Advanced Biochemistry

CHEMICAL EVOLUTION

This is a Tutorial/Silent Lecture

What is a Tutorial/Silent Lecture?

a sequence of "slides" formatted to guide you through the exploration/study of the topic

you are the main actor in this active learning experience
think of it as working with a tutor without having to pay for it

as the slide sequence unfolds, you will get opportunities to engage with the material

- **by thinking about/answering questions**,
(my answer is always provided on the next slide).
- **by completing a "short assignment"**
(it never will take more than a few minutes, if at all that long),
- by **watching a short video/clip**
(the embedded links will take you to my YouTube@VsBCBMB channel;
key moments are shown in the slide-deck as still images, in case you don't want to watch the videos)

of course, you can skip the active learning aspect and look at the answers right away.

Why Give This a Go?

- **benefits: you set the pace** taking as much or as little time as you need.
- you **can turn tutorials/silent lectures into fully immersive experiences** (eg playing your favourite music while working through the content),
- **or invite friends to over the Q&A structured/guided materials together,** discussing the questions before looking at answers.

each of these features help you to hold on to the material.

Advanced Biochemistry

this collection of handouts builds on the "Biochemistry Fundamentals" collection = the chapters assume that you know the basics covered in the "Fundamentals" handouts (free downloads), and have some basic knowledge of molecular/cell biology

while this tutorial can be used stand alone, you will benefit more from the "silent lectures" by

- working through the chapters in the order that they are posted in the "download gallery"
- **reviewing the associated "Biochemistry Fundamentals" chapter** to refresh your memory
- spending 5-10 minutes to **summarize for yourself what you already know/remember** about the topics of the handout you are about to look at.
- **take advantage of the "interactive" elements**

I welcome your thoughts and ideas for further improvements of the chapters. You can submit your comments by contacting me at <u>pdf-comments@vsbcbmbstudy.com</u>

extending what you already know, the chapters of the "Advanced Biochemistry" Collection take a more in depth look at the chemical aspects of life.

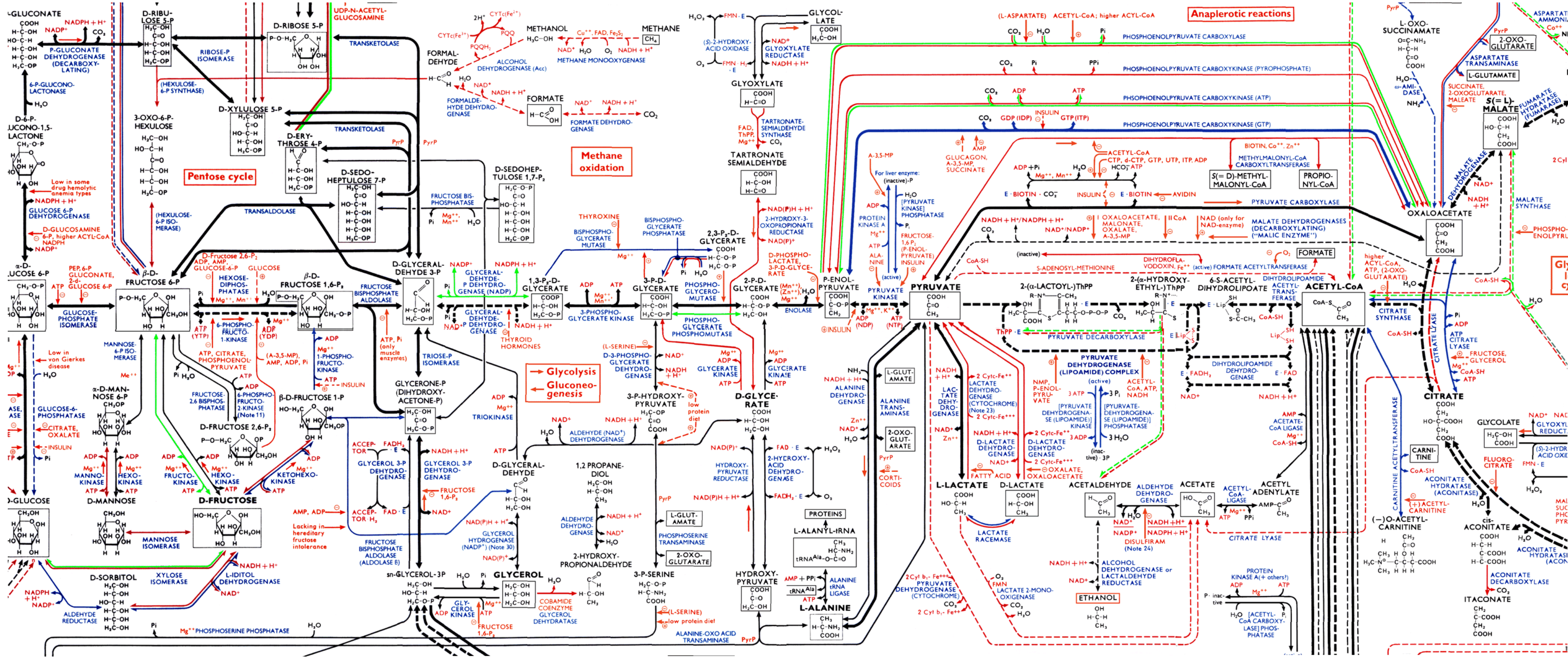
by any measure, the Collection is incomplete = there will be topics that you will need to study on your own; however, filling in the missing parts should neither be difficult nor time consuming if you "own" the materials that are covered in the Collections.

let's begin with a simple questions .....

what "image" forms in your mind if you hear the word "Biochemistry"?

The “Mental Image” of Biochemistry … For Most People

(including many instructors)



Partial View from Boehringers Metabolism Chart (no longer available from Boehringer)

https://github.com/usnish/biochemical-pathways-poster/blob/master/prebuilt_hires.png

admittedly …this looks gruesome (unless you love organic chemistry) … especially if you are expected to memorize any part of it for exams….

…..but as we will discover in later chapters, it looks worse than it is because much of this can be intuitively deduced by combining simple, generic chemical reaction templates into "pathways.

for the time being ….let's start by taking a deep breath and by **putting the general idea of what you see into a definition of the term "biochemistry"** ….

….try …..

Biochemistry - Definition

biochemistry is the science that seeks to understand the chemical basis of cellular functions. It deals with the chemical nature of biological compounds and macromolecules, and how these compounds are formed, interconverted, changed and degraded,

- to capture/generate energy,
- to build/remodel/maintain/destroy supramolecular assemblies/cells/organismal structure,
- to store/retrieve/transmit information, and
- to regulate cellular/organismal functions.

… a lot to unpack, but you will if you stay the course.

the interesting thing here: just like biology itself, biochemistry is an integrated science and its boundaries with molecular and cell biology are blurry (studying the latter two without any biochemistry knowledge is futile as likely will not understand any of it)…..

Biochemistry – The Roadmap…..

starting from this definition of biochemistry, you will get an understanding for the narrative of this Collection by attempting the following exercise:

In <u>one sentence,</u> define "life" from a science perspective

…try!! …even if it takes you a while…you will gain a lot from it by discovering the extent of your knowledge, blind-spots and biases ….it's even more fun to do this exercise with a few friends and see who comes closest, or what you can come up with as a group….

Biochemistry – The Roadmap…..

starting from this definition of biochemistry, you will get an understanding for the narrative of this Collection by attempting the following exercise:

In <u>one sentence,</u> define "life" from a science perspective

life – definition (V's pitch)

life is a process in which an open system maintains a homeostatically controlled directed flow of electrons.

…not poetic, but - in its minimalistic form - provides you with powerful pointers for the flow of the narrative (= where to start learning about biochemistry and how to advance)

<u>and</u>

explains why defining life *per se* should not rely on attributes like "reproduction" (eg: if you were to lose your ability to reproduce, would you still be alive? What about GMO crops or Eunuchs? The latter are examples for life in the absence of reproduction .... that said: reproduction is necessary to propagate life <u>through time</u>…which is different from understanding what life is at its core)

The Narrative

why is the minimalist definition of life helpful for establishing the narrative of a "biochemistry course"?

>>life is a process in which an open system maintains a homeostatically controlled directed flow of electrons<<

provides a natural order/roadmap for exploring biochemistry because:

>>a …..system<< implies boundaries & basic units ➔ best to start with the periodic table asking .."how can you form basic units"?

>> …open…. << whatever the boundaries, they need to be semipermeable → "how can that be accomplished"?

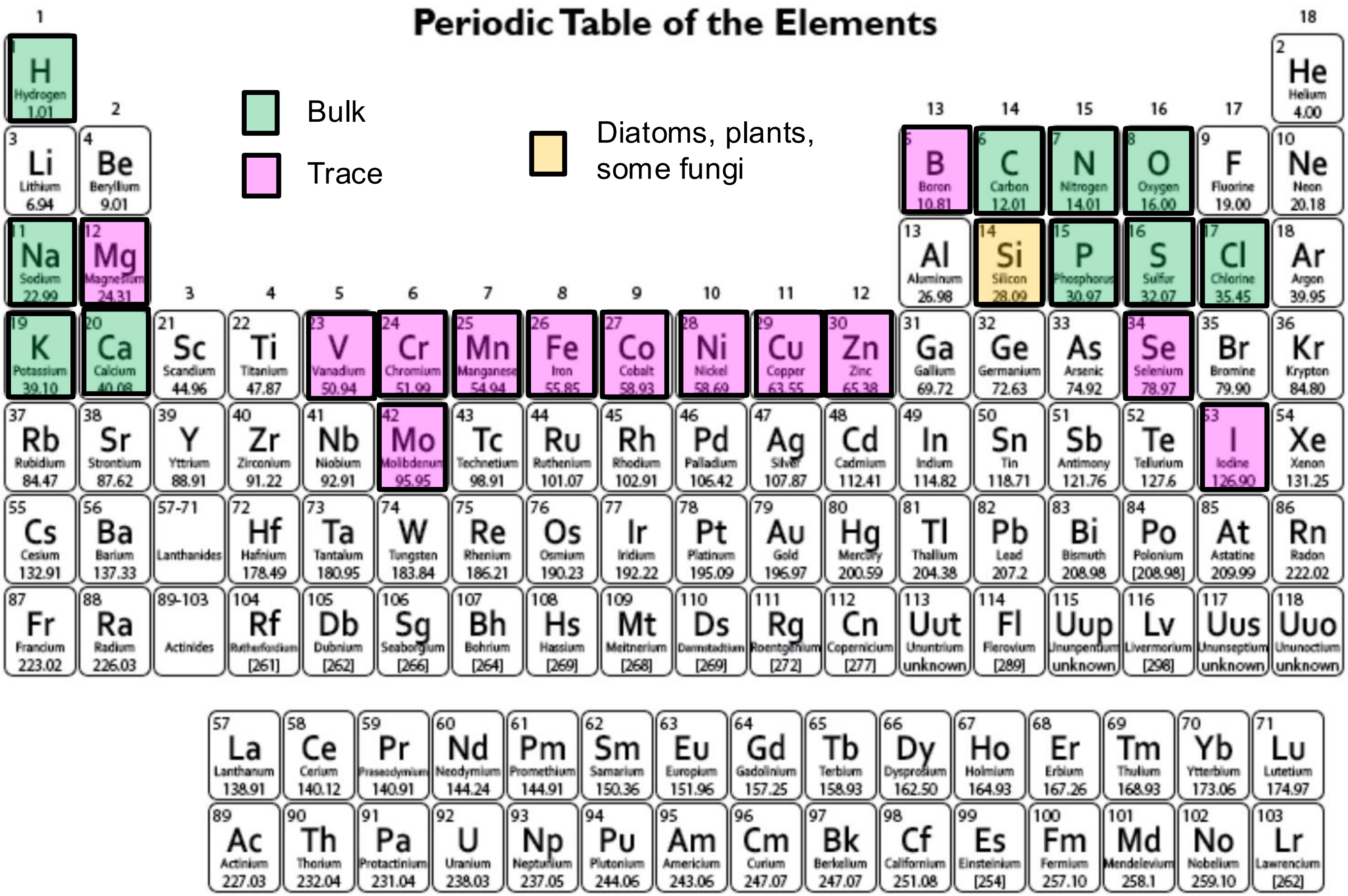
>>process …maintains…directed flux of electrons<< deals with the chemical nature of biological compounds and macromolecules, and how these compounds are formed, interconverted, changed and degraded, to (1) capture/generate energy, (2) to build/remodel/maintain/destroy supramolecular assemblies/cells/organismal structure, and (3) store/retrieve/transmit information.

>>…homeostatically controlled…<< deals with responsiveness of the system → how is that accomplished?

curiously: "open system" = physics; "directed flux of electrons" = chemistry, "homeostatically" = physiology ➔ it is all integrated and part of what you study …

with this in mind…..what did Nature have to work with?

