lineages are assigned arbitrary colors and named, with well-characterized lineage names, in italics. Lineages lacking an isolated representative are highlighted with non-italicized names and red dots. Eukaryotic supergroups are noted but not otherwise delineated due to the low resolution of these lineages. The CPR (Candidate Phyla Radiation) phyla are assigned a single color as they are composed entirely of organisms without isolated representatives, and are still in the process of definition at lower taxonomic levels. The PVC superphylum is a superphylum of bacteria named after its three important members: Planctomycetota, Verrucomicrobiota, and Chlamydiota.

Source: Bakalar N (2016) Earth may be home to a trillion species of microbes. *New York Times* Section D, page 4 March 23, 2016.

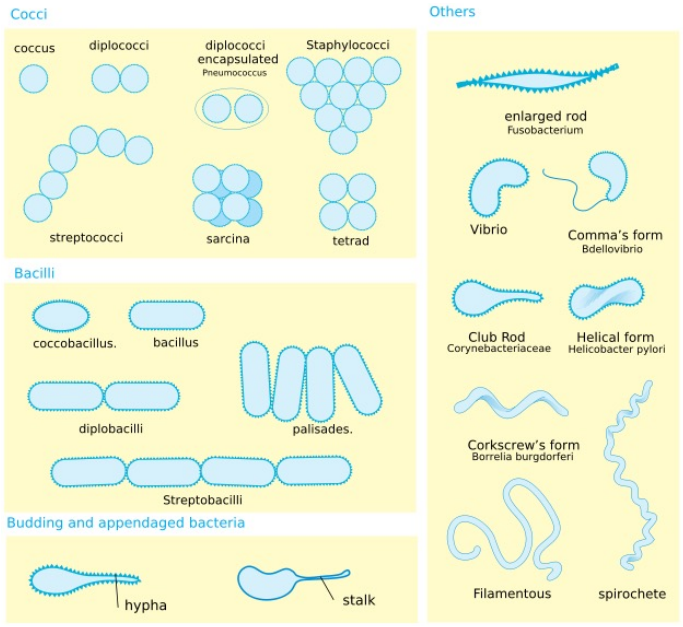
According to a new estimate, there are about one trillion species of microbes on Earth, and 99.999 percent of them have yet to be discovered. As recently as 1998, the number of microbial species was thought to be a few million at most, little more than the number of insect species. But estimates have been growing ever since. Now Kenneth J. Locey and Jay T. Lennon, biologists at Indiana University, have used two techniques to conclude that the number of microbial species is larger than any previous researchers have imagined (Locey KJ et al. 2016 Scaling laws predict global microbial diversity *Proc Natl Acad Sci USA* 113:5970-5975 PMID: 27140646). The first method extrapolates from the available data for microbes, based not on individual organisms but on samples of DNA. “So if we say a million, we mean a million pieces of DNA that we think belong to different organisms and among them represent different species,” Dr. Locey said. The second approach was to use a well-known model of biodiversity as a basis for making predictions. “If you know the number of individuals, you can predict the most abundant species,” Dr. Locey said. ”So we used those two inputs — the number of individuals over all and the number of individuals belonging to the most abundant species — to estimate the total number of species.” The two methods provided numbers that matched: Earth contains 10^11 to 10^12 species of microbes. “We think our approach was rigorous in that we used a theoretical prediction and a statistical estimate,” he said. “We ended up with intersecting predictions based on different methods.” Finding the number of species has broader implications, Dr. Locey said. “How many species could have actually evolved in four billion years? What are the upper constraints of evolution on Earth? How many species have evolved, how many could have evolved? “As far as I know, no one has approached those questions. We’re very far away from discovering what’s really out there.”

Morphology is not a reliable guide to phylogeny



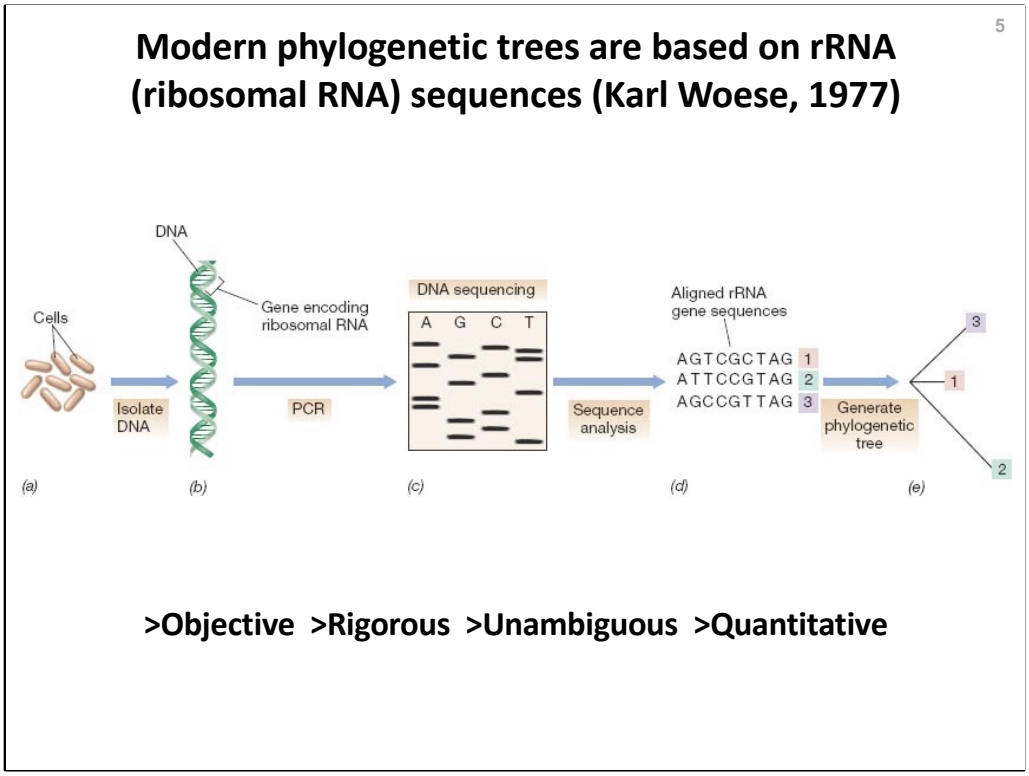
Birds have wings. Bats have wings. Humans do not have wings. So, bats must be more closely related to birds than to humans, right? Sorry, wrong! This is a clear case of convergent evolution. Wings evolved more than once. In the evolutionary lineages leading to birds and bats, wings evolved independently. Humans and bats are both mammals; they are more closely related to each other than they are to birds.

Morphology is not a reliable guide to phylogeny



Bacteria come in all shapes and sizes, as we will see later in this course. However, neither of these parameters (shape or size) is a reliable guide to the evolutionary relationship between different species of bacteria. Bacteria with a similar shape may be only distantly related. Conversely, bacteria with different shapes (e.g., coccus versus bacillus) may be closely related.

Question: if size and shape are not reliable guides to the relatedness between different bacterial species, how can we build phylogenetic "trees" for bacteria? Answer: by basing our phylogenetic "trees" on bacterial ribosomal RNA (rRNA) gene sequencing, as shown on the next slide.

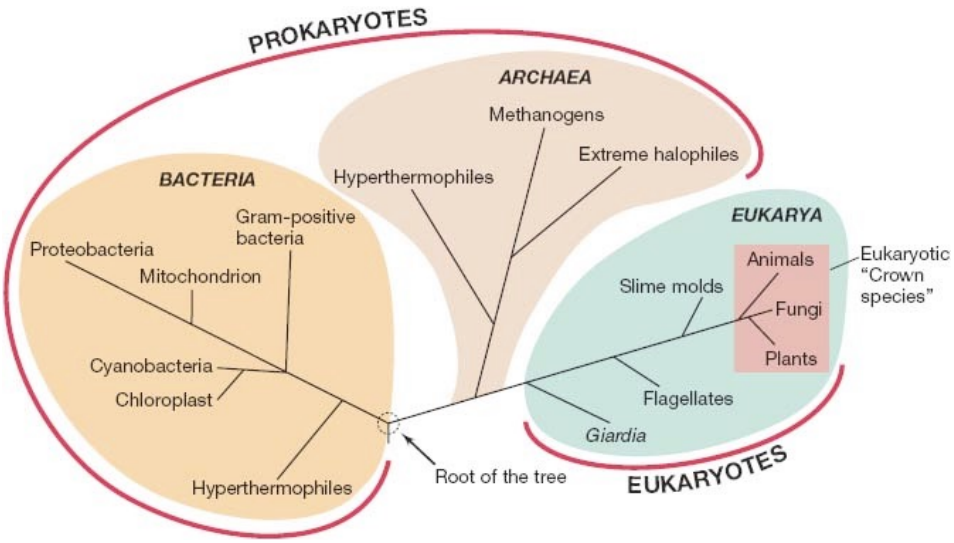


Source: Madigan MT, Martinko JM, Stahl DA, Clark DP (2012) *Brock Biology of Microorganisms [13th edition]*. Pearson Education Inc., San Francisco.

Figure 2.16. Ribosomal RNA (rRNA) gene sequencing and phylogeny. (a) Cells are broken open. (b) The gene encoding rRNA is isolated, and many identical copies are made by the technique called the polymerase chain reaction. (c, d) The gene is sequenced, and the sequence obtained is aligned with other rRNA gene sequences. A computer algorithm makes pairwise comparisons and generates a phylogenetic tree (e) that depicts the differences in rRNA sequence between the organisms analyzed. In the example shown, the sequence differences are as follows: organism 1 versus organism 2, three differences; 1 versus 3, two differences; 2 versus 3, four differences. Thus organisms 1 and 3 are closer relatives than are 2 and 3 or 1 and 2.

Carl Richard Woese (born July 15, 1928; died December 30, 2012) was an American microbiologist and biophysicist. Woese is famous for defining the Archaea (a new domain or kingdom of life) in 1977 by phylogenetic taxonomy of 16S ribosomal RNA, a technique pioneered by Woese that revolutionized the discipline of microbiology. He was also the originator of the RNA world hypothesis in 1977, although not by that name.

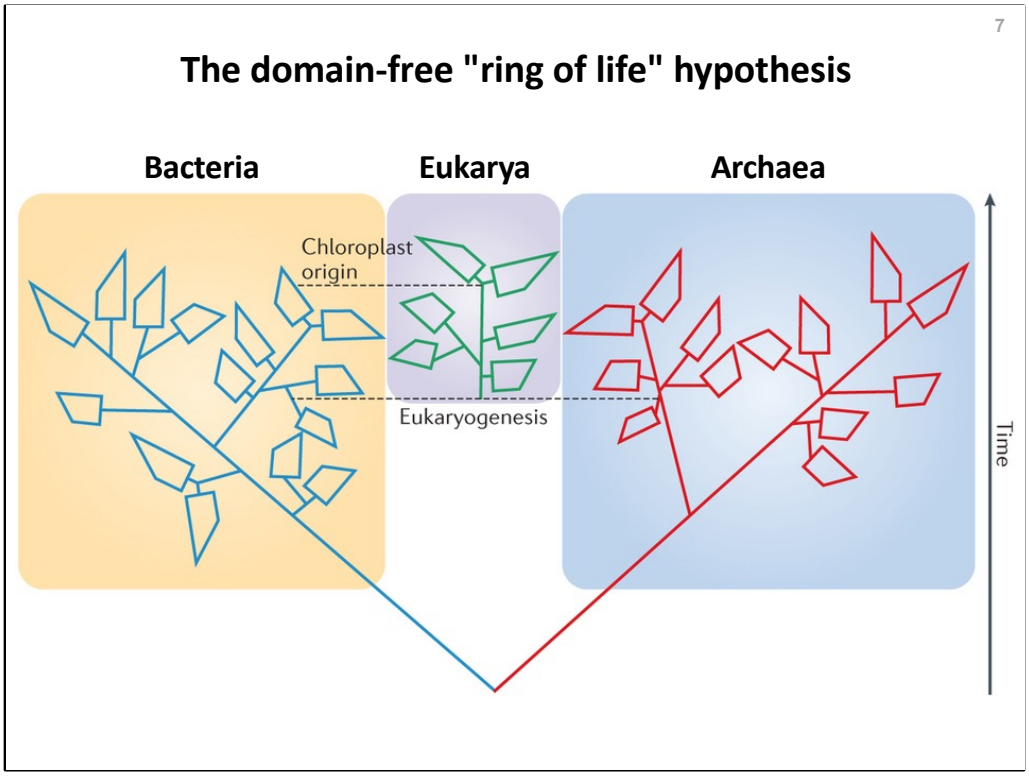
Phylogenetic "tree of life" based on rRNA: microbes account for almost all of the diversity of life on Earth



Three domains of life: Bacteria, Archaea, Eukarya

Source: Madigan MT, Martinko JM, Stahl DA, Clark DP (2012) *Brock Biology of Microorganisms [13th edition]*. Pearson Education Inc., San Francisco.

Figure 2.17. The phylogenetic tree of life as defined by comparative ribosomal RNA gene sequencing. The tree shows the three major domains of organisms (*Bacteria*, *Archaea*, *Eukarya*) and a few representative groups in each domain. All *Bacteria* and *Archaea* and most *Eukarya* are microscopic organisms. Only plants, animals, and fungi contain macro-organisms. Phylogenetic tree of each domain can be found in Figures 2.19, 2.28, and 2.32. LUCA, last universal common ancestor.