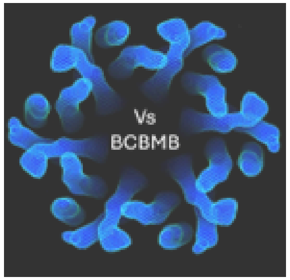
The Chemistry on Early Earth Selected a Subset of Elements From the Periodic Table to Go Ahead and Come Alive



to understand why these were the "winners", we want to go back in time, asking where it all started……….

………on Earth (presumably), some 4 billion years ago…..

what did Earth look like then? Would you spend Spring Break there…Why (not)?



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what did Earth look like then? Would you spend Spring Break there…if not why?

Answer: probably not … terrifying by today's standards: hot, high volcanic activity, extreme lightening, high UV radiation (no ozone!)

chemically … a "horrible" mix of gases: N_2, H_2,CO_2, NH_3, CH_4, H_2S, H_2O (both gas and liquid) but **<u>no free molecular oxygen</u>.**

- ➢ the absence of free molecular oxygen is significant and important because under anaerobic conditions, an underappreciated element was "shining" – can you guess which one it was?

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- this element is iron (Fe), which is one of the most abundant elements on Earth. If abundancy has something to do with likelihood to become a major building block, then **how come that humans are not "metallic"**?

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simplest Answer: **chemically too limited.** Still our bodies contain ~3.5-4g of iron

Best known:

- iron ions in hemoglobin (participate in the transport of oxygen),
- biocatalysts with "iron-sulfur clusters" that participate in redox-reactions (electron transfers)

→ enabled by iron's broad range of oxidation states (-2 to +6). Most common: +2 and +3.

In its "+2" (ferrous iron) state, iron is quite water soluble. Different for "+3" state (ferric iron) - which outside the body is found mostly as iron oxide (rust, Fe_2O_3) if the environment contains free oxygen.

Why bring up Iron?

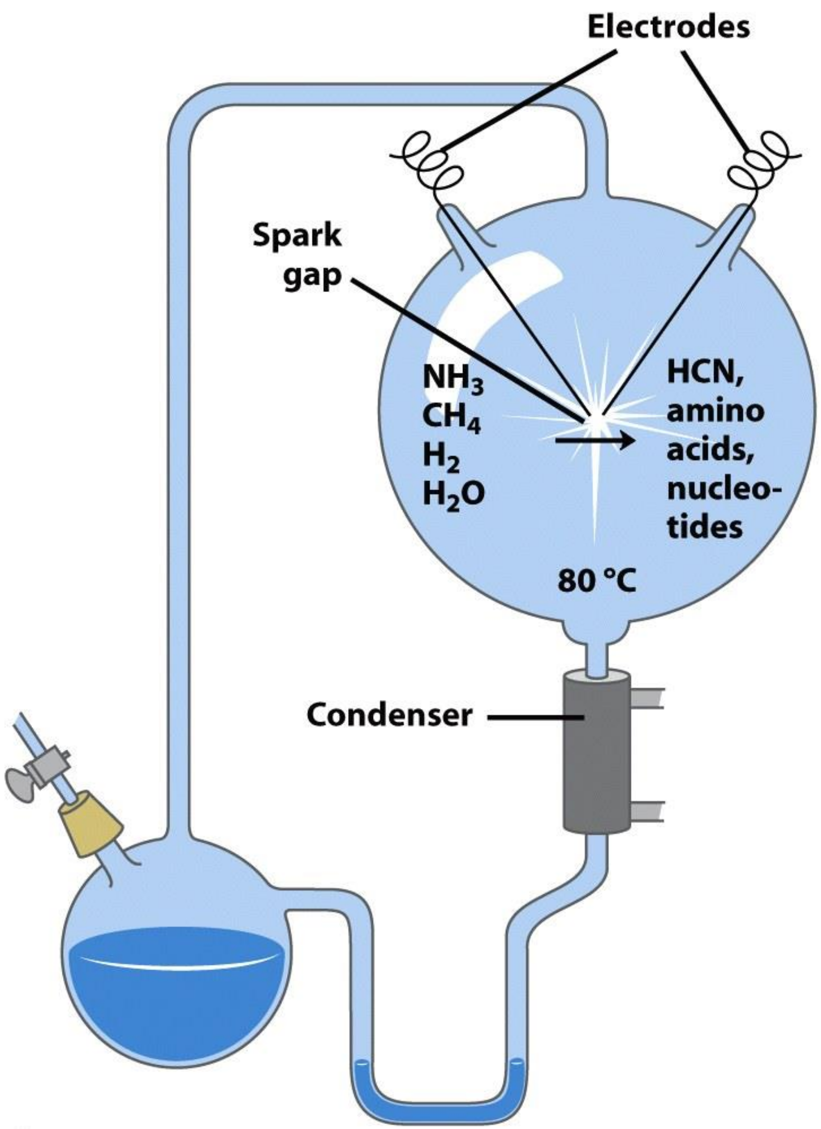
- **Answer:** versatile redox properties of iron (and the presence of sulfur + protons/acid) were great for early "**catalysis**" (remember our **concepts?**) that supported the generation of complex organic molecules from simple inorganic precursors.

- in fact, the hostile environment on the early Earth would warm the heart of an "industrial" chemist - because in this environment, reactions are forced without paying attention to efficiency, resources or the delicate/fragile nature of "life".

- while logical from today's vantage point – Vitalism (= the idea that only biological organisms can produce biologically relevant biomolecules) made life's basic chemistry untouchable …..until….

- ……1828, when the German chemist Friedrich Wöhler synthesized the first organic compound: urea

yet it took until the mid 20th century for somebody to be bold enough to experimentally test whether "biology" could have originated from the conditions that existed in the early atmosphere



1952

Stanley Miller and Harold Urey

conducted the classical experiment that proved that organic, biologically relevant compounds could have arisen in an abiotic environment believed to mimic early Earth.

this work happened at the Univ of Chicago

a brief account can be found at:
http://en.wikipedia.org/wiki/Miller-Urey_experiment

perhaps unexpectedly, these types of experiments are still very much in fashion today and many questions about what exactly happened remain unanswered.

until we know, there remains room for alternative theories like creationism or ...

Alien Influencers ….

How life on Earth really got its start.

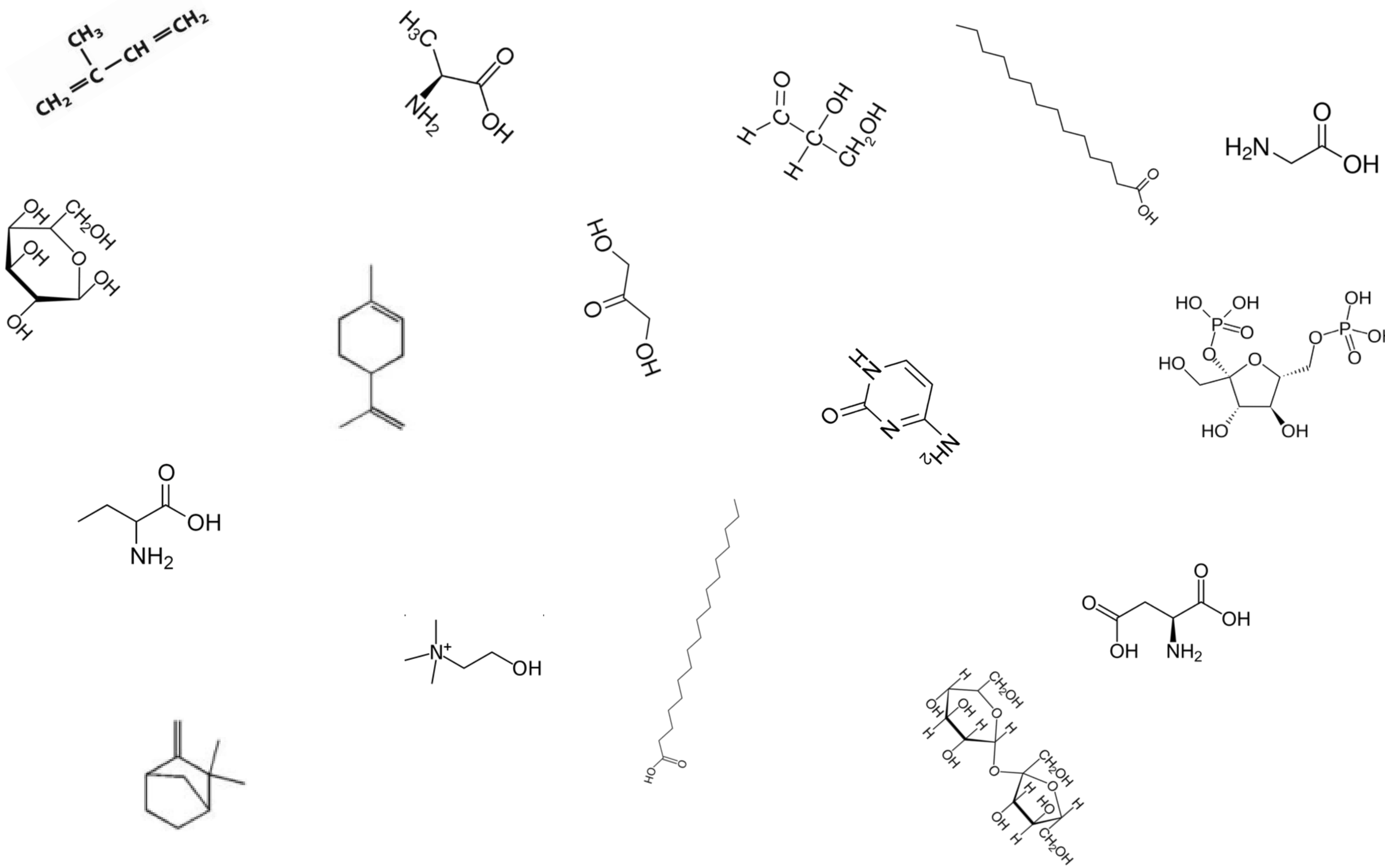
time will show ….

…but seriously…..

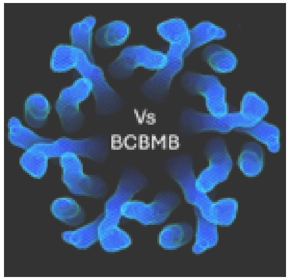
there is growing consensus that a significant amount of organic material reached (and continues to reach) Earth from outer space (meteorites, interstellar dust)

Types Of Compounds That Can Form Spontaneously Under Conditions Assumed To Have Existed On Early Earth

try to classify the molecules that are shown below

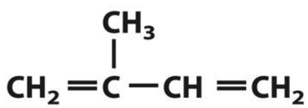


this should be a "piece of cake" …. if you went over the "Biochemistry Fundamentals" Collection

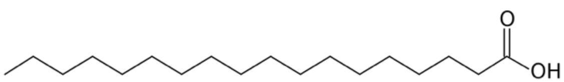


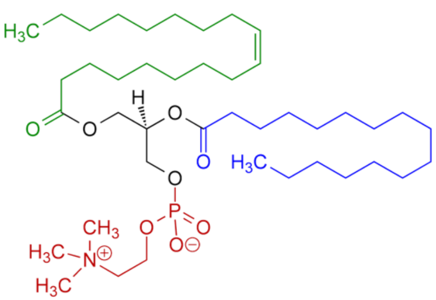
Types Of Compounds That Can Form Spontaneously Under Conditions Assumed To Have Existed On Early Earth

Lipids

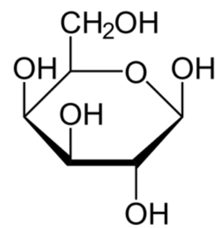


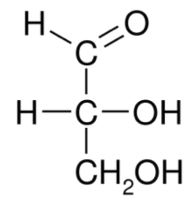
Isoprene

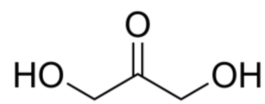




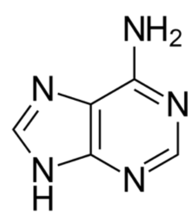
Carbohydrates

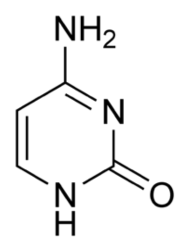




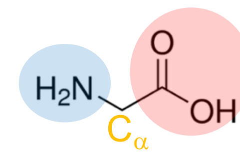


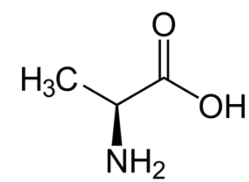
Heterocyclic Aromatic “Nucleobases”

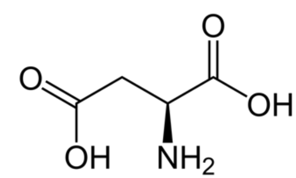


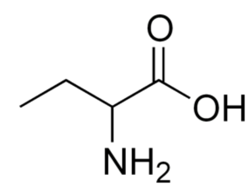


α–Amino Acids









taken together – these types of compounds were sufficient to advance chemical evolution.