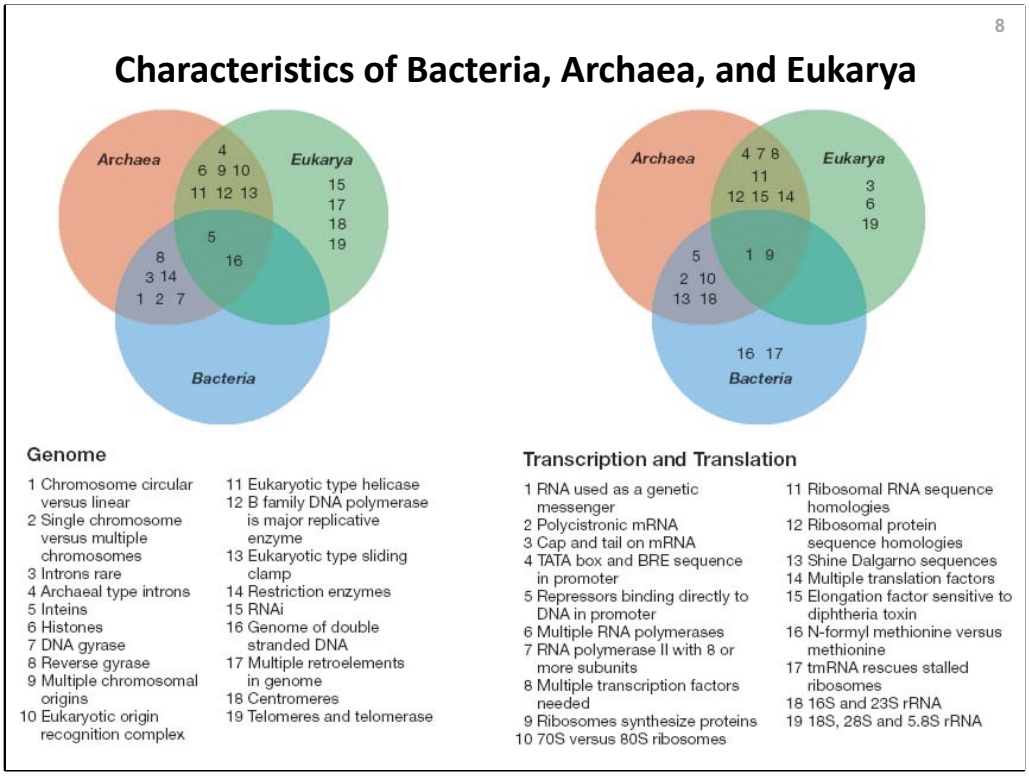


Source: McInerney JO, O'Connell MJ, Pisani D (2014) The hybrid nature of the Eukaryota and a consilient view of life on Earth. *Nature Rev Microbiol* 12(6): 449-455 PMID: 24814066.
Source: López-García P, Moreira D (2020) The Syntrophy hypothesis for the origins of eukaryotes revisited. *Nature Microbiol* 5(5): 655-667 PMID: 32341569.

Figure 2. Schematic representation of the flow of genetic material from the two major prokaryotic groups into the base of the eukaryotes and the separate flow of genetic material from cyanobacteria into plastid-containing eukaryotes.

There is a considerable amount of consilience between the phylogenetic, cell biology, population biology, biochemical and paleontological evidence in favour of a scenario in which a merger of just two prokaryotes - one eubacterial and one archaebacterial - formed the eukaryotic cell relatively late in the history of life. The eubacterial ancestor subsequently became the **mitochondrion**. Many of its genes were transferred to the nucleus as a consequence of selective pressures against the replication of large amounts of DNA, the protein products of which were not needed in such large quantities. Unfortunately, the consideration that this was the relationship of a 'host' and a 'symbiont' has led to the denigration of the role of the eubacterial component of the eukaryotic cell in favor of a focus on the archaebacterial component. However, in most eukaryotes (including animals), the eubacterial gene components are numerically greater and span all major functions, including both informational and operational functions. Therefore, a proper view of eukaryotic origins needs to take account of both ancestries of eukaryotic genes.

The chimeric nature of the eukaryotes has profound implications for our understanding of the nature of life on Earth. If the Eukaryota have hybrid origins, then strictly speaking, both the extant Eubacteria and Archaebacteria are paraphyletic, whereas the Eukaryota, as a lineage, is monophyletic, despite its symbiogenic ancestry. The ring of life hypothesis has been described by some experts as a two-domain hypothesis. This characterization is inherently flawed owing to the chimeric nature of the eukaryotes. Given that the current evidence suggests that there is a single highest-level category of life with two (ancient) primary groups (Eubacteria and Archaebacteria) followed by a secondary group of more recent origins (Eukaryota), we advocate a 'domain-free' view of life on Earth. It is time to get used to depicting life on this planet as a graph with rings.



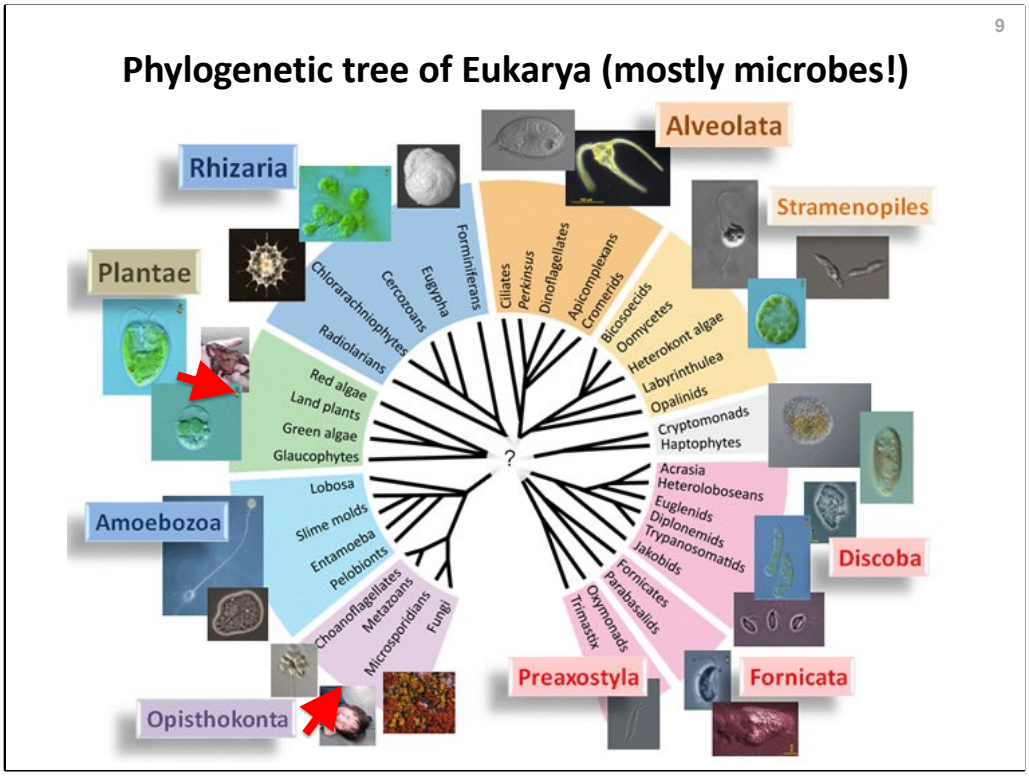
Source: Madigan MT, Martinko JM, Stahl DA, Clark DP (2012) *Brock Biology of Microorganisms [13th edition]*. Pearson Education Inc., San Francisco.

Figure 7.5. Molecular features of the three domains. Venn diagrams show which features are shared by the domains and which are unique. (a) Genomic features. (b) Features of transcription and translation. Before the advent of rRNA-based phylogeny in the late 1970s (pioneered by Carl Wose), no distinction was made between *Bacteria* and *Archaea*.

You do not need to memorize the information on this slide! The main concepts that you should take from this slide are that, in terms of fundamental biological processes (like genome structure and function, RNA transcription, protein translation):

1. There is a lot of overlap between Archaea and Bacteria that is not shared with Eukarya.
2. There is a lot of overlap between Archaea and Eukarya that is not shared with Bacteria.
3. There is very little overlap between all three Domains: Archaea, Bacteria, Eukarya.
4. There is no overlap between Bacteria and Eukarya except for those few traits that are also shared with Archaea.

Again, it is worth noting that morphology is not a reliable guide to phylogeny! Under the microscope, it is difficult or impossible to distinguish between Archaeal cells and Bacterial cells. However, at the molecular level, they are radically different organisms that diverged from each other **several billion years** ago! Specifically: the timelines of divergence suggest that Bacteria diverged from a common ancestral species between 2.5 and 3.2 billion years ago, whereas the Archaea diverged earlier, between 3.1 and 4.1 billion years ago.

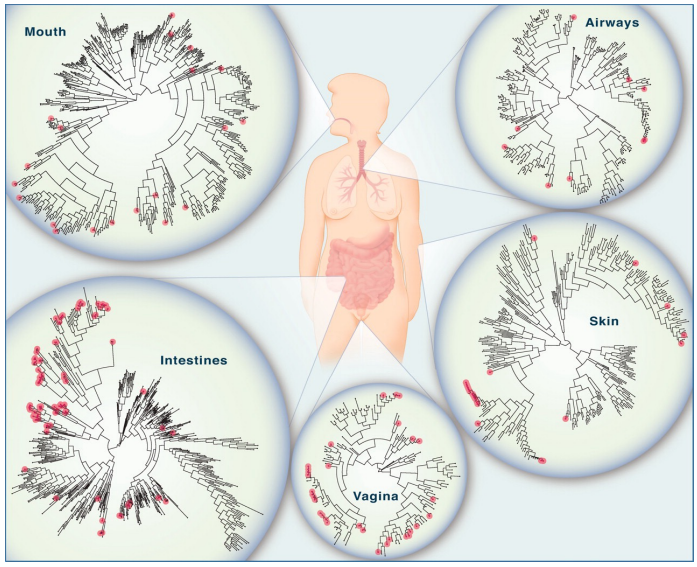


Source: Madigan MT, Martinko JM, Stahl DA, Clark DP (2012) *Brock Biology of Microorganisms [13th edition]*. Pearson Education Inc., San Francisco.

Figure 2.32. Phylogenetic tree of some representative *Eukarya*. This tree is based only on comparisons of genes encoding ribosomal RNA (rRNA). Some early-branching species of *Eukarya* lack organelles other than the nucleus. Note that plants and animals branch near the apex of the tree. Not all known lineages of *Eukarya* are depicted.

Groups containing multicelluar organisms (animals, plants, fungi) are indicated by red arrows.

Human microbiome: your body contains 10X more prokaryotic cells than eukaryotic cells…



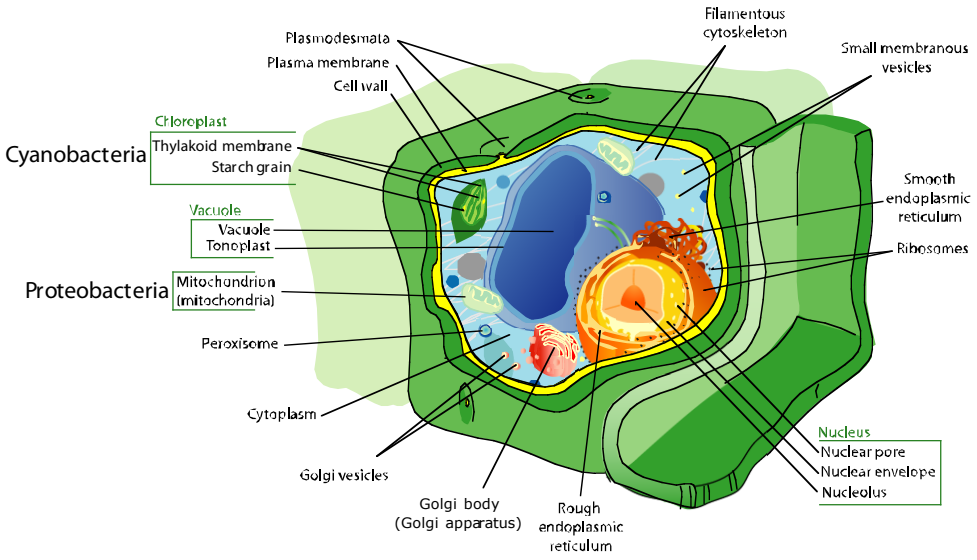
…and 150X more non-redundant prokaryotic genes!

Source: Lee YK, Mazmanian SK (2010) Has the microbiota played a critical role in the evolution of the adaptive immune system? *Science* 330(6012): 1768-1773 PMID: 21205662.

Figure 1. The microbiome of various anatomical locations of the human body. Numerous bacterial species colonize the mouth, upper airways, skin, vagina, and intestinal tract of humans. The phylogenetic trees show the speciation of bacterial clades from common ancestors at each anatomical site. Although the communities in different regions of the body share similarities, they each have a unique site-specific "fingerprint" made of many distinct microbes. Each site has a very high level of diversity, as shown by the individual lines on the dendrograms. Data are from the NIH-funded Human Microbiome Project; circles represent bacterial species whose sequences are known.

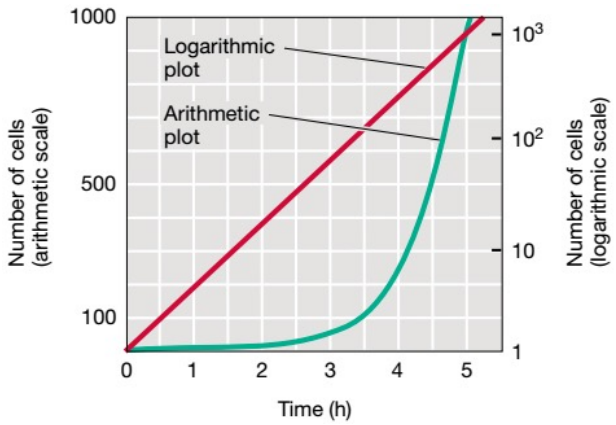
Some interesting facts:
1. The human body contains 10X more microbial cells (about 10^14 = 100 trillion) than human cells (about 10^13 = 10 trillion).
2. The human microbiome contains more than 150 times as many non-redundant genes as in the human genome.
3. The aggregate human microbiota likely contains 1,000 to 1,150 bacterial species (spread among all people sampled), with each person harboring about 160 bacterial species on average (actually, these numbers are badly out of date already, and the "newest numbers" are much, much larger!).

Mitochondria and chloroplasts in eukaryotic cells originated from endosymbiotic bacteria



Chloroplasts evolved from endosymbiotic cyanobacteria. **Mitochondria** evolved from endosymbiotic alpha-proteobacteria.

The "miracle" of exponential growth



$$N = N_0 e^{kt}$$

This is the equation used to calculate the exponential growth of cell populations. You should recognize this equation because it is basically the same equation that is used to calculate radioactive decay, which is also an exponential process. The only difference is that the "decay rate constant" (-k) is negative whereas the "growth rate constant" (k) is positive.

N = number of cells after some time interval (t)
N_0 = number of cells at t = 0
e = base of the natural logarithm (~ 2.718)
k = instantaneous growth rate constant
t = time elapsed

The exponential growth equation is a "primary concept" – you should memorize it and you should feel comfortable using it.

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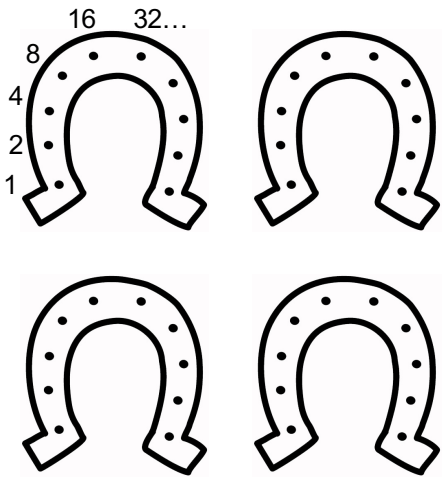
You are paying a farrier (maréchal-ferrant) to put new shoes on your horse. There are 10 nails per shoe. He makes two offers: **(a)** You pay 10,000 Sfr for the job, or **(b)** You pay 1 Sfr for the 1st nail, 2 Sfr for the 2nd nail, 4 Sfr for the 3rd nail, 8 Sfr for the 4th nail…(etc.). Which offer is the best deal for you?

A. (a) costs more than (b)

B. (b) costs more than (a)

C. (a) and (b) are equal

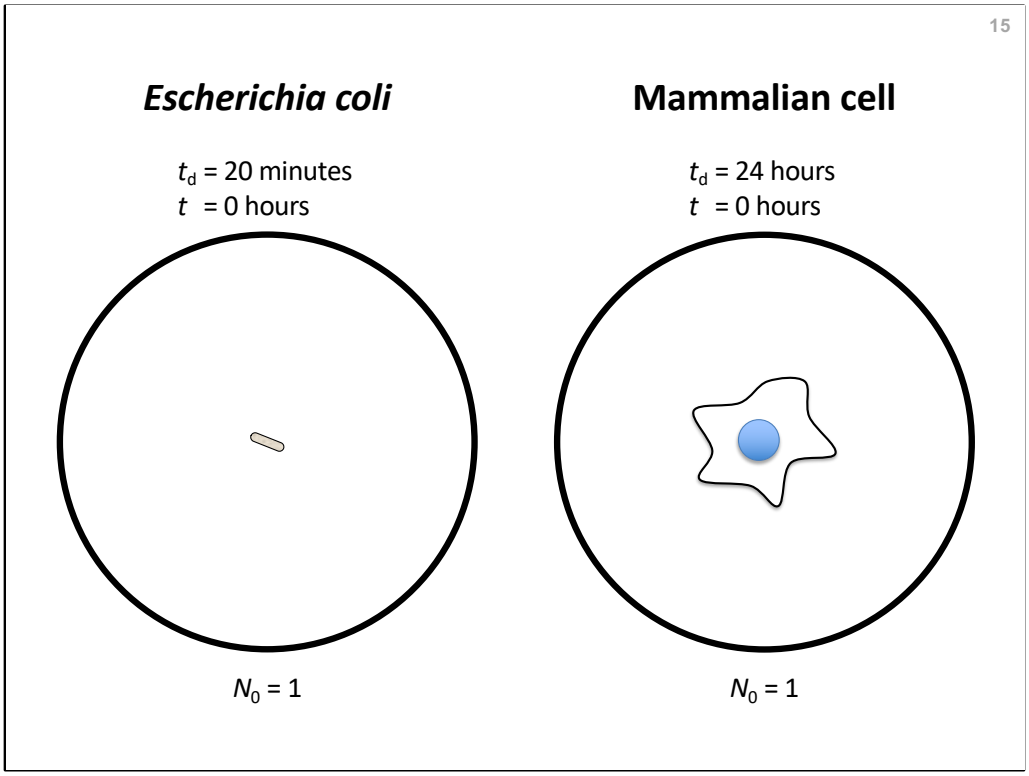


Answer: (B)

Indeed, (B) would equal about 1,100,000,000,000 or 1.1 & 10^{12} Sfr! That's **1.1 trillion Sfr**!

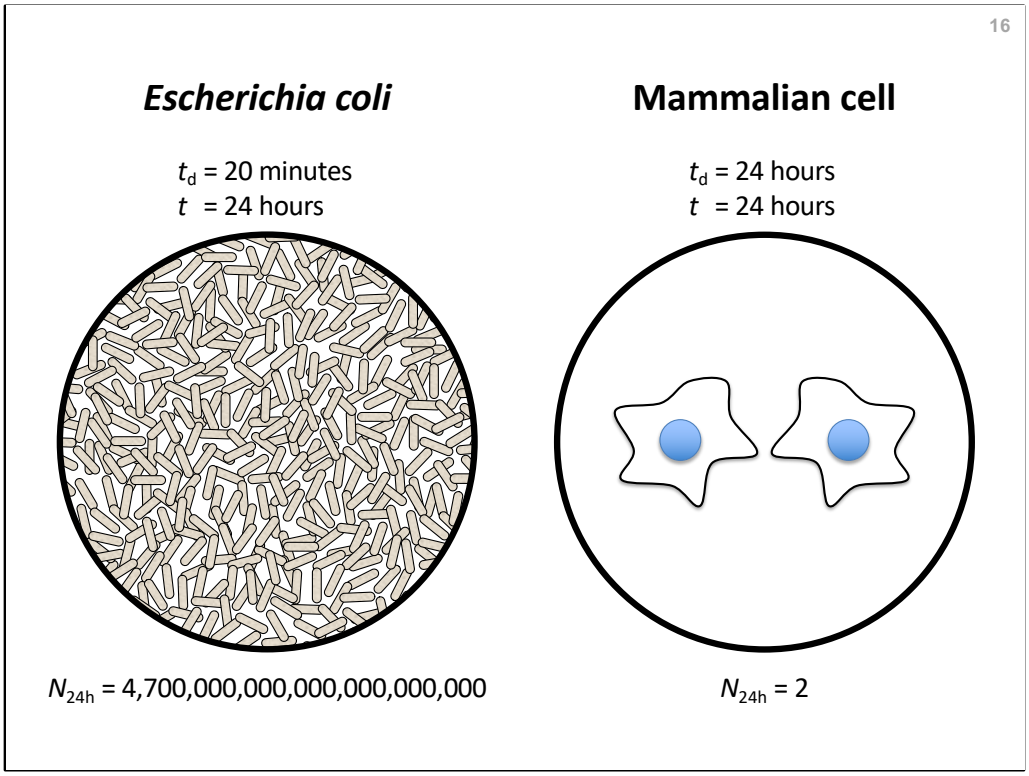
How to calculate (k), the "instantaneous growth rate constant" of a cell population

1. Measure (experimentally) the culture's doubling time (t_d)
2. Rearrange the growth equation to $k = 1/t * \ln(N/N_0)$
3. Use the special case where $t = t_d$ so $k = 1/t_d * \ln(N/N_0)$
4. If $t = t_d$ then $N = 2 * N_0$…
5. …so $k = 1/t_d * \ln(2)$
6. Et voilà…



t_d = doubling time of a bacterial culture (i.e., the time required to double the cell number or cell mass).
t = time elapsed since the culture was initiated.
N_0 = the number of cells in the culture at t = 0 hours.

In a steady-state culture, the doubling time for cell number and the doubling time for cell mass are the same.



t_d = doubling time of a bacterial culture (i.e., the time required to double the cell number or cell mass).
t = time elapsed since the culture was initiated.
N_{24h} = the number of cells in the culture at t = 24 hours.

The mass of an *E. coli* cell is 6.65×10^{-13} grams; for simplicity, let's call it 10^{-12} grams (1 picogram).
If the cellular doubling time of *E. coli* (T_d) is 20 minutes (or 0.333... hours), and we start with a culture of one cell, then:
After 24 hours the number of cells will be about $4.7*10^{21}$ cells with a total mass of $4.7*10^{9}$ grams.
After 48 hours the number of cells will be about $2.2*10^{43}$ cells with a total mass of $2.2*10^{31}$ grams.
Note that if growth were linear rather than exponential, then:
After 24 hours the number of cells will be just 73
After 48 hours the number of cells will be just 145!

The mass of the Earth is $5.98*10^{27}$ grams; for simplicity, let's call it $6*10^{27}$ grams.

So if we start a culture with one *E. coli* cell, which grows thereafter with a doubling time of 20 minutes, the total cell mass after 48 hours of growth would be equivalent to more than 3,000 Earths!!! Of course, this doesn't actually happen because cells grow only until they exhaust the nutrients available in their immediate environment, at which point they make the transition from the "exponential phase" of growth to the "stationary phase" of growth.

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Size and volume of prokaryotic cells

Organism	Characteristics	Size (μm)	Volume (μm^3)	*E. coli* Volumes
Thiomargarita namibiensis	Sulfur chemolithotroph	750	200,000,000	100,000,000
Epulopiscium fishelsoni	Chemoorganotroph	80 X 600	3,000,000	1,500,000
Achromatium oxaliferum	Sulfur chemolithotroph	35 X 95	80,000	40,000
Lyngbya majuscula	Cyanobacterium	8 X 80	40,000	20,000
Human lung macrophage	—	~20 (sphere)	5,000	2,500
Thiovulum majus	Sulfur chemolithotroph	18	3,000	1,500
Staphylothermus marinus	Hyperthermophile	15	1,800	900
Titanospirillum velox	Sulfur chemolithotroph	5 X 30	600	300
Human red blood cell	—	~6-8 (disk)	100	50
Magnetobacterium bavaricum	Magnetotactic	2 X 10	30	15
Escherichia coli	**Chemoorganotroph**	**1 X 2**	**2**	**1**
Pelagibacter ubique	Chemoorganotroph	0.2 X 0.5	0.014	0.014
Mycoplasma pneumoniae	Pathogenic	0.2	0.005	0.0025

Source: Madigan MT, Martinko JM, Stahl DA, Clark DP (2012) *Brock Biology of Microorganisms [13th edition]*. Pearson Education Inc., San Francisco.

Table 3.1. Cell size and volume of some prokaryotic cells, from the largest to the smallest.

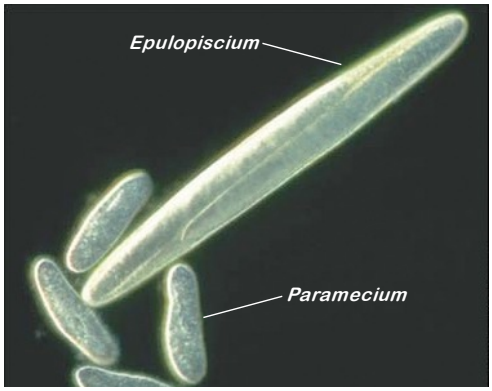
A spherical eukaryotic cells with a radius of 10 μm would have a volume of about 4,200 μm^3. A spherical eukaryotic cells with a radius of 20 μm would have a volume of about 33,500 μm^3.

Sizes of various human cell types for comparison (μm^3):

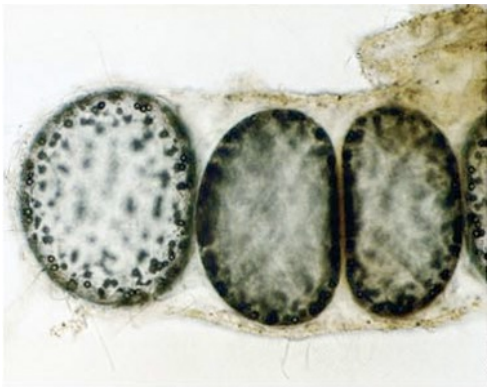
Sperm cell	30
Red blood cell	100
Lymphocyte	130
Neutrophil	300
Beta cell	1,000
Enterocyte	1,400
Fibroblast	2,000
HeLa cell (cervix)	3,000
Hair cell (ear)	4,000
Osteoblast	4,000
Macrophage (alveolar)	5,000
Cardiomyocyte	15,000
Megakaryocyte	30,000
Fat cell	600,000
Oocyte	4,000,000

Not all prokaryotic cells are 'micro' cells!

(a) ***Epulopiscium fishelsoni***
(surgeonfish symbiont)
80 µm X 600 µm

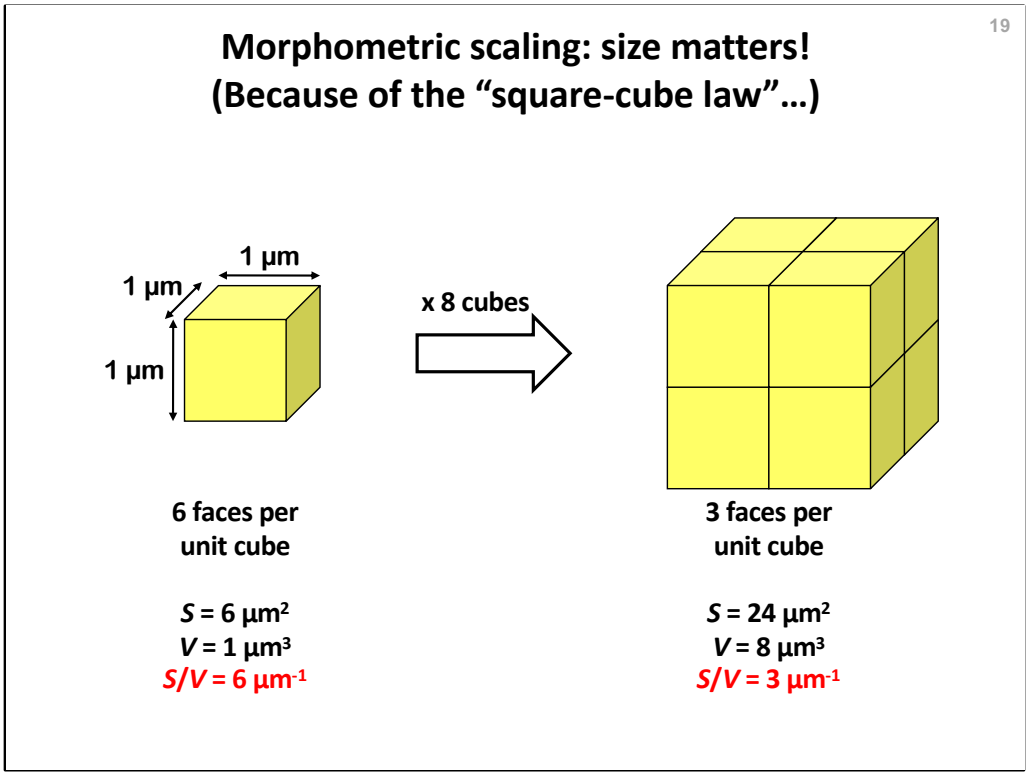


(b) ***Thiomargarita namibiensis***
(sulfur chemolithotroph)
750 µm diameter



Source: Madigan MT, Martinko JM, Stahl DA, Clark DP (2012) *Brock Biology of Microorganisms [13th edition]*. Pearson Education Inc., San Francisco.