Simple Organic Compounds Are Nice. Excitingly, As Their Concentrations Increase … What Does Also Increase, And Why Is This “Good” For Life?

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Answer: rate of diversification; is good because diversity increases as “chemical space” is explored (chemical kinetics v ~ [compound(s)]). And along with this diversity come more complicated composite organic compounds (= molecules that combine different types of simple organic precursor molecules) and polymers

Why are they good?

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Answer: the increased structural complexity, at some point, is likely to impart new functional properties that are advantageous over just the single constituent.

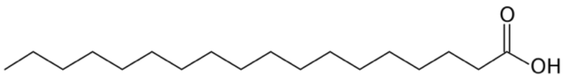
Let's take a look at a few examples

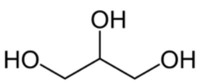
http://en.wikipedia.org/wiki/Cofactor_(biochemistry)

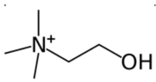
(great resource, fully annotated and cross-referenced)

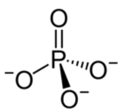
Combinatorial Chemistry Explores Chemical Space

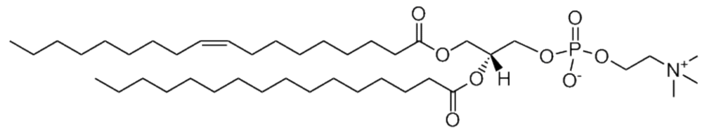
let's call this "LATERAL" if building a complex molecular template
involves precursors from different molecular families

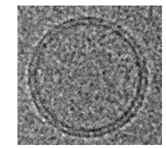


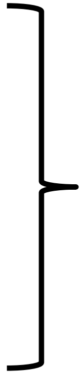




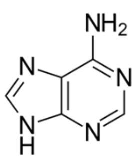


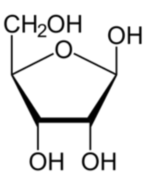


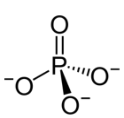


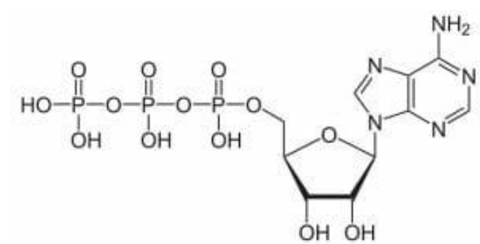


2 long chain acids + glycerol + phosphate + "headgroup" = phospholipid
➔ membrane formation (*Biochemistry Fundamentals Collection – Lipids, Advanced Biochemistry Collection – Lipids and Membranes)*





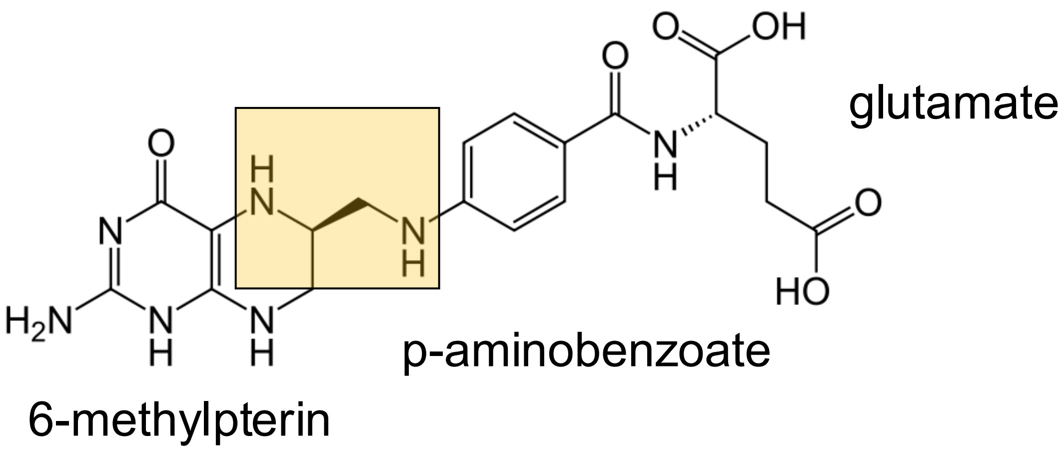




adenine + ribose + (1, 2) or 3 phosphates = adenosine triphosphate
➔nucleic acids, energy, signaling, protein modification
(*Biochemistry Fundamentals Collection – Nucleic Acids, Advanced Biochemistry Collection, various chapters)*

"Lateral" Exploration Of Chemical Space Also Created Less Familiar Looking Compounds

Tetrahydrofolate



function: Transfer of C1-carbon units (–CHO, $-CH_3$, $-CH_2OH$)
(carried by/between N-atoms in region highlighted in orange)

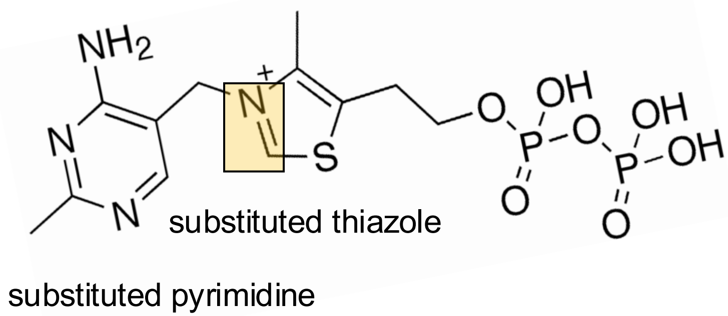
"lateral" - in this case: an amino acid + benzene derivative + pterin derivative
(which by itself is the combination of a pyrimidine and pyrazine)

don't get frustrated by these … there is no expectation that you know what they do, how they do it, and what part of the molecule serves as staging area for supporting the cell's chemistry (we will cover this in later chapters)

introducing them here is just to give you a sense for the spectrum of things that appeared … and to prime your "chemistry hat" to catch on to (some of) the interesting parts of the molecule's chemistry

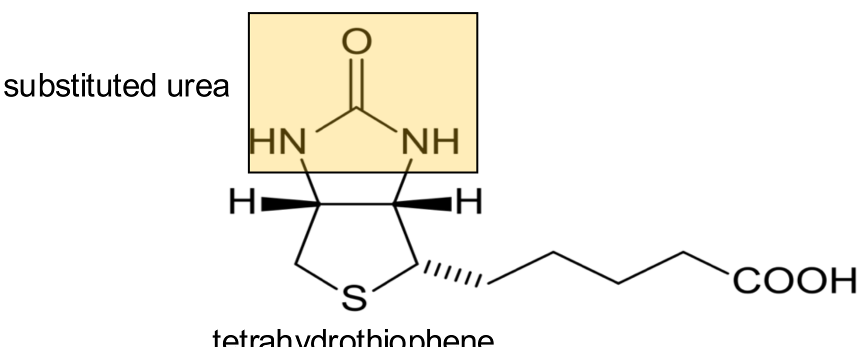
➔ looking at molecules asking yourself "**what could they do and why?"** is an important part of learning and understanding biochemistry

Thiamine Pyrophosphate



function: Transfer of groups with 2-carbon atoms and decarboxylation (removal of CO_2)

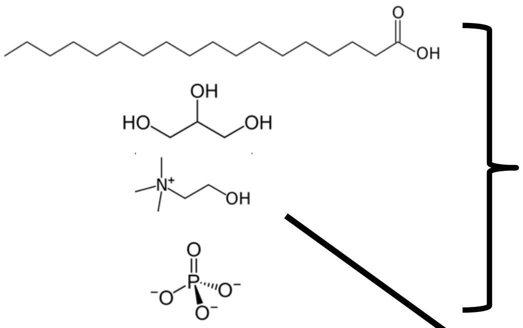
Biotin

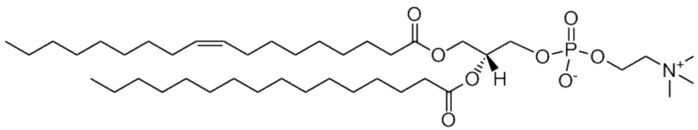


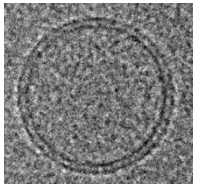
carboxylation (addition of CO_2 to a molecule)

"Vertical" Exploration Of Chemical Space Creates
REDUNDANCY

Redundancy refers to the use of molecular or process variants to modulate/tweak the function or output of a common molecular scaffold or process.

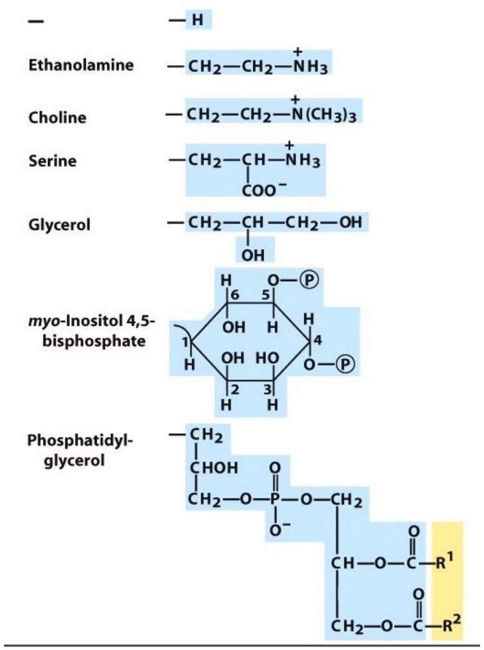






2 long chain acids + glycerol + phosphate + "headgroup" = phospholipid
➔ mostly membrane formation

Headgroups:

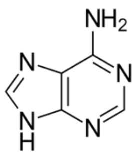


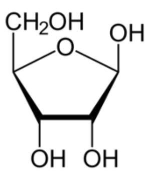
"vertical" = swapping a singular component in a complex molecular template molecule

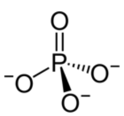
here: template = 2 fatty acids + glycerol + phosphate + headgroup

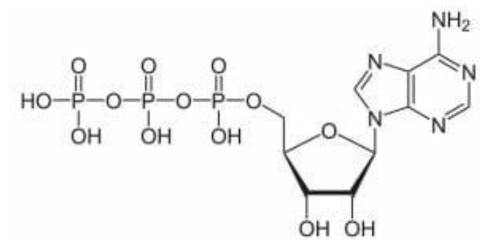
➔ swapping of headgroups creates a closely related family of molecules that have similar overall characteristics but are also different enough to contribute to biochemical solutions in very specific ways.

REDUNDANCY - CONTINUED

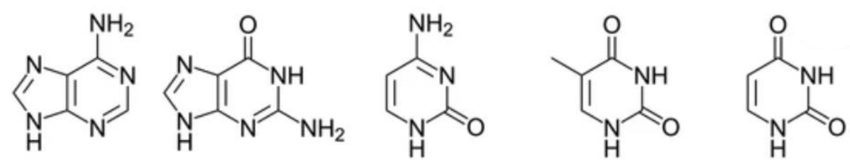








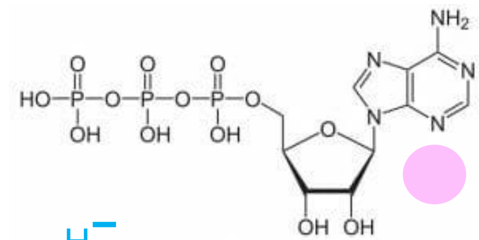
adenine + ribose + (1, 2) or 3 phosphates = adenosine triphosphate

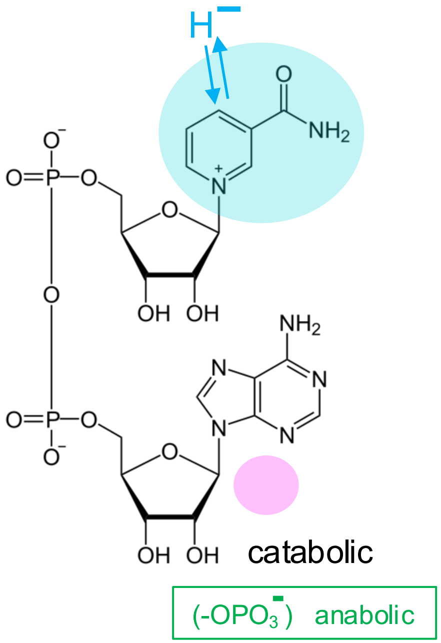


A Adenine **G** Guanine **C** Cytosine **T** Thymine **U** Uracil

swapping of the nucleobase creates a small group of related molecules that we discovered to be uniquely suited for information storage and transmission (*Biochemistry Fundamentals – Nucleic Acids*)

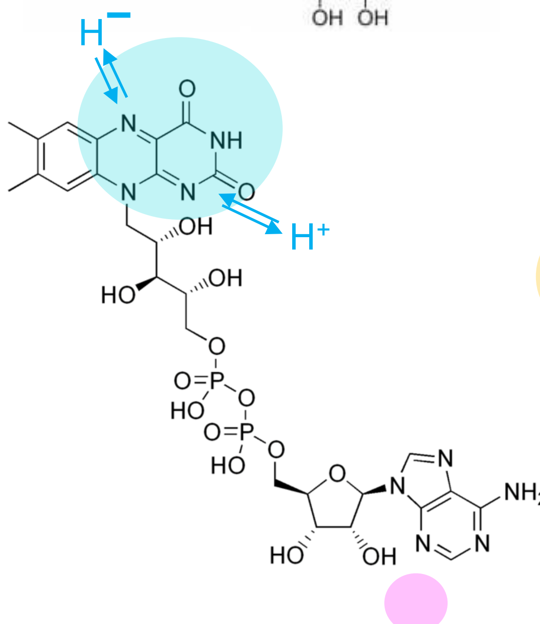
And Some More Tweaking …….





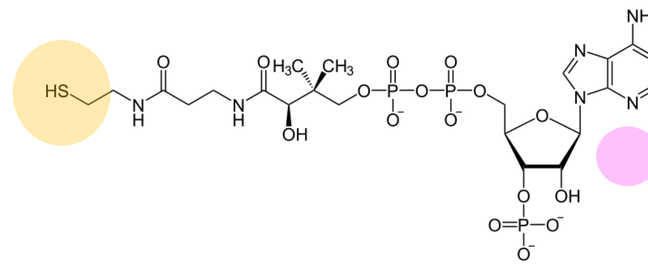
Nicotinamide Adenine Dinucleotide (NAD(P)$^+$)

Redox Coenzyme



Flavine Adenine Dinucleotide (FAD)

Redox Coenzyme



Coenzyme A

Acyl-Carrier

there are endless possibilities how to mix things up – yet only a finite number of compounds survived the culling….why?

Abundance, Stability, and Modularity

while randomness played big in the auditioning (and the diversification of life later), final casting of molecular inventory was driven by "hard" boundary conditions like

- abundance of a chemical species
- chemical stability/reactivity

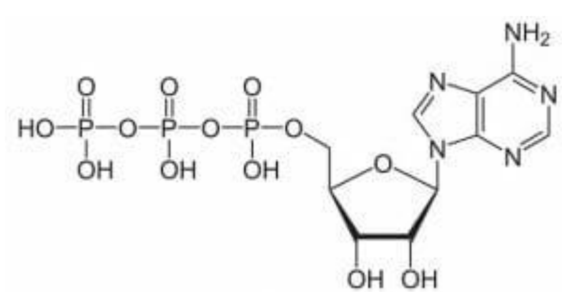
And, more significantly:

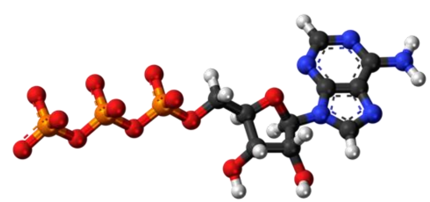
- efficiency/cost : benefit ratio, and
- modularity

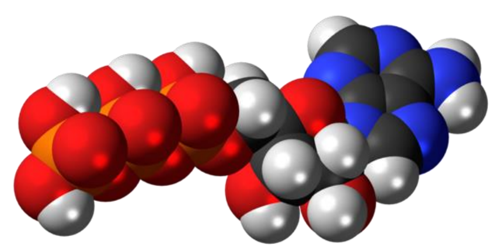
modularity: use of structurally/functionally **identical** building blocks, molecules or processes in the implementation of **different** biological contexts.

(you develop this molecule once and it is super successful because you can use it in many ways = very high benefit without additional "cost")

example: ATP Adenosine Triphosphate



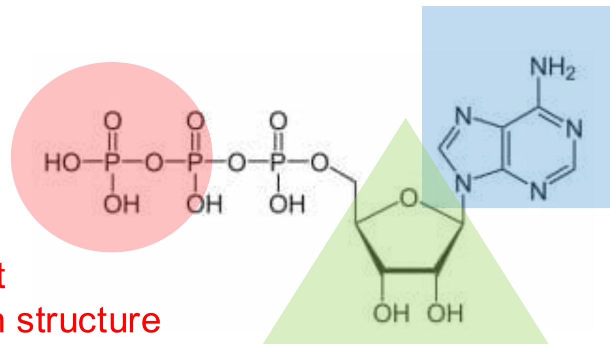




ATP Modularity Is Exceptional

- **Molecular Recognition**
  - ✓ information storage
  - ✓ allosteric regulation of enzymes
  - ✓ signaling molecule

- **chemical activation**
- **motor protein function**
  - ✓ muscle contraction
  - ✓ motility cell
  - ✓ vesicle transport
  - ✓ cilia movement
  - ✓ chromosome movement
  - ✓ remodeling of chromatin structure
  - ✓ transcription
  - ✓ translation



- **Polymerization (nucleic acids)**

- **active transport across membranes**
- **chemical modification**
  - ✓ Modification of enzyme activity
  - ✓ Modification of organelle dynamics
  - ✓ Modification of small molecules

many more miscellaneous functions that involve various mixes of the components

this is as close to a perfect molecule as you can get. EVERY part of it can serve at least one function

.... ATP Is Everywhere.....

...let's get some sense of what that means .....

the ubiquitous use of ATP raises an interesting question: **how much ATP does a human body use every day?**

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Answer: your own body weight!

→ how much is that in a 75-year life time? How does that number compare to real world objects you can think of?

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Answer: ~150 lbs of ATP **per day** ➔ in 75 years =1.8 million kg (~400 elephants / ~40 Boeing 737 aircrafts)

How much it would cost to order a day's supply of ATP from a chemical reagent suppplier.

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how much it would cost to order a day's supply of ATP from a chemical reagent suppplier?

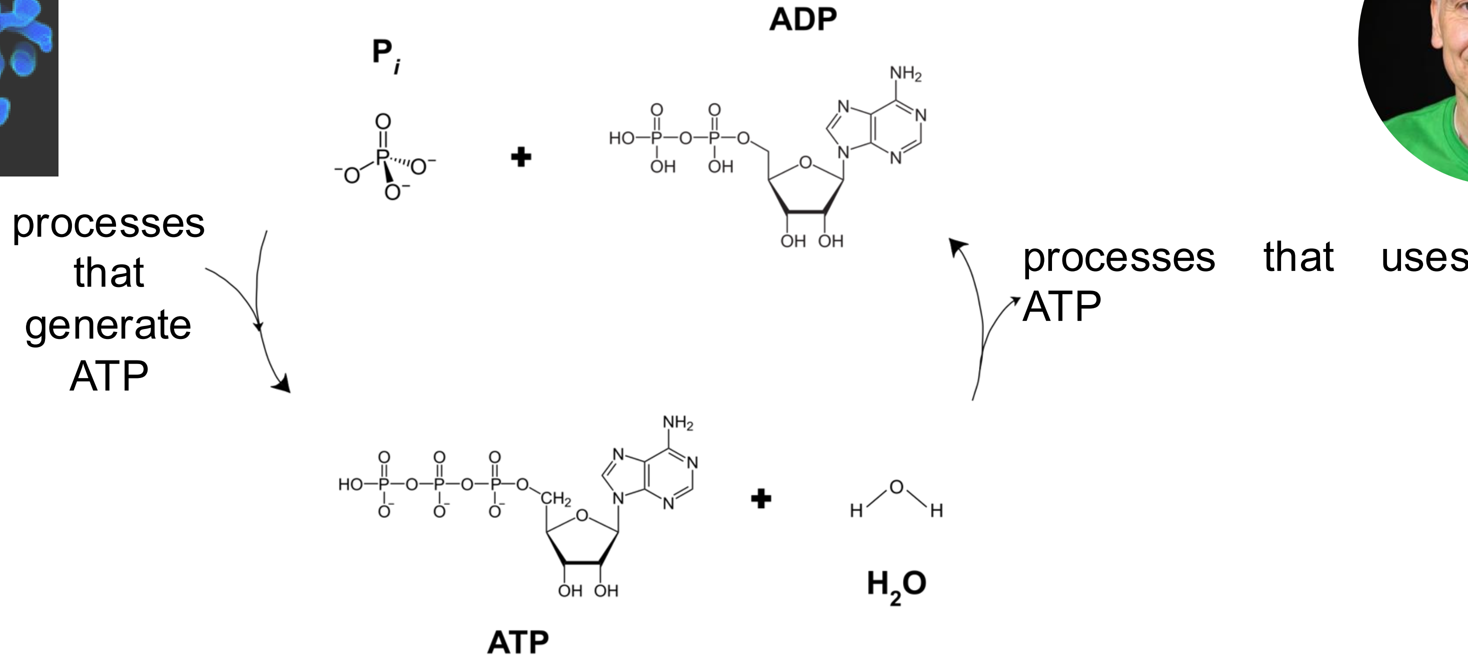
Answer: can't buy 100% pure ATP ➔ best approxmation is the highest purity available off the shelf (99%): ~1 million/day

Conclusions:

- by human standards and considering that ATP is one of the simplest molecules inside us, the pure chemical value and output of a body are staggering.

- Recycling efficiency is exceptionally high because
at steady state, the body contains 0.2 mol of ATP ~100g

ATP – Recycling (Overall Reaction)

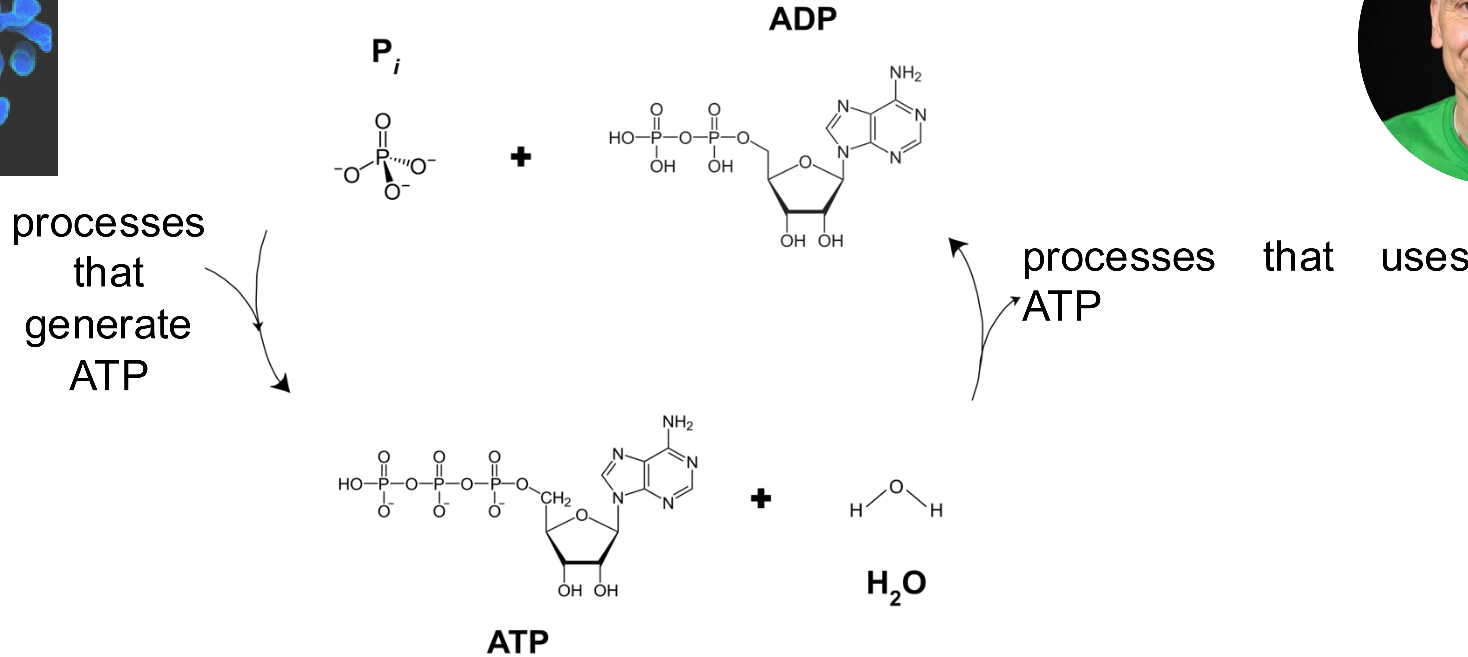


https://en.wikipedia.org/wiki/Adenosine_triphosphate#/media/File:ATP-ADP.svg

forgetting the explicit details for a moment: If you worked through the "Molecular and Cell Biology" Collection (Chapter on Molecular Signaling), then this should trigger a memory ….. **what are you looking at?**