Figure 3.2. Some very large prokaryotes. **(a)** Dark-field photomicrograph of a giant prokaryote, the surgeonfish symbiont ***Epulopiscium fishelsoni***. The rod-shaped *E. fishelsoni* cell in this field is about 600 µm (0.6 mm) long and 80 µm wide and is shown with three cells of the protist (eukaryote) *Paramecium*, each of which is about about 150 µm long. *E. fishelsoni* is a member of the Bacteria and phylogenetically related to *Clostridium* species. **(b)** ***Thiomargarita namibiensis***, a large sulfur chemolithotroph (phylum Proteobacteria of the Bacteria) and currently the largest known prokaryote. Each ovoid-shaped cell can be up to 750 µm in diameter.

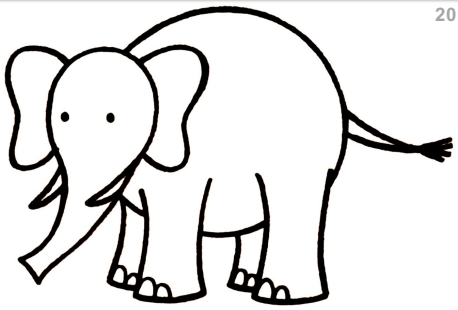


Imagine a cube-shaped cell that measures 1 micron on each side. By definition, a cube has 6 faces. The surface area (*S*) of this small cell is 6 microns squared and the volume (*V*) is 1 micron cubed so the ratio of surface area to volume is 6. Now imagine building a large cell of the same shape by putting together 8 of these "unit masses". The surface area (*S*) of this large cell is 24 microns squared and the volume (*V*) is 8 microns cubed so the ratio of surface area to volume is 3. In other words, the small cell has an *S*/*V* ratio twice that of the large cell. This shrinkage of the *S*/*V* ratio as the object gets larger can be explained both mathematically and geometrically. Mathematically, because as an object gets larger while maintaining the same shape the surface area increases as the square of the characteristic length while the volume increases as the cube of the characteristic length. Geometrically, because as the object gets larger by adding unit masses, more and more "faces" of the unit masses are "hidden" inside the object where they no longer contribute to the surface area; thus, in the small cell on the left each unit mass contributes 6 faces to the surface area, whereas in the large cell on the right each unit mass contributes only 3 faces to the surface area.

20

Drawings are not to scale! ☺

Etruscan shrew (1.8 grams) consumes the equivalent of 300% of its body mass daily



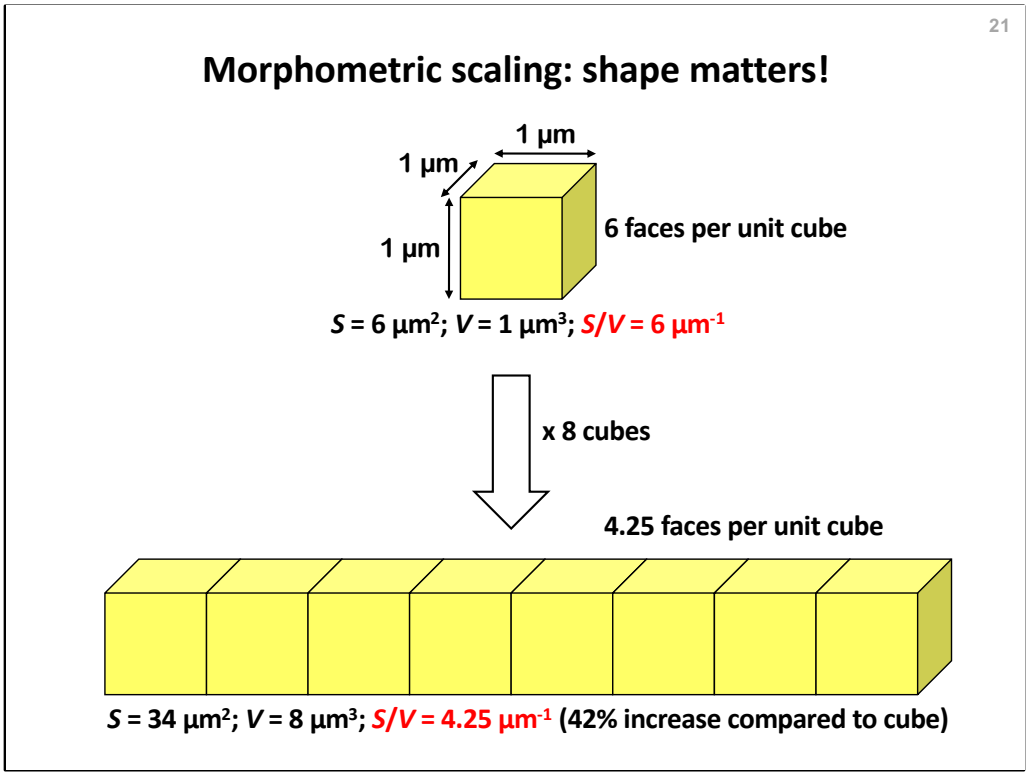
Elephant (5,500 kilograms) consumes the equivalent of 5% of its body mass daily

A. Shrew food is less nourishing than elephant food.

B. Shrew metabolism is less efficient than elephant metabolism.

C. Shrews lose body heat faster than elephants.

Answer: (C)



Imagine a cube-shaped cell that measures 1 micron on each side. By definition, a cube has 6 faces. The surface area (*S*) of this small cell is 6 microns squared and the volume (*V*) is 1 micron cubed so the ratio of surface area to volume is 6. Now imagine building a large cell of a different shape (elongated like a typical rod-shaped bacterial cell) by putting together 8 of these "unit masses". The surface area (*S*) of this large cell is 34 microns squared and the volume (*V*) is 8 microns cubed so the ratio of surface area to volume is 4.25. In other words, the small cell has an *S*/*V* ratio roughly 1.412 times that of the large cell.

Compare this example with the example on the previous slide. On the previous slide the small and large cells have the same shape whereas on this slide the small and large cells have different shapes. On both slides, the "unit mass" of the small cell contributes 6 faces to the surface area. On the previous slide, the "unit masses" of the large cell each contribute only 3 faces to the surface area because 3 faces of each "unit mass" are "hidden" inside the object. On this slide, the "unit masses" of the large cell each contribute 4 faces to the surface area (or 5 faces for the two "unit masses" at the ends of the cell) while 2 faces are "hidden" inside the object (or 1 face for the two "unit masses" at the ends of the cell).

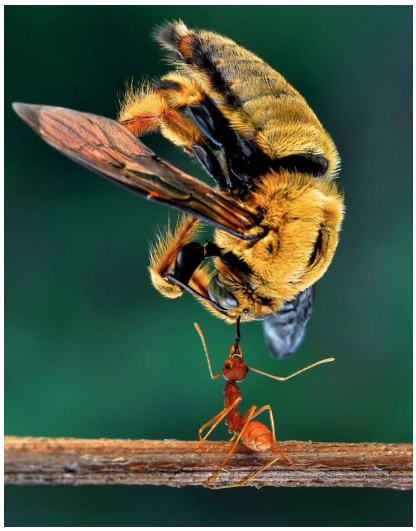
22

Ants are “stronger” than humans (in proportion to their size) because:

A. Ants have more muscle mass than humans do (relative to body mass).

B. Ant muscles are stronger than human muscles (per gram of muscle mass).

C. Ants are smaller than humans.

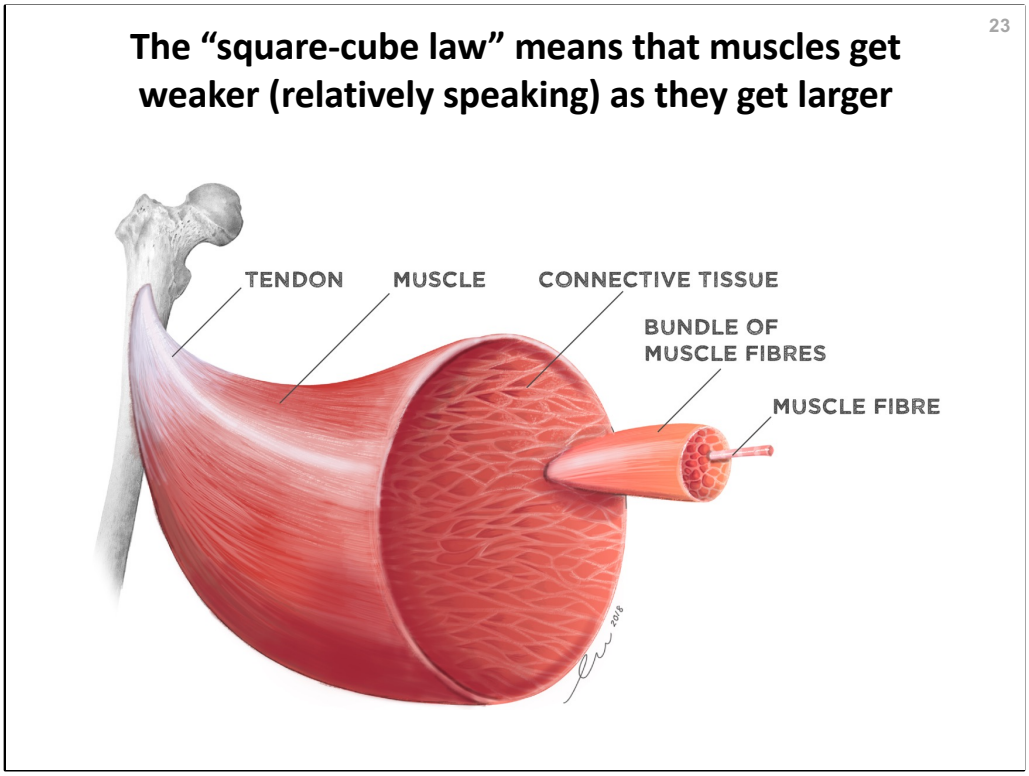


Answer: (C)

Source: https://engineering.osu.edu/news/2014/02/study-ants-remarkable-strength-may-lead-powerful-micro-sized-robots
Source: Nguyen V, Lilly B, Castro C (2014) The exoskeletal structure and tensile loading behavior of an ant neck joint. J Biomech 47(2): 497-504 MPID: 24287400.

The neck joint of a common American field ant can withstand pressures up to 5,000 times the ant’s weight. “Ants are impressive mechanical systems—astounding, really,” says Carlos Castro, assistant professor of mechanical and aerospace engineering at The Ohio State University. “Before we started, we made a somewhat conservative estimate that they might withstand 1,000 times their weight, and it turned out to be much more.”

One day, this research could lead to micro-sized robots that combine soft and hard parts, as the ant’s body does. Much work in robotics today involves assembling small, autonomous devices that can work together. But a difficult problem will emerge if the researchers try to create large robots based on the same design. Ants are super-strong on a small scale because their bodies are so light. Inside their hard exoskeletons, their muscles don’t have to provide much support, so they are free to apply all their strength to lifting other objects. Humans, in contrast, carry comparatively heavy loads due to our body weight. With our muscles supporting our body weight, we don’t have as much strength left over to lift other objects. On a human-sized scale, though, ants are overcome by basic physics. Their weight increases with their overall volume (dimensions cubed), while the strength of their muscles only increases with the cross-sectional surface area (dimensions squared); this is because the number of muscle fibers that can be packed into a muscle is proportional to the area of the muscle’s cross-section. So, a human-sized ant would not be so successful in carrying extreme loads at a human scale. (Actually, a human-sized ant would probably collapse under the weight of its own exoskeleton.) A large robot based on that design might be able to carry and tow cargo in microgravity, though, so it’s possible that we may one day employ giant robot ants in space, “or, at least, something inspired by ants” (according to Castro).



The square-cube law can be stated as follows: When an object undergoes a proportional increase in size, its new surface area is proportional to the square of the multiplier and its new volume is proportional to the cube of the multiplier.