(if you didn't work through Molecular and Cell Biology, then please skip forward to slide 43 because the next few slides (in all likelihood) won't make any sense to you whatsoever)

ATP – Recycling (Overall Reaction)



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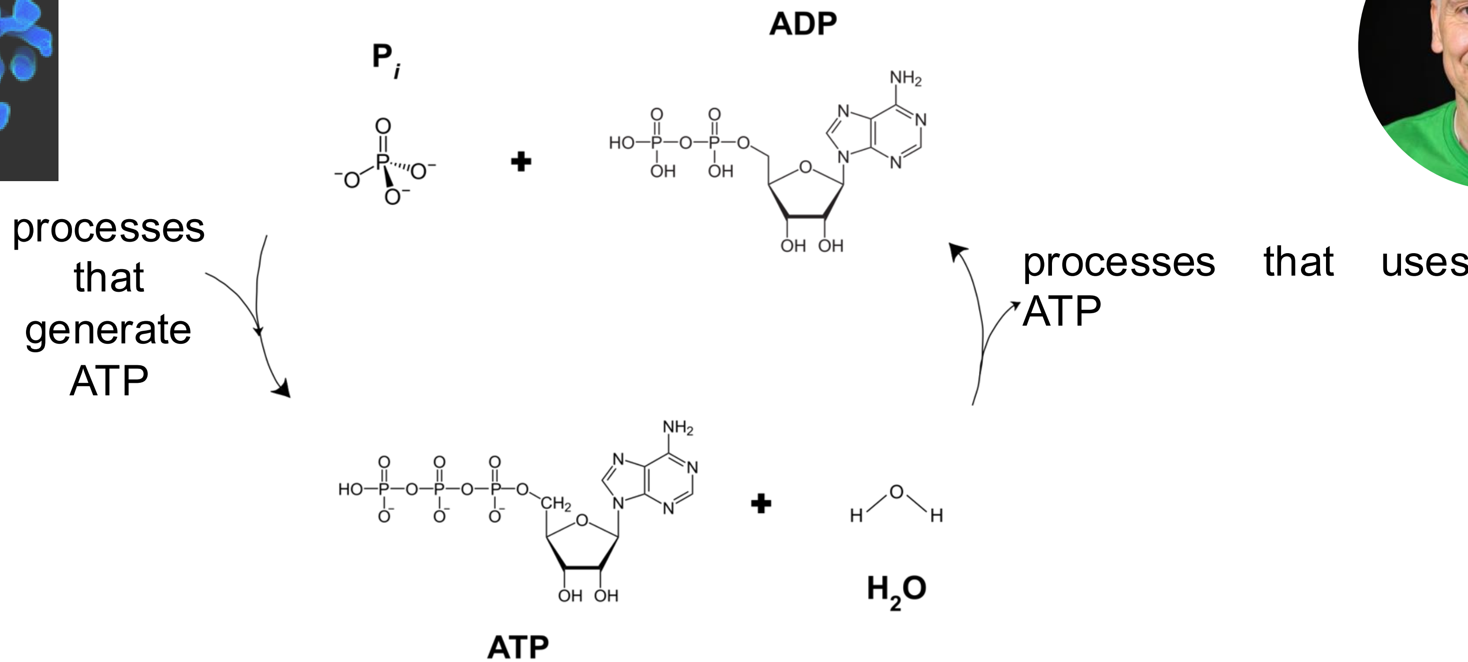
Answer: a "substrate cycle" …. what properties do they have based on what we covered in the Molecular Signaling Chapter of the "Molecular and Cell Biology" Collection?

ATP – Recycling (Overall Reaction)



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Answer: a "substrate cycle" …. what properties do they have based on what we covered in the "Molecular Signaling" Chapter of the "Molecular and Cell Biology" Collection?

Answer: they impart **hypersensitivity** and through that can be **used to build bistable switches + oscillators (by feedback regulation of either or both branches).**

recall: hypersensitivity = increased detectability of changes in net flux through a paired set of reactions (forward – backward) that are catalyzed by independently controllable enzymes)

while it is a detour - **let's think about the significance of "substrate cycles" in this particular context** because it allows you to get a glimpse of why chemical evolution picked a few favorites….

ATP Cycling – Exploiting The Good, While Avoiding Evil

What about Hypersensitivity?

given the myriad processes that consume ATP, you definitely want the cell to pay VERY close attention to [ATP]. To keep a stable steady state requires the system to take note of even small changes, and to correct accordingly

➔ **hypersensitivity is a good thing in principle**!

there is **one problem though** … given the large number of processes that impact [ATP] usage and generation, there is **zero likelihood** that this substrate cycle has **zero feedback loops** = need to think about possible **emergence of bistable or oscillatory behavior.**

bistability would be fatal: a sudden, extreme drop in [ATP] will irreversibly crash the chemical engine because ATP is needed for so many things.
(a sudden increase would also be bad because the energy yield from ATP would be lower, making reactions that need ATP less favorable/impossible)

fortunately, bistability **cannot establish itself because**

- ✓ **ATP synthesis and breakdown occur in more than one paired set of reactions** whose regulatory feedback loops are different from each other.

→ the many **positive/negative feedback loops largely cancel each other out**

= **integration of the large number of feedback loops leaves a small regulatory oscillation, which – incidentally – is more helpful than simple hypersensitivity. Why?**

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- ✓ The many **positive/negative feedback loops largely cancel each other out**

= integration of the large number of feedback loops leaves a small regulatory oscillation, which – incidentally – is more helpful than simple hypersensitivity. Why?

Answer: simple hypersensitivity is meant to turn a small input signal into a significantly larger output response.
since such changes occur ALL the time, plain hypersensitivity poses the danger of a "runaway" response.

➔ to avoid a "runaway" situation [ATP] is controlled by pitting acceleration against braking at ALL times. The resulting small oscillations about the steady state threshold [ATP] do exactly that = they keep the system close to its most sensitive state while preventing runaway situations.

➔ the small/dampened oscillations create an exceptionally robust system!
(that because of the many inputs remains constantly aware of and integrates EVERYTHING that happens)

Robustness Is Great….But Still Deadly Without Failsafe

the cursory look at the systemic ATP-ADP substrate cycle left us with the insight that the chemical engine's energy supply is phenomenally robust.

yet, without a "failsafe" the very same design WOULD KILL YOU if - despite all control - [ATP] becomes depleted because of an extreme boundary condition, such as starvation (low/no glucose intake), hypoxia, or toxins blocking the respiratory chain that powers most of ATP synthesis

Alas, **how do you avoid getting killed by the "robustness" that normally keeps you alive?**

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Alas, **how do you avoid getting killed by the "robustness" that normally keeps you alive?**

Answer: First protective measure is "ATP buffering" ("Gen Chem" anyone??) by exploiting the enzyme adenylate kinase that catalyzes the reaction: 2ADP --> AMP + ATP

➔ first clue why ATP was chosen as energy currency instead of ADP. More importantly, however the **action of adenylate kinase generates an independent signal that is outside the basic ATP:ADP substrate cycle….and that signal is AMP**

TABLE 15–4 Relative Changes in [ATP] and [AMP] When ATP Is Consumed

Adenine nucleotide	Concentration before ATP depletion (mM)	Concentration after ATP depletion (mM)	Relative change
ATP	5.0	4.5	10%
ADP	1.0	1.0	0
AMP	0.1	0.6	600%

Note: adenylate kinase <u>amplifies</u> the amplitude of [ATP] change!

➔ **Second protective measure:** rise of [AMP] signals that an "energy-supply failure" is imminent

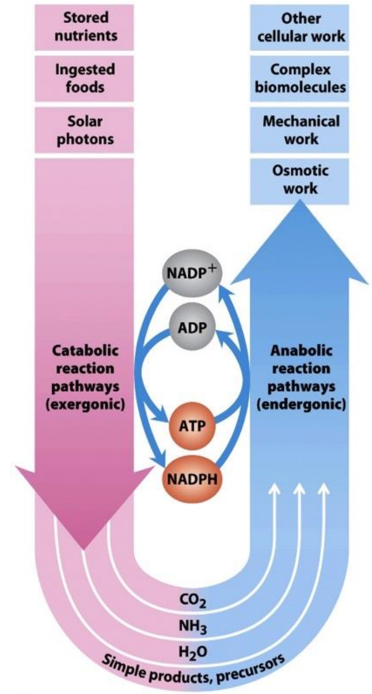
➔ triggers the "failsafe": activation of AMP-activated protein kinase (AMPK) ➔ metabolic switching in most organs. For instance: skeletal muscle: switch to fatty acid oxidation and formation of mitochondria; liver stops to make glucose, fatty acids, and cholesterol. In addition, cell-cycle progression is arrested, and most biosynthetic processes will cease until [ATP] returns to normal.

Coming Back To The "Why's" in Chemical Evolution

the detour about ATP-cycling gave you a glimpse of why this molecules became so central, but ….things do not stop at ATP…..

…the overall design of the chemical engine pitches two antagonistic branches against each other

- **Catabolism:** breaks down organic matter to useful metabolic intermediates and a common set of energy depleted end-products (most important: H_2O; CO_2)
- **Anabolism:** builds complex organic molecules and polymers from smaller precursors



Catabolism and Anabolism are coupled to each other through:

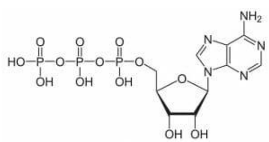
- energy currency (catabolism yields; anabolism consumes)
- redox equivalents (catabolism yields; anabolism consumes)

What is **interesting here is that the branches are integrated through use of three redundant ADP-derivatives**:

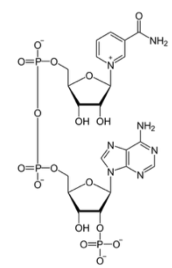
- ✓ ATP – universal energy currency in both branches
- ✓ NAD^+ - redox currency in catabolism (see slide 28)
- ✓ $NADP^+$ - redox currency in anabolism

Why can energy metabolism thrive on just one compound, ATP, while redox management requires two currencies?

Answer: always want [ATP] high – but for the redox equivalents the oxidized form of the carrier needs to be high in catabolism while the reduced form must be high in anabolism ➔ short of physically separating these two branches completely, the conflict can only be resolved through chemically different carriers.



ATP



$NADP^+$

Chemical Evolution – Redundancy And Modularity Summary

The previous arguments were dense … what are the take home messages?

- ✓ Harsh climate and metal/acid dependent catalysis on early Earth promote the formation of a complex mix of (primitive) organic molecules
- ✓ Over time, combinatorial chemistry results in a diverse repertoire of complex organic molecules that combine >1 kind of compound type ("lateral" diversification)
- ✓ Combinatorial chemistry also results in the emergence of redundant classes of compounds ("vertical" diversification), some of which are the chemical evolution equivalent of the "Goldilocks zone" in astronomy. Meaning: a few select compounds emerged in which just every single aspect of their structure and reactivity profile was right to promote the emergence of life.

  - ❖ Most prominent example for a "Goldilocks compound" is ATP, which not only is part of a redundant cluster of nucleotides (that later turn into informational molecules ..among other things), but also displays extreme modularity (ability to drive a large spectrum of different processes)

- ✓ The modularity of ATP will establish a "systemic" substrate cycle that becomes an exceptionally robust cornerstone for the integration of metabolism.
- ✓ At the same time – $NAD(P)^+$ - emerge as master regulators and integrators of redox chemistry. Notably – both are members of the ADP-centric redundant cluster of compounds, further explaining how a small number of key components became integral for all further increase in chemical complexity.

Beyond Compound Molecules

“composite compounds” are VERY USEFUL … but…keeping an eye on “life”, they are not enough. **Why?**

…your thoughts?...

Beyond Compound Molecules

"composite compounds" are VERY USEFUL … but…keeping an eye on "life", they are not enough. **Why?**

- **several answers** to that question:
- these more complex compounds do not carry (much) information,
- **single copies** of them are very small compared to the scale of cells = can't implement a macroscopic feature
- they also seem to be reaction-partners rather than able to actively drive reactions themselves.

- ➔ in order to "build life from scratch" we need more complicated compounds → oligomers/polymers….

What Oligo-/ Polymers Can We Make From Biologically Relevant Building Blocks?

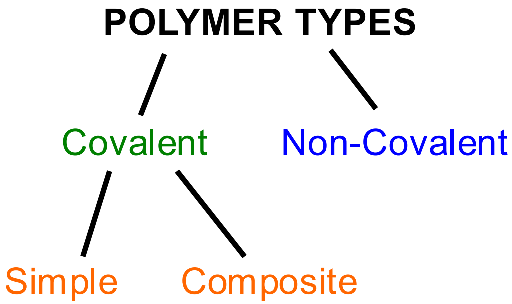
let's briefly review what you remember…..

based on their backbones (=repetitive structure that is found in every unit of the oligo-/polymer), biology is build on **two fundamentally different types of organic oligo-/polymers:**

➢ **Covalent and Non-Covalent**

✓ Covalent oligo-/polymers can be subdivided into two subtypes: simple (backbone is formed by a single component) and composite (backbone is formed by two chemically different components).

✓ **Non-Covalent:** these polymers have no defined backbones and are held together by weak interactions only



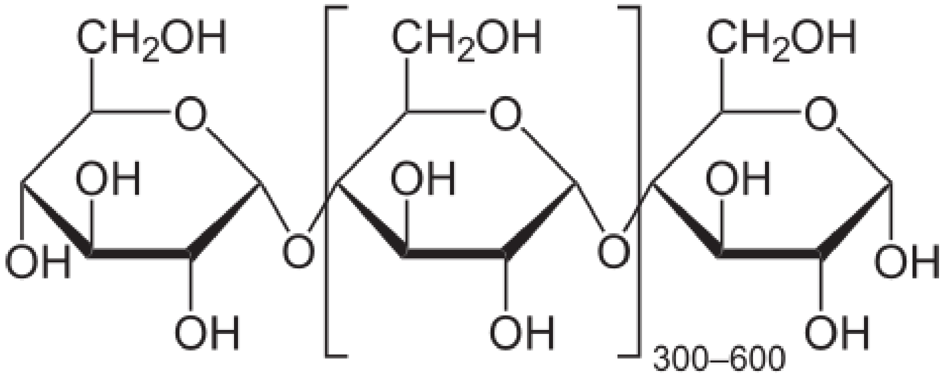
➢ **Examples (oligomer, polymer classes)**

✓ **Covalent (simple):** protein, polysaccharides, porphyrins

✓ **Covalent (composite):** nucleic acids

✓ **Non-covalent:** membranes, non-covalent protein aggregates like microtubules (formed from α/β tubulin dimers) or F-actin (formed from G-actin).

Review: What About Polymers Made From Sugars? (= Polysaccharides)

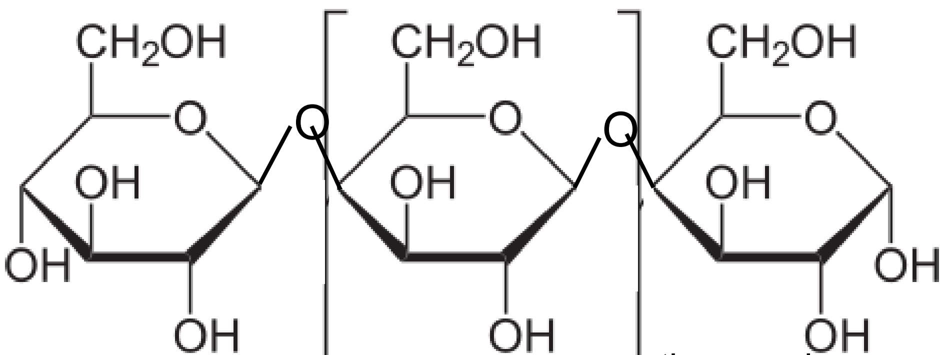


Poly-α(1-->4)-D-glucose

forms spirals

amylose (starch)

Both polymers are water insoluble, which is counterintuitive given the large number of hydroxyl groups that should be able to hydrogen bond with water (see: Fundamentals - CARBS)



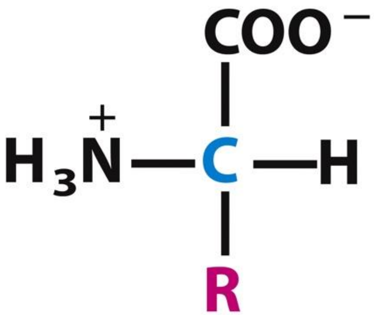
Poly-β(1-->4)-D-glucose

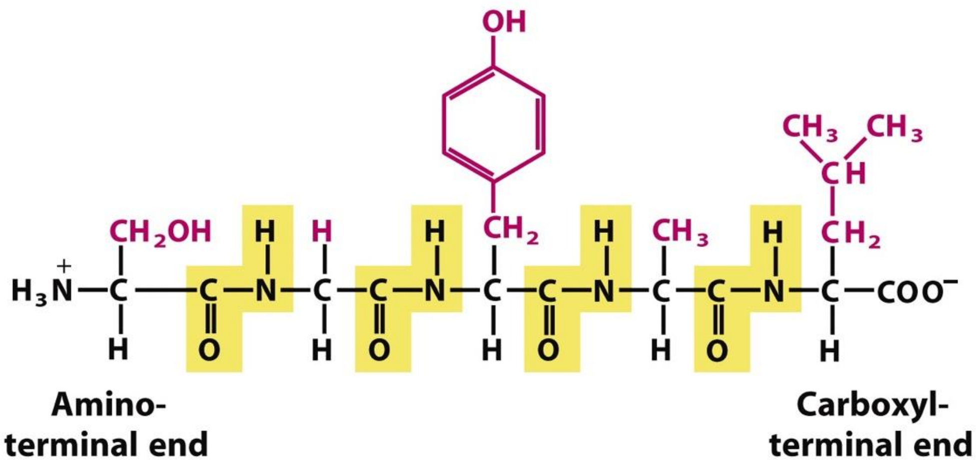
forms linear polymers that laterally assemble into sheets

cellulose (wood)

this goes into the right direction: each of them is large, and can perform one very useful function: nutrient/energy storage (amylose) or mechanical support (cellulose)
(**review more detail:** "Biochemistry Fundamentals – CARBOHYDRATES")

What About Polymers Made From Amino Acids? (= Proteins)





Amazing - why?

the redundant set of amino acids contains 21 members (20 common + selenocysteine) with different chemistries in the sidechains. Depending on sequence, proteins can assume almost any 3D-shape. Add the option of varying size (n=3 to over 2000), this seems to be incredibly **versatile and perfect …but for what?**

Catalysis

but also **good for structural/mechanical support** (Fundamentals – Proteins). On the flip side, proteins are **bad for information storage - why is that?**

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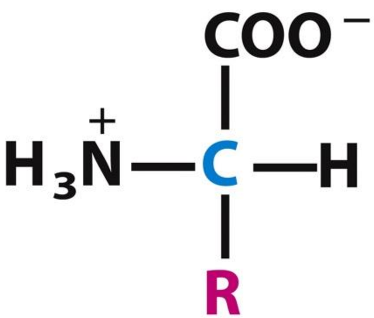

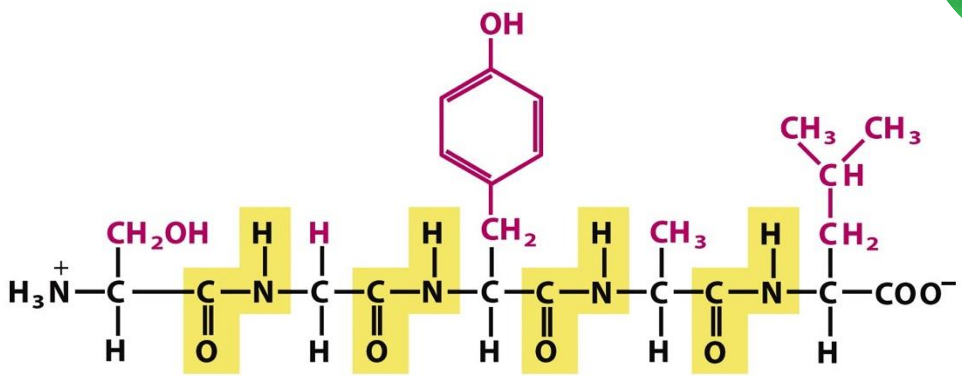

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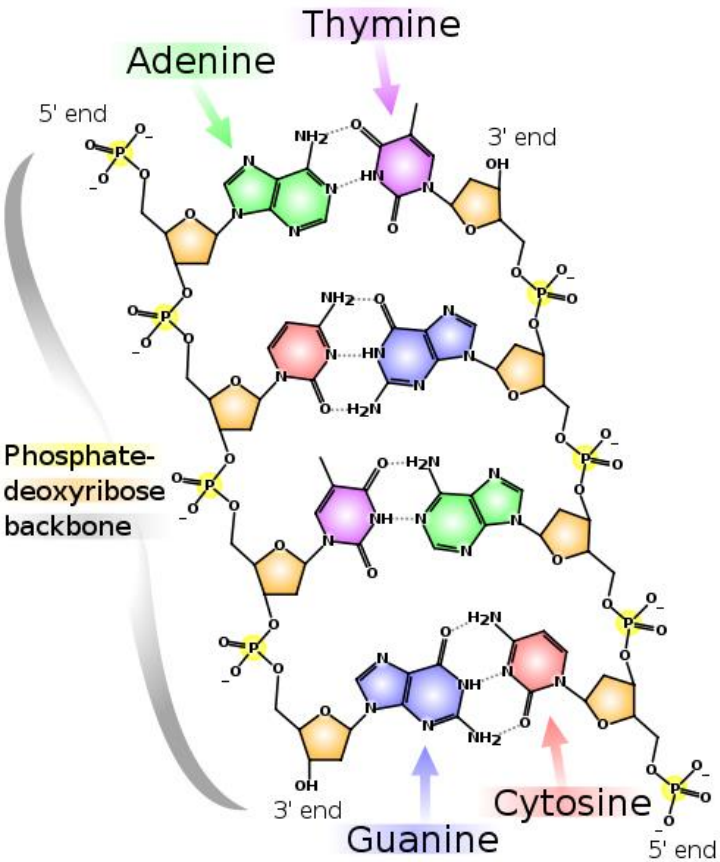
Answer: proteins, are too complex for information storage, in part because there is no clearly predictable correlation between sequence and overall shape

(which ultimately would carry the information; more detail "Biochemistry Fundamentals - PROTEINS)

This Leaves
Us With "Composite" Polymers …The Most Important Of Which Are… Nucleic Acids

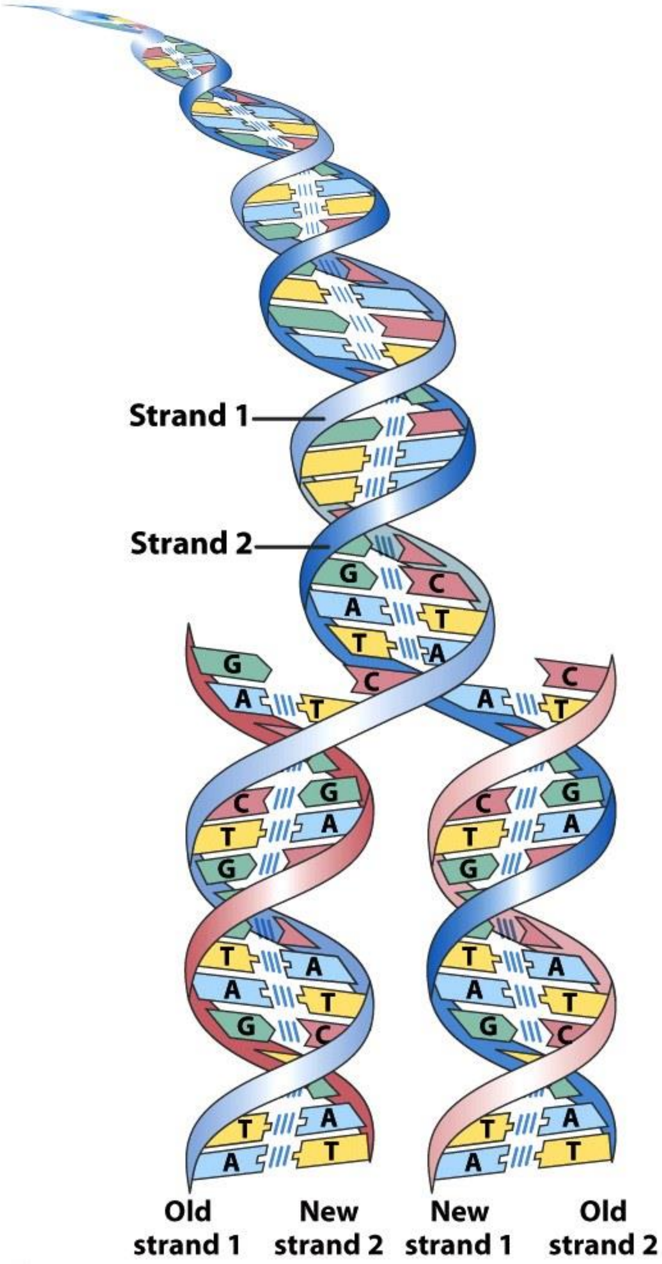


two varieties: DNA, RNA - vary in the sugar (deoxyribose, ribose respectively) and one of the bases (thymine, uracil).

opposed to proteins, these polymers are perfectly suited for information storage
(review "Fundamentals – Nucleic Acids"
if you don't remember why)
they are just complex enough to encode information, and the complementarity between bases allows for transcription, translation and heredity because it makes base pair interactions specific.

note: **complementarity and specificity** are another two **really important concepts**
(see also "How Do Molecules See? – Pt1; slide 15)

FIGURE 1–29



INTEGRATION

based on the "Molecular and Cell Biology" Collection you should….