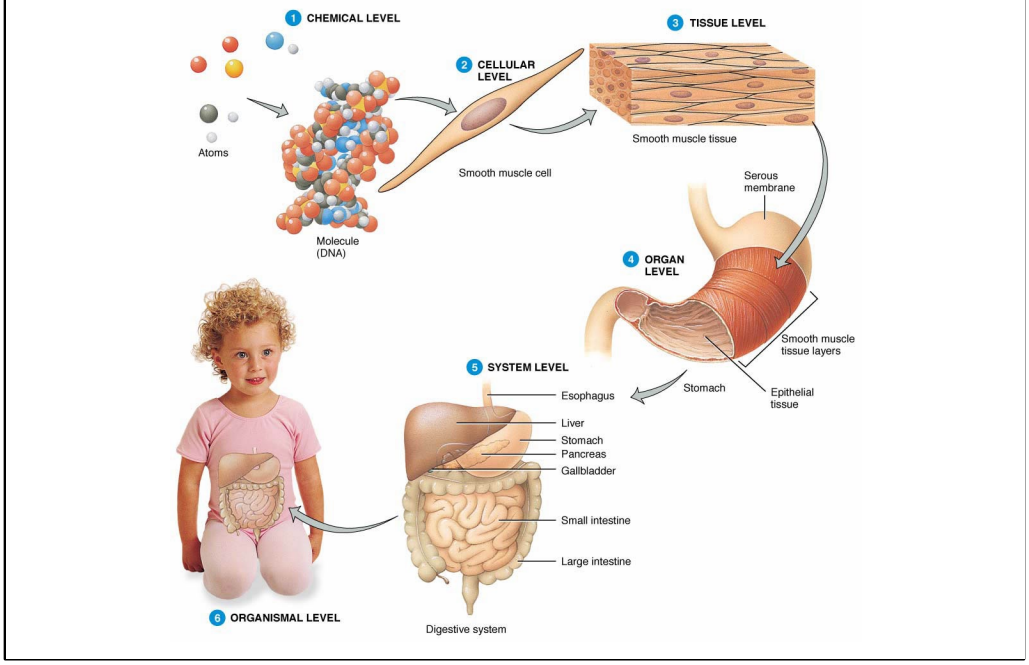
The strength of a muscle is determined by the number of elongated muscle fibers that can be packed into the muscle’s cross-section.

As the size of the muscle increases, the number of muscle fibers scales as the square (x^2) of the multiplier, because the cross-sectional area is proportional to the square of the characteristic dimension.

As the size of the muscle increases, the overall mass of the muscle increases as the cube (x^3) of the multiplier, because the volume of the muscle is proportional to the cube of the characteristic dimension.

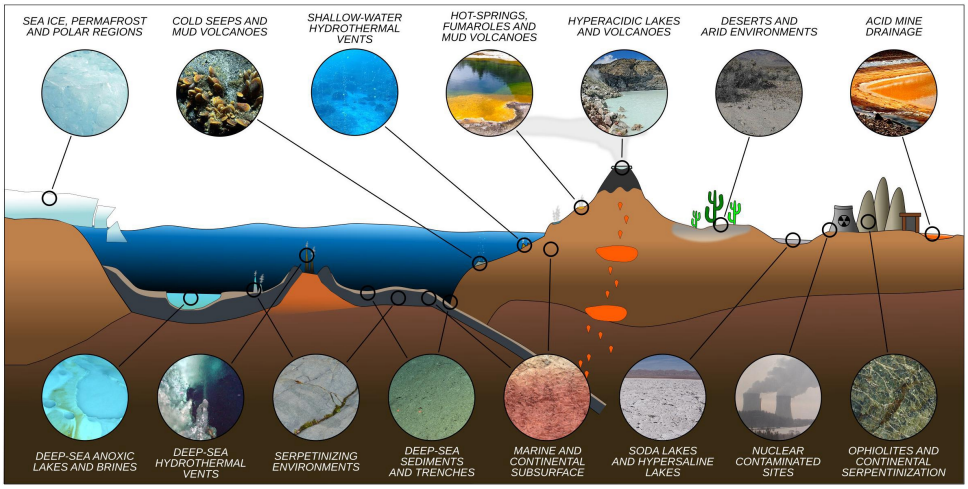
Therefore, as the size of the muscle increases, the muscle’s mass (proportional to x^3) increases exponentially faster than the number of muscle fibers (proportional to x^2). This means that, as the size of the muscle increases, its absolute strength increases but its relative strength decreases. Conversely, as the size of the muscle decreases, its absolute strength decreases but its relative strength increases. Small animals are strong, relative to their size, precisely because they are SMALL!

Cellular homeostasis in multicellular organisms



Large multicellular animals (like us humans) have evolved complex tissue and organ systems to maintain the physical and chemical parameters that cells experience within very tight boundaries. This process of "leveling out over time and fluctuating conditions" is called "homeostasis". Relevant environmental parameters include pH, salinity, pressure, and temperature. Bacteria, being single-celled organisms, typically do not have this luxury. Instead, bacteria have evolved to occupy specific niches with specific physicochemical parameters. This is a large part of the reason for the astonishing diversity of microbial species: genetic adaptations that optimize a species for survival in one niche may severely disadvantage the survival of that species in a different niches. Thus, bacteria have had to undergo extensive speciation (evolution) in order to "fill up" all of the available environmental niches on Earth.

Evolutionary adaptation of bacteria to vastly different environmental niches



Source: Merino N et al. (2019) Living at the extremes: extremophiles and the limits of life in a planetary context. *Front Microbiol* 10: 780 PMID: 31037068.

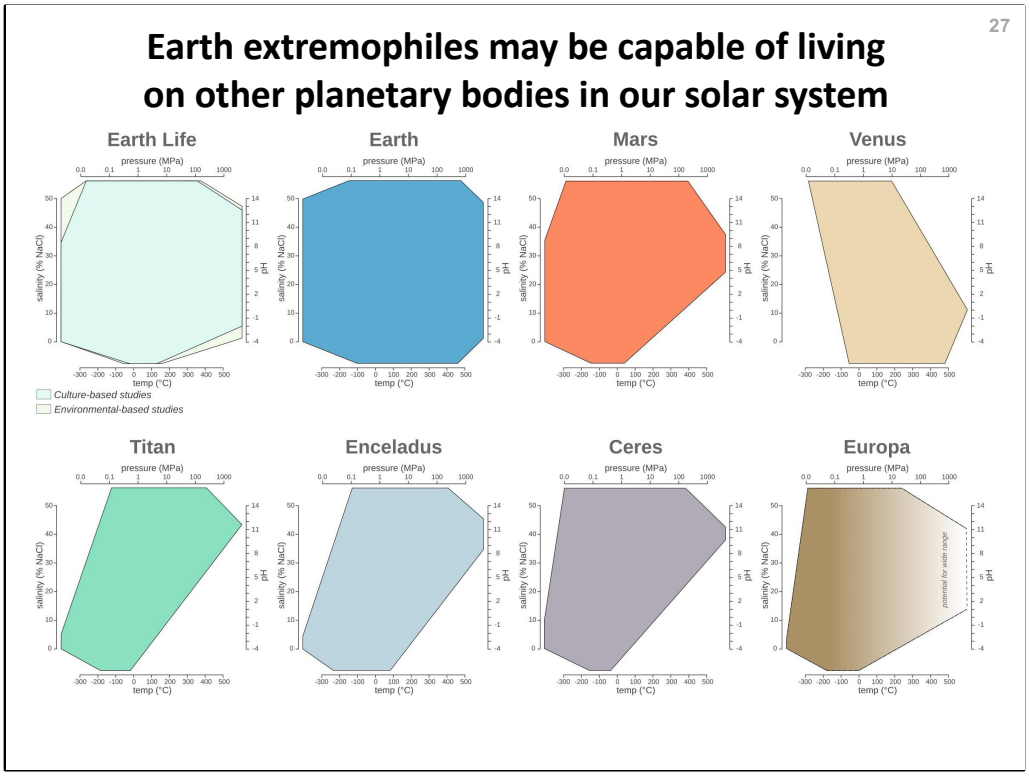
Figure1. Representative idealized cross section of Earth's crust showing the diversity of extreme environments and their approximate location. Live bacteria can be found in all of these environments.

Extremophiles: life at physical-chemical extremes

Extreme	Description	Species	Domain	Habitat	Optimum	Max/Min
Temp (high)	Thermophile	*Methanopyrus kandleri*	Archaea	Deep-sea seafloor hydrothermal vents	106˚C	122˚C
Temp (low)	Psychrophile	*Psychromonas ingrahamii*	Bacteria	Sea ice	5˚C	–12˚C
pH (high)	Alkaliphile	*Natronobacter gregoryi*	Archaea	Soda lakes	pH 10	pH 12
pH (low)	Acidophile	*Picrophilus oshimae*	Archaea	Acidic hot springs	pH 0.7	pH -0.06
Pressure	Barophile	*Moritella yayanosii*	Bacteria	Deep ocean sediments	700 atm	>1,000 atm
Salt (NaCl)	Halophile	*Halobacterium salinarum*	Archaea	Salterns	25%	32% (saturation)

Source: Madigan MT, Martinko JM, Stahl DA, Clark DP (2012) *Brock Biology of Microorganisms [13th edition]*. Pearson Education Inc., San Francisco.

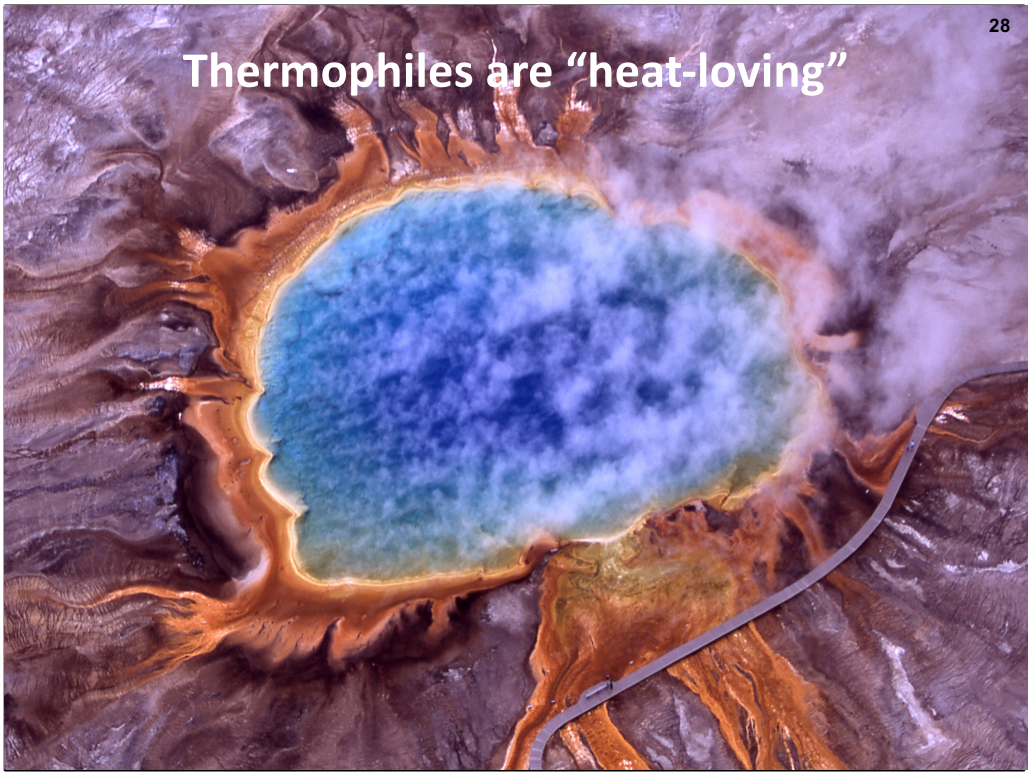
Table 2.1. Classes and examples of extremophiles. The organisms listed are the current "record holders" for growth at a particular extreme condition. ***Methanopyrus kandleri*** is an anaerobe and shows growth at 122˚C only under several atmospheres of pressure. ***Natronobacterium gregoryi*** is also an extreme halophile, growing optimally at 20% NaCl. ***Picrophilus oshimae*** is also a thermophile, growing optimally at 60˚C. ***Moritella yayanosii*** is also a psychrophile, growing optimally near 4˚C.



Source: Merino N et al. (2019) Living at the extremes: extremophiles and the limits of life in a planetary context. *Front Microbiol* 10: 780 PMID: 31037068.
Source: "Subglacial Microbial Life on Earth and Beyond" https://asm.org/Articles/2024/February/Subglacial-Microbial-Life-on-Earth-and-Beyond

Figure 2. The temperature, pressure, pH, and salinity boundaries observed for life on Earth compared to the phase space observed on selected planetary bodies. Polygon charts are designed to represent ranges in multidimensional space. Each edge represents the range for the specific variables. Single values (e.g., when min = max) are represented by a single vertex on an axis, while missing values (e.g., NA or NR) are represented by the absence of the corresponding polygon edge on the corresponding axis.

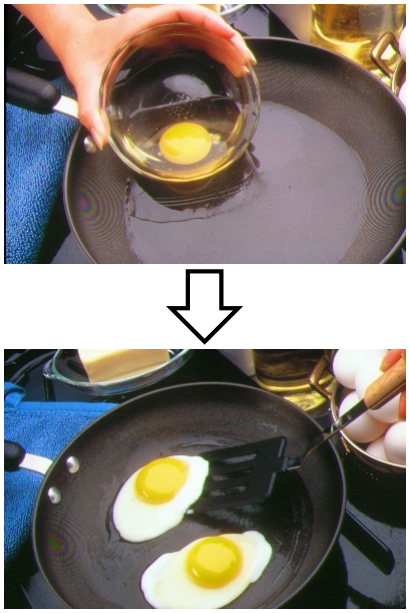
Titan is a moon of Saturn. It is about 5,150 kilometers in diameter.
Enceladus is a moon of Saturn. It is about 500 kilometers in diameter.
Ceres is a dwarf planet in the middle main asteroid belt between the orbits of Mars and Jupiter. It is about 940 kilometers in diameter.
Europa is a moon of Jupiter. It is about 3,120 kilometers in diameter.

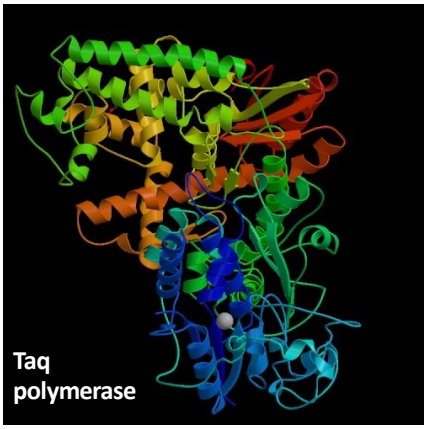


Thermophiles, a type of extremophile that thrives at very high temperatures, produce some of the bright colors of Grand Prismatic Spring, Yellowstone National Park, United States of America.