- know approx. size of a bacterial genome + how it compares to eukaryotic genome
- be able to explain how a bacterium can fit all this DNA into the small cell (dimensions?) + how this chromosome structure differs from chromatin structure in eukaryotes.

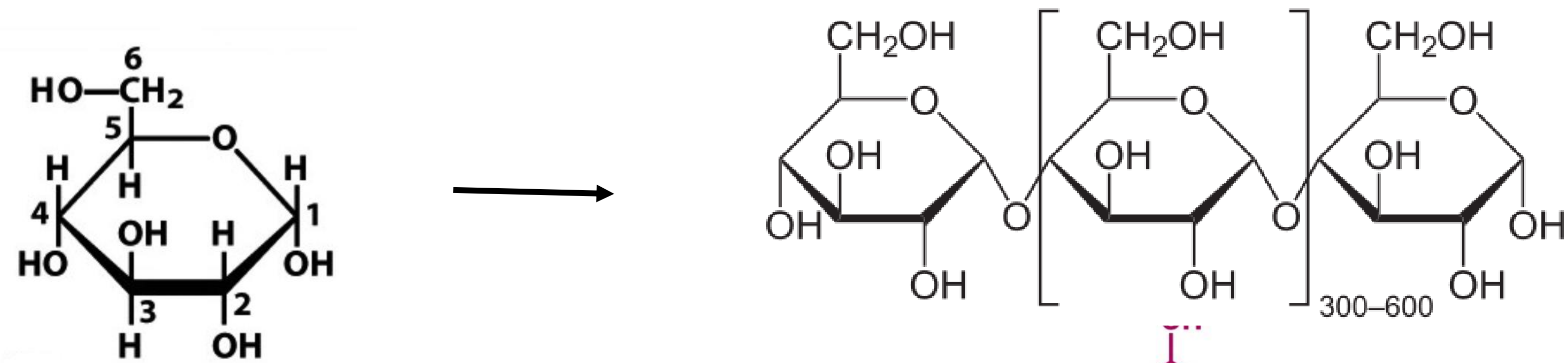
If you do not remember – review the "Chromatin" Chapter of the "Molecular and Cell Biology Collection)

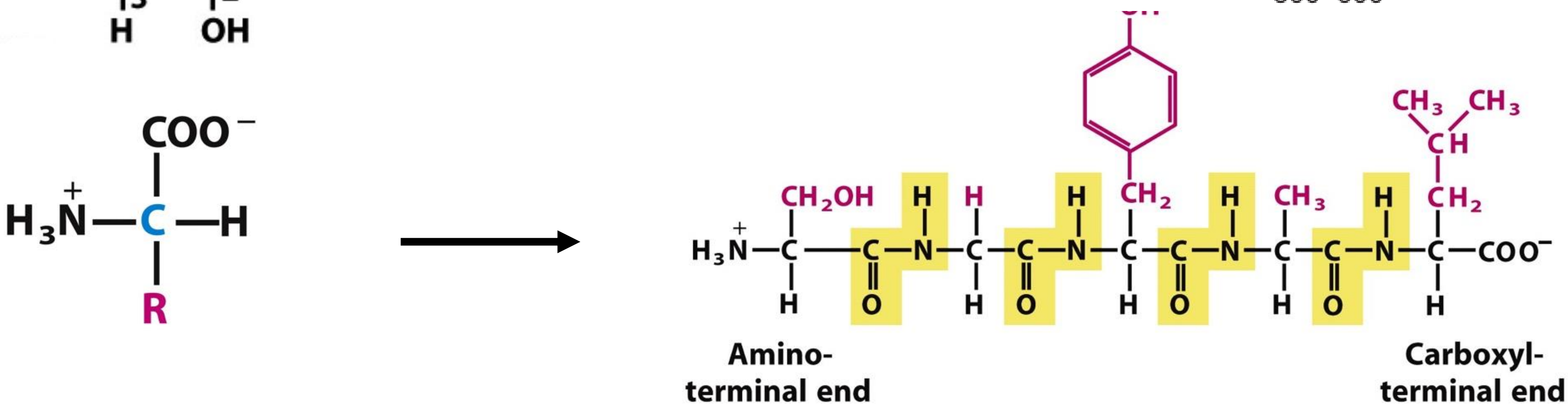
the three classes of covalent polymers can meet requirements for **energy storage, information storage, mechanical support and catalysis** but they are **<u>endpoints</u>** = … going back to beginnings - we still owe an answer/need a theory of how these polymers could emerge in the first place.

under the conditions of the early atmosphere, some small oligomers may have arisen just by chance. But to get bona fide polymers, and to make their synthesis reproducible and controllable, some “breakthroughs” had to happen to advance beyond the stage of small(ish) molecules - **why?**

a big part of the answer comes from the chemistry of how the polymers form

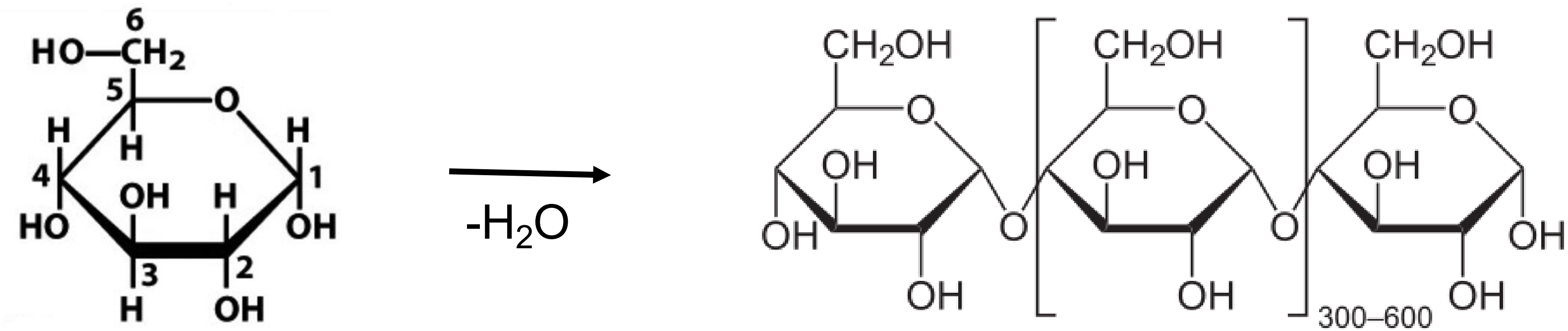
Let's Look At Them Again

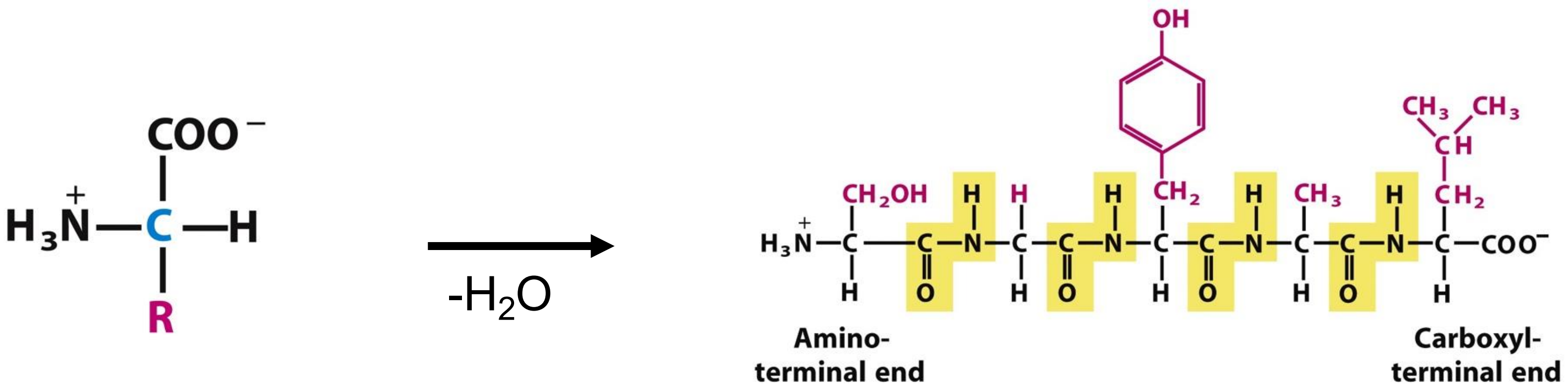




what type of reaction underlies polymer formation in these cases?

Let's Look At Them Again





what type of reaction underlies polymer formation in these cases?

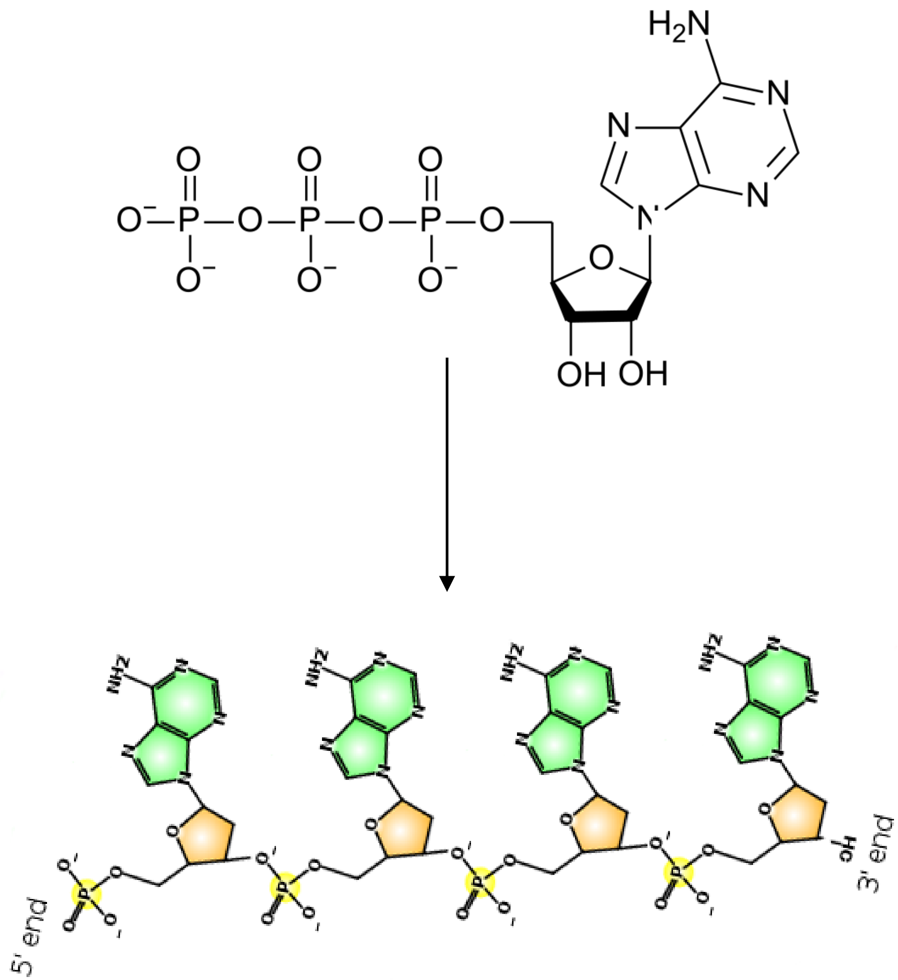
condensation

= formation of bond through elimination of a small molecule

(often, but not always water)