29

Usually, proteins + heat = disaster!!!
(but thermophiles are an exception)

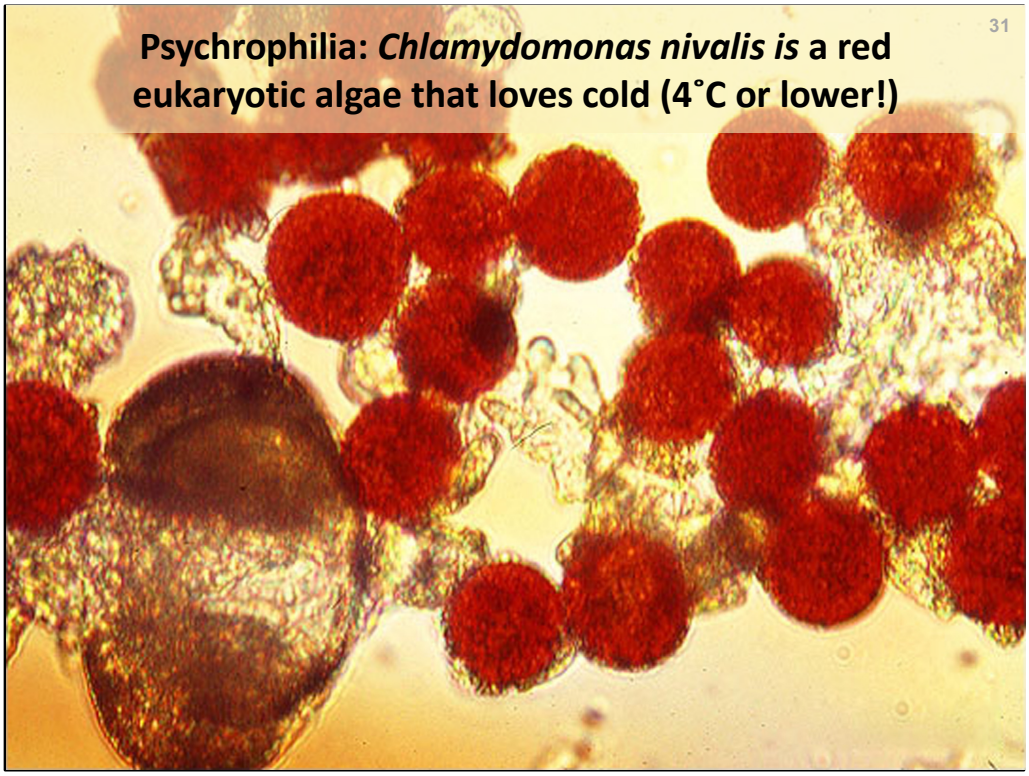




Hyperthermophilic proteins:
- **Use the normal set of amino acids**
- **Are intrinsically heat-resistant**
- **>10% are unique (new to science)**

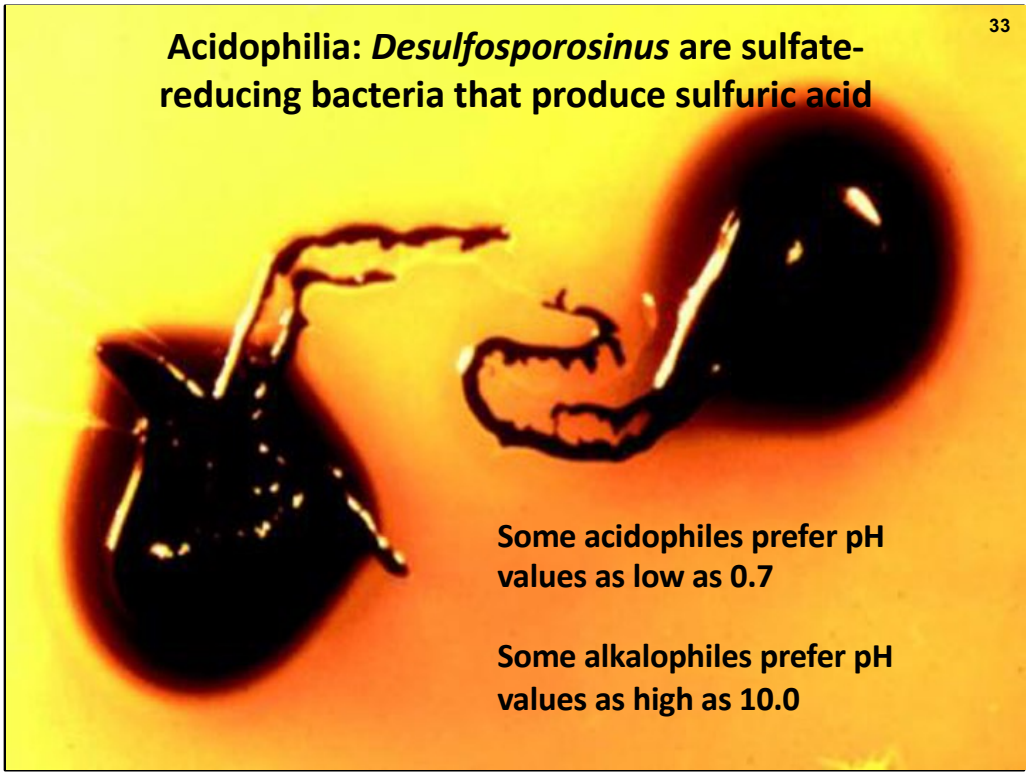
Thermophilus aquaticus, the source of Taq polymerase used in the polymerase chain reaction (PCR), thrives at 70° C but can survive at temperatures of 50° C to 80° C. This bacterium is a chemotroph - it performs chemosynthesis in order to obtain food. (We will talk more about **chemolithoautotrophic** bacteria later in BIO-372.) However, since its range of permissible temperatures overlaps somewhat with that of the **photolithoautotrophic** cyanobacteria that share its ideal environment, it is sometimes found living in close association with its cyanobacterial neighbors and obtaining energy for growth from cyanobacterial photosynthesis. (We will talk more about **photolithoautotrophic** bacteria, especially cyanobacteria, later in BIO-372.)


30
Psychrophiles are “cold-loving”



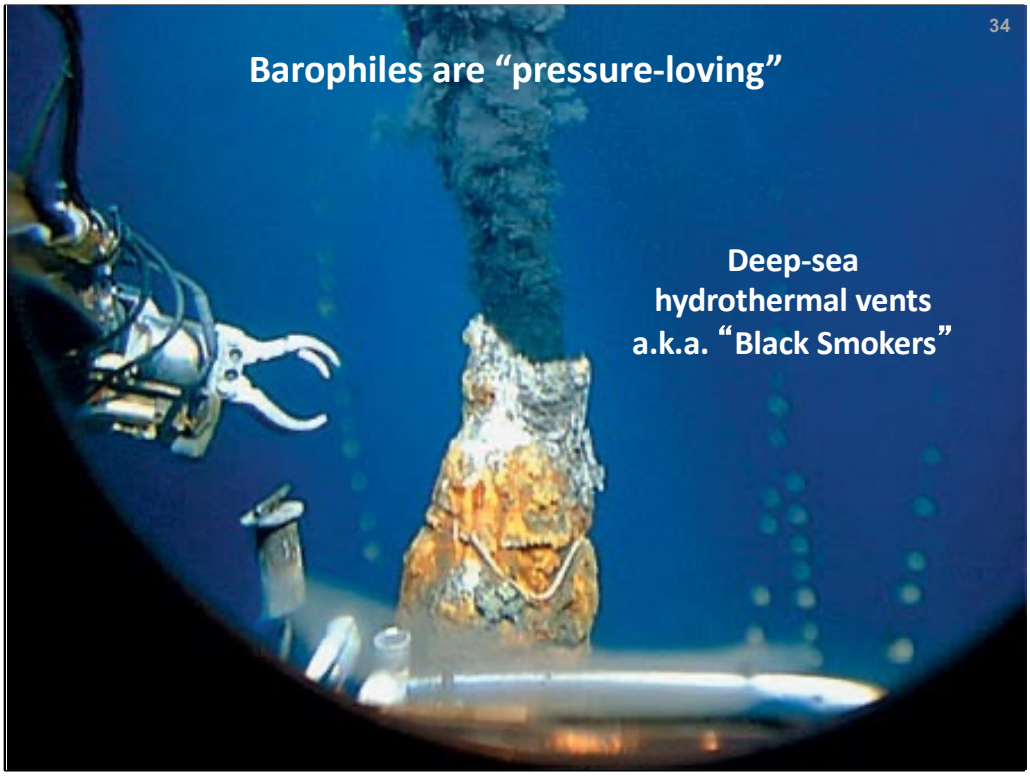
Chlamydomonas nivalis.

Acidophiles are “acid-loving”



Colonies of acidophilic sulfate-reducing bacteria (*Desulfosporosinus*-like isolates) isolated from water draining the former Wheal Jane tin mine in Cornwall. The diameter of the colonies is about 3 mm.

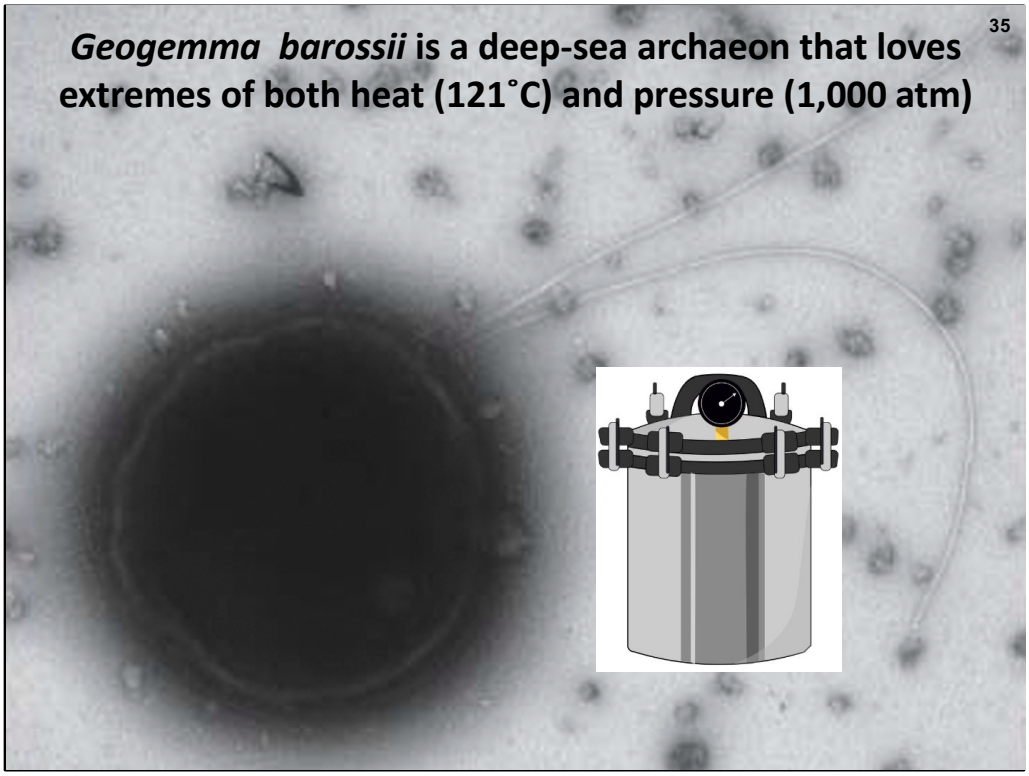
The pH of your stomach acid is pH 1 to 3, which is a strong acid. That's when your stomach is empty. Your stomach pH rises to about pH 5 when your stomach is full (after a meal).



Source: http://www.whoi.edu/oceanus/viewArticle.do?id=2400

The photo shows the deep-sea submarine “Alvin” reaching a manipulator toward a black smoker chimney, seen through the submarine’s viewport, at 17 degrees South on the East Pacific Rise. Hot hydrothermal fluids surge through the chimney at velocities of 1 to 5 meters per second. The "black smoke" consists of an abundance of dark, fine-grained, suspended particles that precipitate when the super-heated fluid upwelling from the deep ocean subfloor mixes with cold seawater. Photo by Meg Tivey, Woods Hole Oceanographic Institution.

We will revisit microbial ecosystems associated with “black smokers” later in BIO-372.



Source: Madigan MT, Martinko JM, Stahl DA, Clark DP (2012) *Brock Biology of Microorganisms [13th edition]*. Pearson Education Inc., San Francisco.

Figure 19.19. *Desulfurococcales* with growth temperature optima >100˚C. Negative stain of a cell of "Strain 121" (*Archaea*), capable of growth at 121˚C. A cell is about 1 µm wide. Strain 121 has an optimal growth temperature of 106˚C. However, Strain 121 actually shows weak growth at 121˚C, the temperature at which microbiological materials and media typically are sterilized by autoclaving, and cells remain viable for 2 hours at 130˚C. Only *Methanopyrus*, a hyperthermophilic methanogen (*Archaea)* can grow at higher temperatures (122˚C). Strain 121 consists of coccoid, flagellated cells. The organism is also a strict anaerobe and grows chemolithotrophically and autotrophically with Fe^{3+} as electron acceptor and formate or H_2 as electron donors. Phylogenetic analysis based on ribosomal RNA gene sequencing places Strain 121 in the family *Pyrodictiaceae*, along with *Pyrodictium* and *Pyrolobus*, probably as a new genus of this family. It is thus clear that this group of *Archaea* collectively contains the most hyperthermophilic examples of all known prokaryotes.

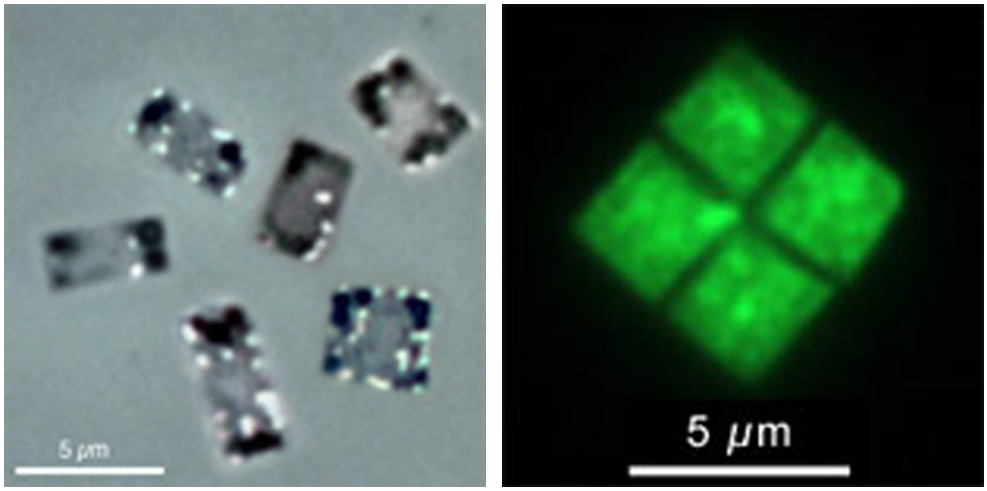
Note that an autoclave (pictured on the slide) used for sterilizing scientific equipment and supplies typically runs at 121ºC and 1.02 atm.

Remember: A pressure (*P*) of 1 atmosphere (atm) equals about 10^5 pascals (Pa), expressed in kg m^{-1} s^{-2}. (More precisely, 1 atm = 101,325 Pa but 10^5 is close enough for our purposes – this is a biology course, not a physics course… ;-)

Saltern pond.

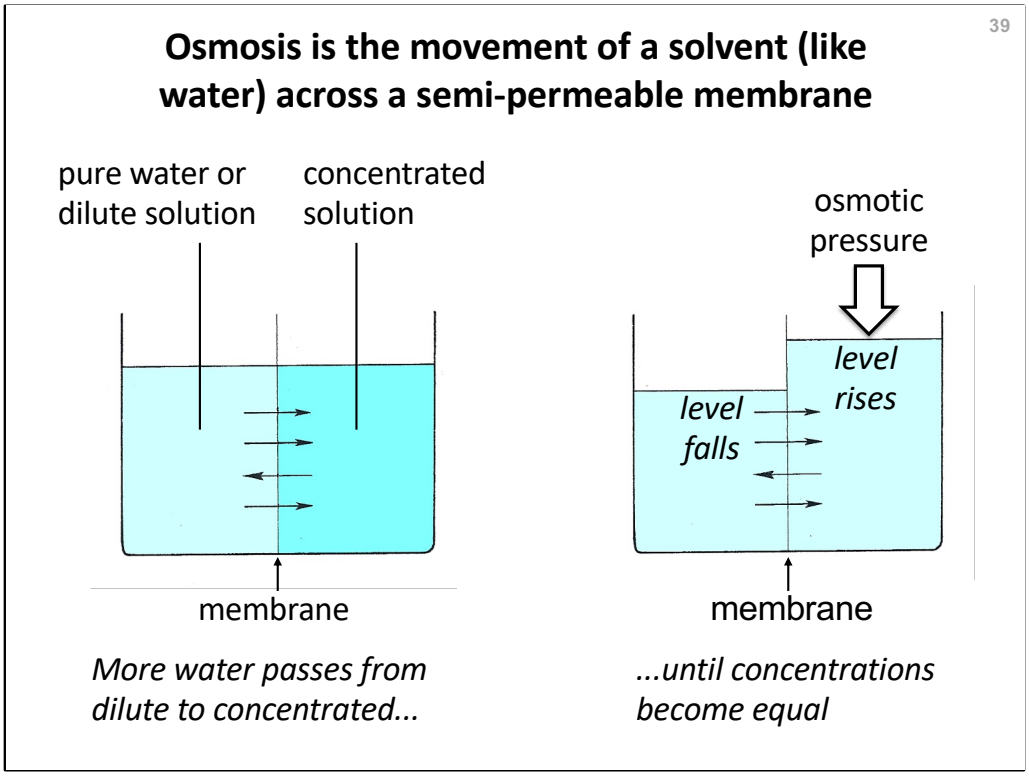
38

Halophilia: *Haloquadratum walsbyi* loves very high salt concentrations (up to 2 M!)



Cellular dimensions: 3 µm × 3 µm × 0.1 µm

Question: Why is the Archaeon organism (*Haloquadratum walsbyi*) able to tolerate right angles? We will return to this question later in BIO-372, so stay tuned…



Source: http://www1.lsbu.ac.uk/water/osmotic_pressure.html

Osmotic pressure is a pressure difference existing at equilibrium between two solutions separated by a semi-permeable membrane that is permeable to water but impermeable to solutes. It is defined in terms of the hydrostatic pressure that must be exerted to the solution to prevent the inflow of water through a semi-permeable membrane. An osmotic pressure is a physical quantity dependent only on the concentration(s) and temperature of the solution. Water moves from a solution with low osmotic pressure (high water action) to a solution with high osmotic pressure (low water action) due to osmosis and, if allowed, would equalize the pressure on both sides of the membrane.

Osmotic pressure is equal to the **hydrostatic pressure** that must be exerted to the solution to prevent the flow of water through the semi-permeable membrane. This **pressure** is generally created by the solute present in the solution. ... Thus, the **osmotic pressure** of a solution will always be **positive**.

Osmotic potential is a measure of the tendency of a solution to withdraw water from pure water by osmosis across a differentially permeable membrane. Net diffusion of water occurs from regions of less negative potential to ones of more negative (or lower) potential and continues until the potentials become equal. The osmotic potential (Ψ_π) of pure water is defined to be zero (= 0 Pa), with all solutions having negative values, in pressure units. Thus, the **osmotic potential** of a solution will always be **negative**.

If a living cell is surrounded by a more concentrated solution (with more negative osmotic potential), the cell (which in this case would be **hypotonic** relative to the external environment) will tend to lose water to the surrounding environment. If the living cell is surrounded by a less concentrated solution (less negative osmotic potential), the cell (which in this case would be **hypertonic** relative to the external environment) will tend to gain water from the surrounding environment. Water flows from higher osmotic potential to lower osmotic potential. In osmosis, water molecules move down the water potential gradient, from a higher water potential to a lower water potential.

Osmosis is the movement of a solvent (like water) across a semi-permeable membrane

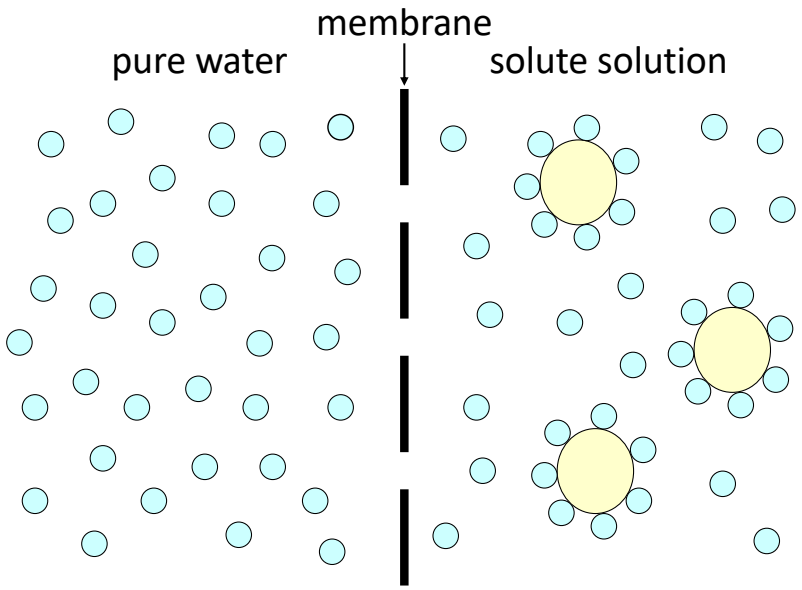
There are microscopic pores in the cell plasma membrane.

Molecules below a certain size can diffuse through the pores.

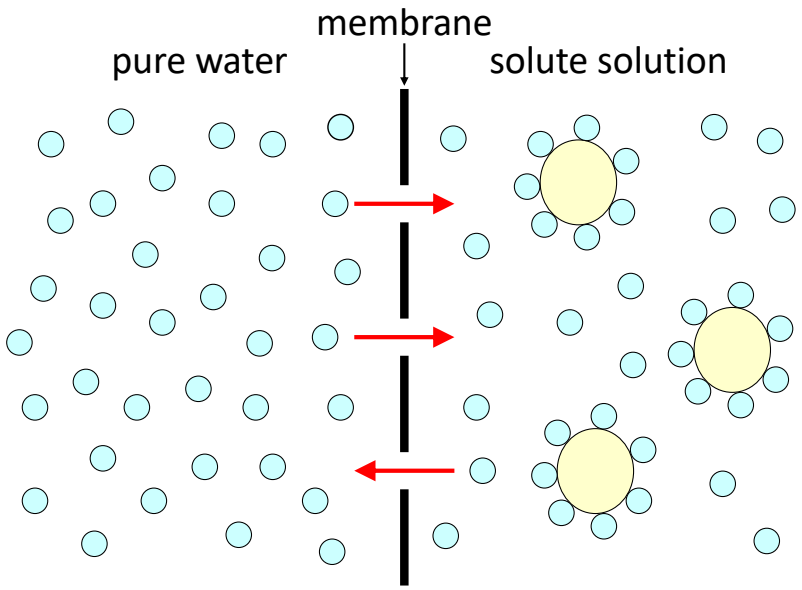
Water molecules can easily diffuse through the pores.

In the next slides,  represents a water molecule

and  represents a solute molecule.



There are just as many water molecules on the right as there are on the left but many of them are attached to solute molecules so they are not free to move.



Because there are more freely-moving water molecules on the left, more of them can diffuse through the pores of the membrane from left-to-right than from right-to-left.

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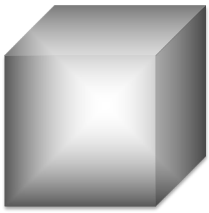
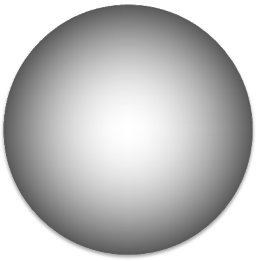
Imagine two cells of *equal volume*. One is sphere-shaped. One is cube-shaped. Which shape allows faster exchange of solutes across the cell membrane?





A. Solute movement across the membrane is faster for cuboidal cells (left) than for spherical cells (right).

B. Solute movement across the membrane is faster for spherical cells than for cuboidal cells.

C. Solute movement across the membrane is equally fast for spherical and cuboidal cells.

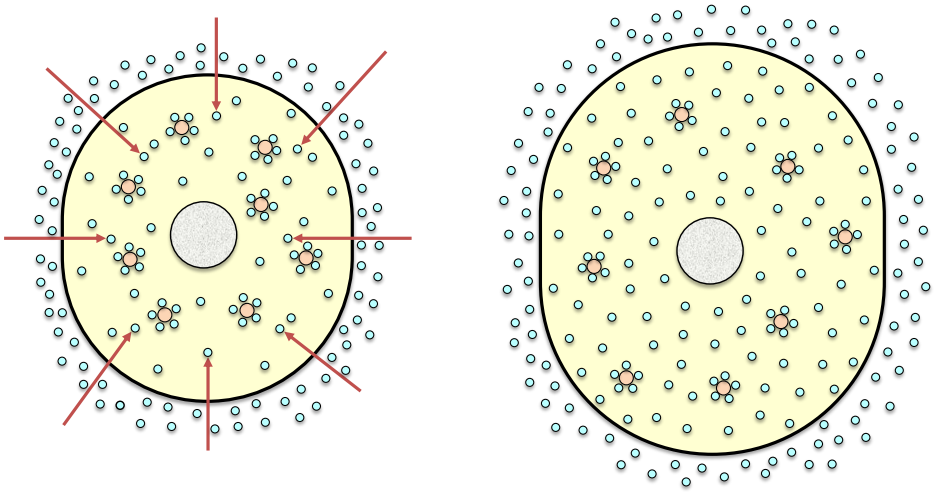
Answer: (A)

This is because the surface area of the cube is greater than the surface area of the sphere. Remember: the sphere has the lowest ratio of surface area to volume of any shape, so any deviation of an object from the spherical shape will increase the surface area relative to the volume.

In cells without a supporting cell wall:

There is a higher concentration of free water molecules outside the cell than inside the cell…

…so water diffuses into the cell by osmosis and the cell swells up (and may eventually rupture).



van't Hoff equation: $P = \Delta n * R * T * V^{-1}$

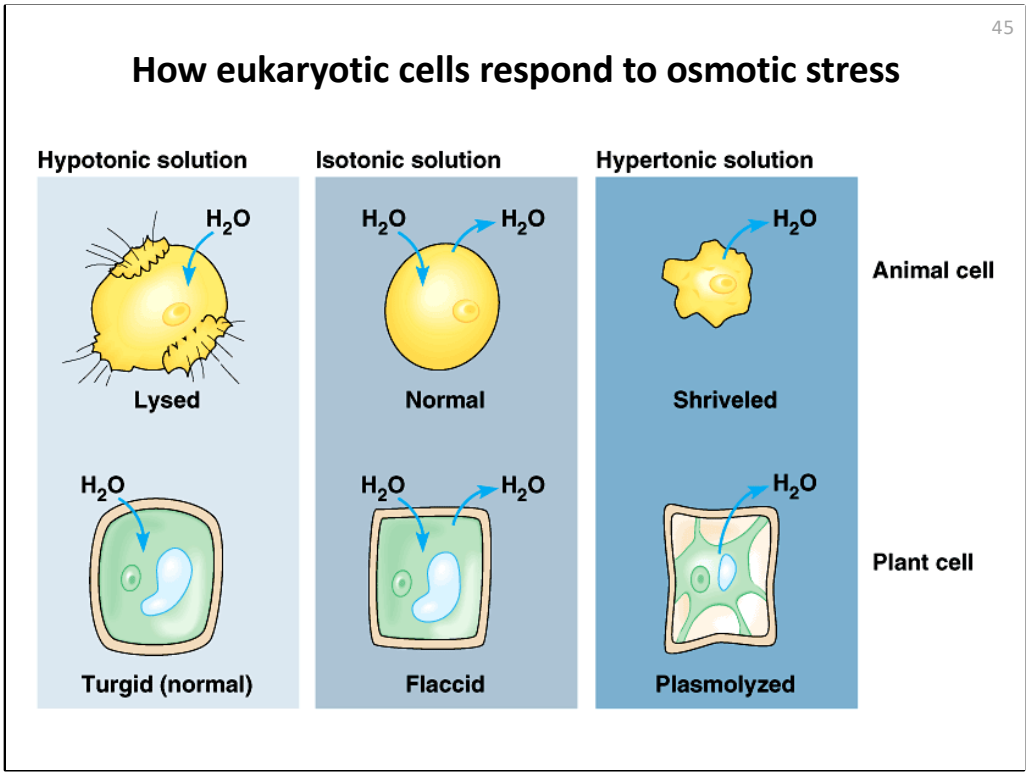
Osmosis can be a big problem for bacteria, which mostly live in **hypotonic** environments where the concentration of solutes outside the cell is lower (**hypotonic**) relative to the concentration of solutes inside the cell (**hypertonic**). Put another way, the "free water concentration" outside the cell is higher than the "free water concentration" inside the cell. Consequently, there is a net flux of water from the external environment into the cell, which cause the cell to swell up and eventually burst, like an over-inflated balloon. This process – the movement of water molecules from an area of higher free water concentration to an area of lower free water concentration through a semi-permeable membrane – is called **osmosis**.

Osmotic pressure is the minimum external pressure required to inhibit the movement of water molecules across a semi-permeable membrane, resulting in no net movement of solvent (water) molecules. Osmotic pressure depends on the molar concentration of the solution outside vs. inside the cell.

Intracellular solutes (ions, metabolites, and macromolecules such as proteins, nucleic acids, etc.) play an important role in establishing the **osmotic pressure** across the cell membrane. The cell membrane is a semi-permeable interface that enables the free passage of water molecules while preventing (partially or totally) the transport of solutes. The osmotic pressure is the extra pressure that is sustained by a semi-permeable barrier that has a higher concentration of solutes on one side of the barrier. In the dilute limit, the osmotic pressure obeys a relation similar to the **ideal gas law** where the osmotic pressure P is given by the **van't Hoff equation**: $P = n * R * T * V^{-1}$

Abbreviations:
P = pressure in $kg * m^{-1} * s^{-2}$
Δn = the number of moles (mol) of intracellular solute in excess of extracellular solute
V = volume in m^3
R = the universal gas constant in 8.3 $J\ mol^{-1}\ K^{-1}$ or 8.3 $kg * m^2 * s^{-2} * mol^{-1} * K^{-1}$
T = the absolute temperature in degrees Kelvin (K)
K = degrees Kelvin
mol = moles

The equation for the van't Hoff relation is a "primary concept" – you should memorize it (and all of its terms) and you should feel comfortable using it. Indeed, you should already be familiar with this equation from your physics courses.



In **hypotonic solution**, the concentration of free water molecules outside the cell is higher than the concentration of free water molecules inside the cells. Consequently, there is a net influx of water into the cell and the cell may expand until it undergoes cytolysis (animal cells) unless the resulting turgor pressure is countered by a rigid cell wall (plant cells, bacteria, archaea).

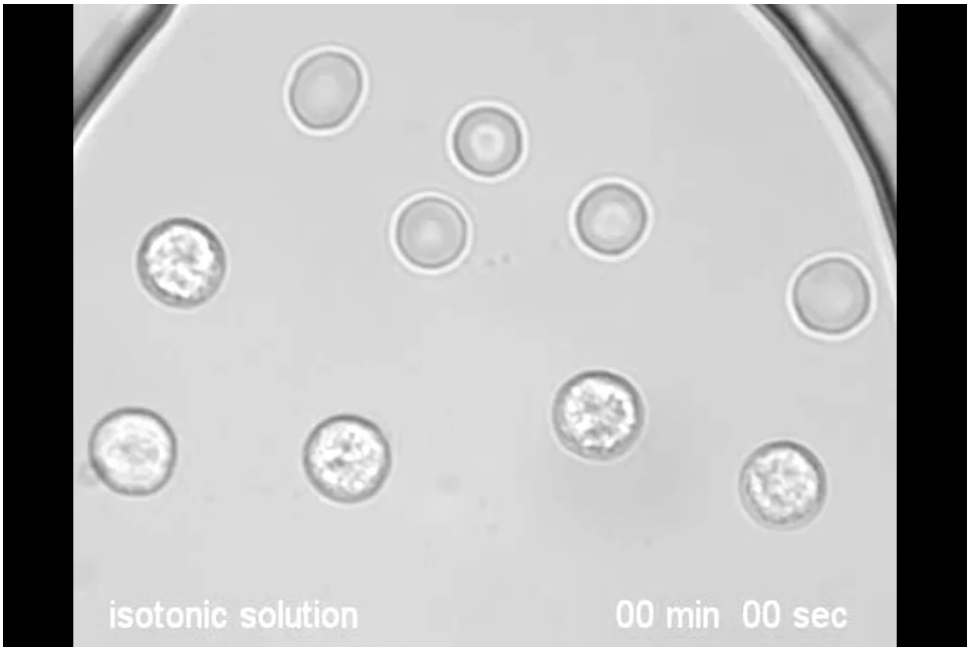
In **isotonic solution**, the concentration of free water molecules outside the cell is equal to the concentration of free water molecules inside the cell. Consequently, there is no net flux of water into or out of the cell (animal or plant cells).

In **hypertonic solution**, the concentration of free water molecules outside the cell is lower than the concentration of free water molecules inside the cells. Consequently, there is a net influx of water out of the cell and the cell may undergo plasmolysis (animal or plant cells).

Plasmolysis can occur if the cell is placed in a hypertonic solution resulting in a net flow of water out of the cell and a reduced turgor pressure within the cell. **Cytolysis**, the reverse process, can occur if the cell is placed in a hypotonic solution resulting in a net flow of water into the cell and therefore an increased turgor pressure within the cell.

In the "real world" of microbes the surrounding medium is usually **hypotonic** relative to the bacterial cytoplasm, which contains a very high concentration of solutes with bound water molecules (i.e., water molecules that are not "free" to move across the cell membrane). I say "usually hypotonic" but not "always hypotonic" - remember, for example, the halophilic microbes like *Haloquadratum walsbyi*!

How human blood cells respond to osmotic stress



Source: http://www.youtube.com/watch?v=OYoaLzobQmk

Erythrocytes (top) and leukocytes (bottom) subjected to a periodic osmotic change: from isotonic to hypertonic, back to isotonic, and finally to hypotonic. When exposed to **isotonic solution**, the cells assume their normal shape. When exposed to **hypertonic solution**, the cells shrivel (**plasmolyze**). When exposed to **hypotonic solution** (distilled water), the cells swell up and burst (**cytolysis**): the erythrocytes become "ghosts" (they expel hemoglobin and lose contrast) and the leukocytes swell and burst like popcorn, releasing their intracellular contents.

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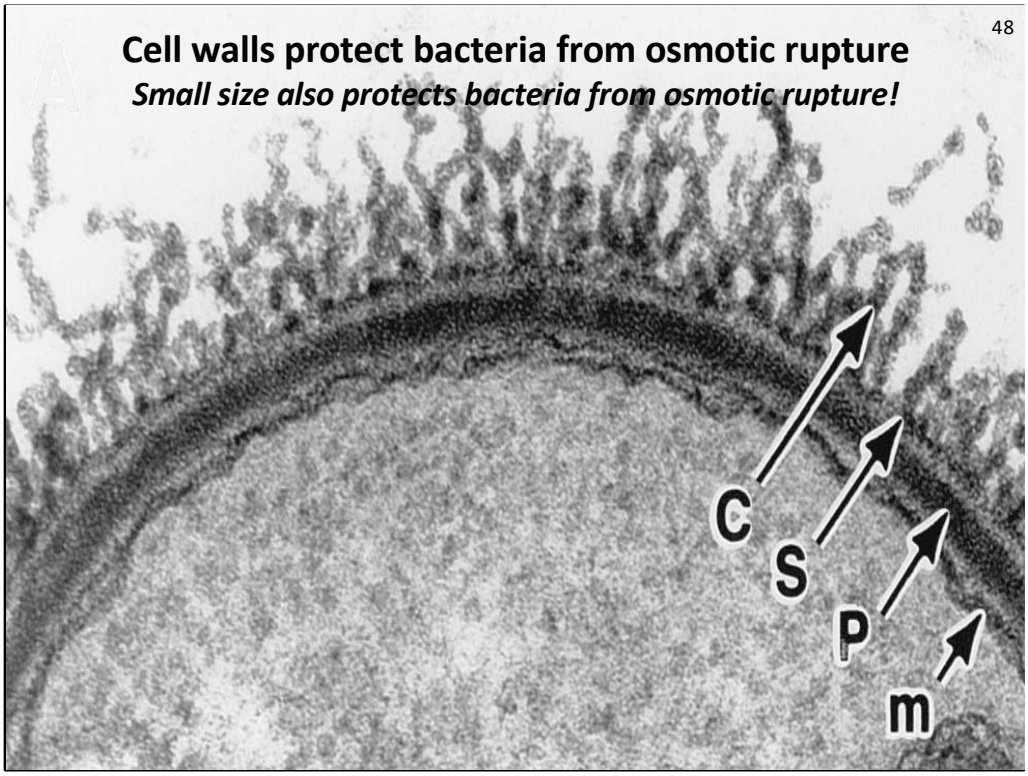
Paramecium uses contractile vacuoles to expel water and prevent osmotic rupture

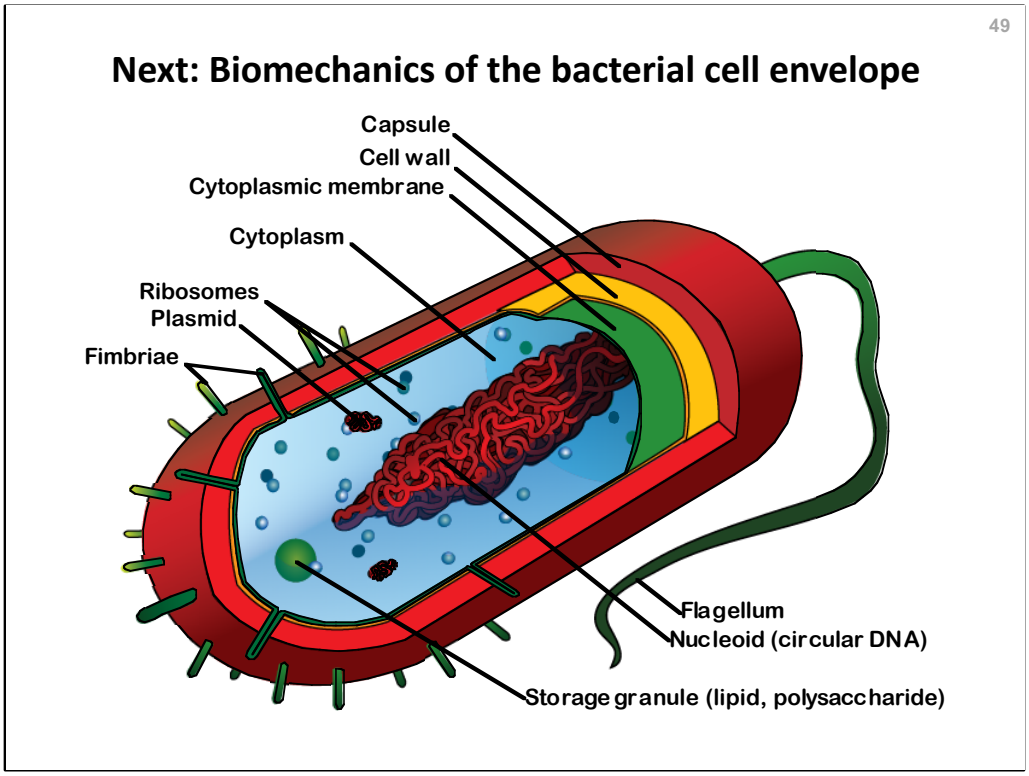
Source: http://g11-bioa-2011-12.wikispaces.com/file/detail/CONTRACTILE+VACUOLES+IN+PARAMECIUM+CAUDATA+HI+DEF+VIDEO+CAS+2811+-+YouTube.flv
Source: https://www.youtube.com/watch?v=vk5NFSvp8lY

Question: Human cells do not have cell walls or contractile vacuoles. Why not? Answer: multicellular organisms (like us) expend a lot of energy to maintain the extracellular medium bathing our cells at an osmotic strength similar to the cell cytoplasm. In other words, the intracellular fluid is isotonic with respect to the cell cytoplasm, so there is no net influx or efflux of water into or out of the cell.



Source: Mesnage S, Tosi-Couture E, Gounon P, Mock M, Fouet A (1998) The capsule and S-layer: two independent and yet compatible macromolecular structures in *Bacillus anthracis*. *J Bacteriol* 180(1): 52-58 PMID: 9422592.

Abbreviations:
C, capsule
S, S-layer
P, periplasmic space
M, cytoplasmic membrane



The bacterial cell wall is the most important load-bearing structure that allows bacteria to survive during osmotic stress, particularly when there is a positive internal **turgor pressure** (outward-facing pressure). This occurs when the bacteria are growing in a **hypotonic** environment where the osmolarity of the cytoplasm exceeds the osmolarity of the surrounding liquid, resulting in the net influx of water into the bacterial cell. Mammalian cells do not have cell walls. As a consequence, mammalian cells are highly vulnerable to changes in the osmolarity of the surrounding medium.