this is very inefficient if not catalyzed, especially for proteins

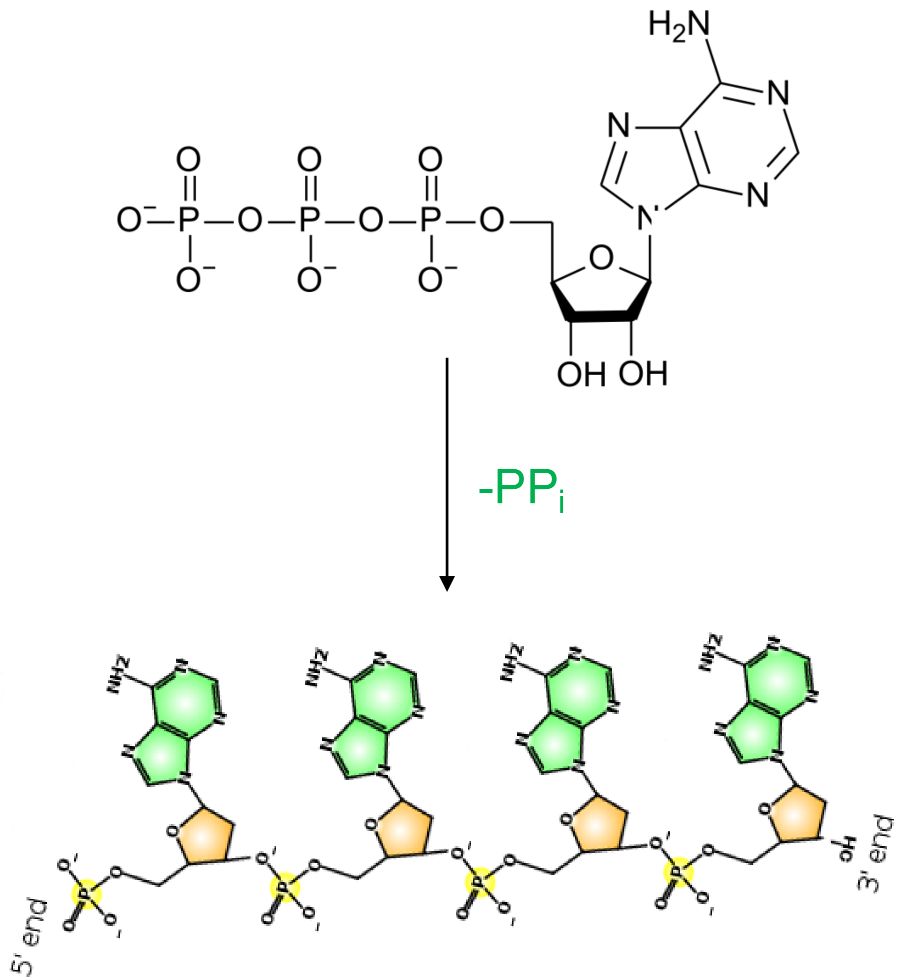
And Nucleic Acids:



in principle the same but with one **BIG** difference:

..try to verbalize ….

And Nucleic Acids:



In principle the same but with one **BIG** difference:

in this condensation the precursor contains a “high-energy bond” (phospho-anhydride), and additional energy could come from hydrolyzing pyrophosphate that is also released.

as a consequence, the **formation of oligonucleotides was easier and more efficient under the conditions of early earth,** which gave rise to the first RNAs (and later DNAs). As we will see shortly, the easier access to oligonucleotides - combined with other breakthroughs - was key for things to go forward

Barebone
SUMMARY

starting with very primitive compounds, chemical environment of early Earth promoted the formation of

✓ Simple organic molecules
✓ Complex/composite organic molecules
✓ Primitive polymers

Examples for
primitive organics: sugars, amino acids, lipids, nucleobases

Complex/composite organics: ATP, NAD(P), Biotin, Tetrahydrofolate, Thiamine Pyrophosphate

Simple Oligo-/Polymers
porphyrins --> carriers for metal ions
amylose, cellulose --> energy storage, mechanical support
proteins/polypeptides --> catalysis, mechanical support

Composite Polymers:
nucleic acids --> information storage

implementation of these early players was driven by:
Catalysis
Recycling
Modularity & Redundancy
Complementarity and Specificity
all of which represent fundamental concepts that are realized throughout the molecular design of life

From Molecules/Polymers To Cells

polymers were a necessity to advance towards life. Yet, **making polymers by pure chemistry is not enough – some additional breakthroughs are needed. What are they?**

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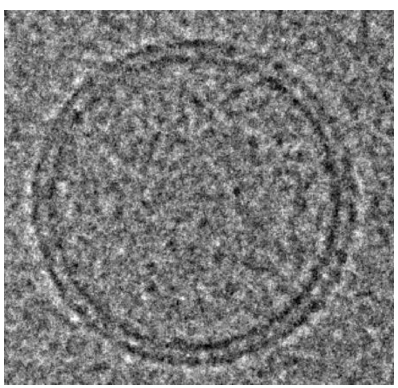
why is compartmentalization (yet another concept) **an enormous advantage?**



EM micrograph of a liposome
Recall: Fundamentals - Lipids

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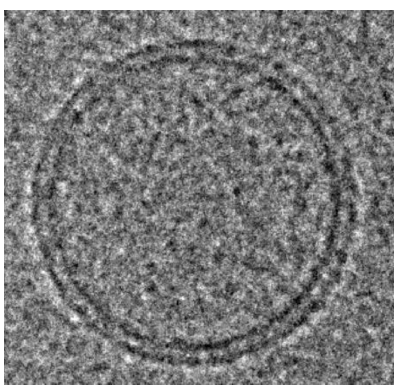

EM micrograph of a liposome
Recall: Fundamentals - Lipids

1. Sequestration increases the likelihood of chemical reactions to occur
2. Sequestration allows to establish/maintain non-equilibrium conditions, which is a hallmark of life
3. Establish compartments inside compartments, keeps different chemistries apart from each other.

The "Dark Side" Of Compartimentalization

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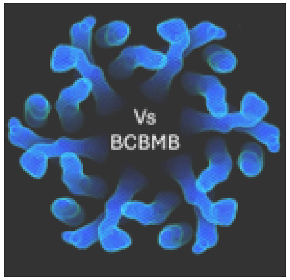

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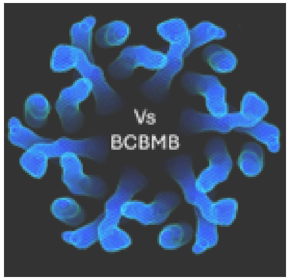
1. develop strategies for solute/material exchange between compartments

2. require mechanisms that allow communication both within and with the surrounding environment, and

3. develop a sense of “self” and a mechanism to retain + propagate information about identity

how can you address these downsides of boundary-associated compartmentalization from an engineering point of view?



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Answer: boundaries are impermeable to polar molecules and large molecules. Why is that problematic?

Answer: need to

1. develop strategies for solute/material exchange between compartments

→ make semipermeable, selective "holes"

2. require mechanisms that allow communication both within and with the surrounding environment, and

→ develop inter- and intracellular signaling mechanisms

3. develop a sense of “self” and a mechanism to retain + propagate information about identity

→ develop an instruction manual that can easily be propagated and is capable to produce readily interpretable instructions … and that is where things started….

how can you address these downsides of boundary-associated compartmentalization from an engineering point of view?

RNA

The “Trojan Horse” of Chemical Evolution

of all polymers – a nucleic acid, ribonucleic Acid (RNA), emerged as the critical juncture that had what was needed to make life advance.
Why is that?

Answer: because the more favorable chemistry of polymerization compared to proteins and polysaccharides created a macromolecule that could meet two essential needs at once:

(1) complementarity between nucleobases allowed for **information storage** (heredity) **and transmission** (instructional), and

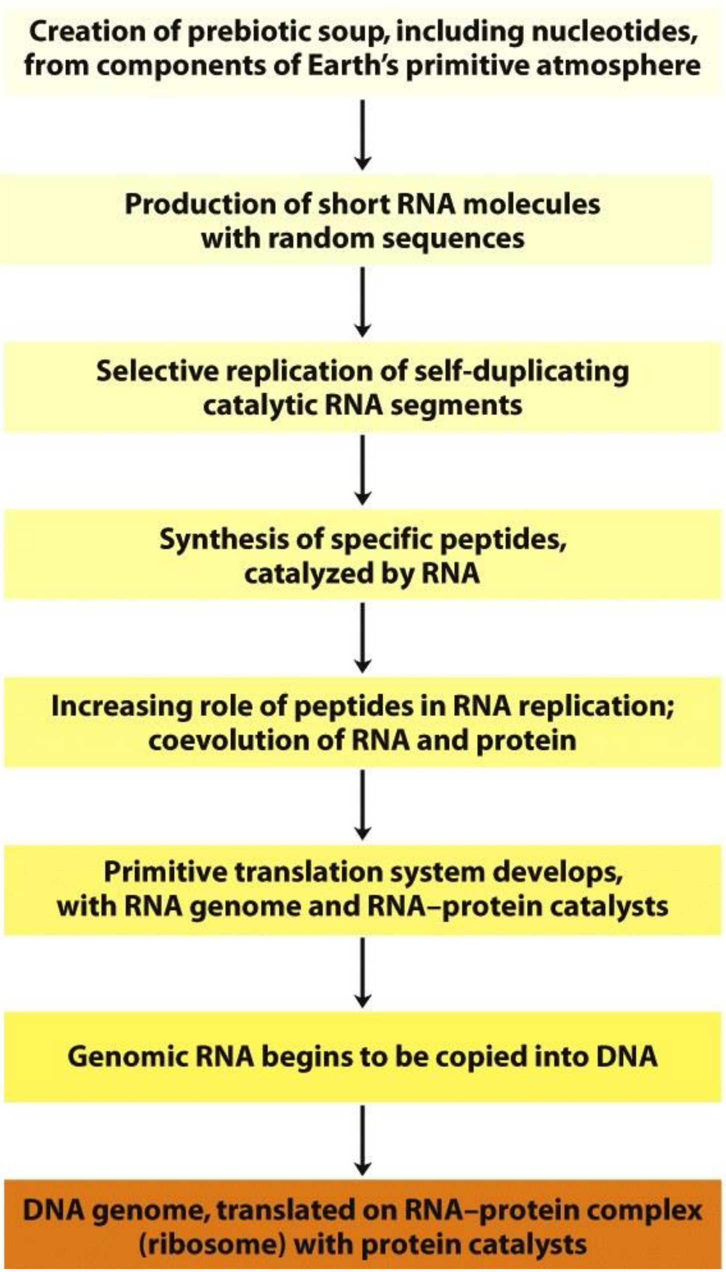
(2) the chemistry of RNA + its ability to adopt complex 3D-structures allowed for **primitive catalytic properties** of some RNA molecules, most notably: amino acid condensation (note: still true today… - catalytic center of ribosomes is RNA)

the powerful emerging catalytic properties of primitive oligopeptides/proteins in turn improved the efficiency of RNA replication and interpretation ….
→ a feed-forward loop was created.

in other words: take lipid enclosed compartment, self-replicating RNA, some primitive catalytic RNAs and small proteins, as well as a mix of organic small compounds ==> equivalent of a very primitive protobiont

A first SUMMARY:

insert compartmentalization somewhere here!

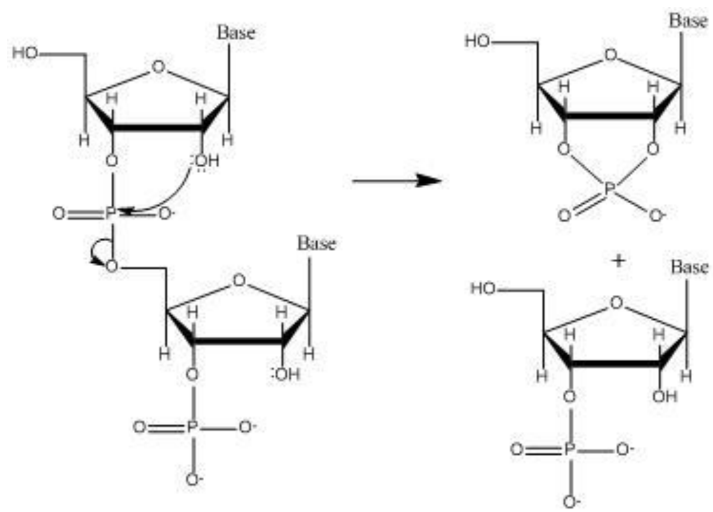


The Age of DNA

if RNA was so great - why did it later get replaced/overtaken by DNA?

Answer: DNA is chemically stable at pH closer to neutral or even basic, RNA is not because the additional -OH group in ribose allows spontaneous intramolecular backbone cleavage

(= genetic material would be prone to spontaneous self-destruction)



➔ as oceans/cells turned less acidic (~pH5.8 → 7), there was an advantage of storing information in DNA and to explore use of less stable RNA as messengers to guide protein synthesis → creates the first primitive prokaryote.

➔ add a nuclear envelope to protect your “chromosomes” and you are looking at the first primitive eukaryotic cell (from greek eu =true, karyon = nucleus).

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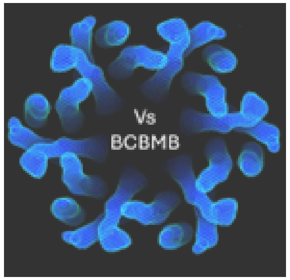
Answer: typically ~2μm prokaryotes vs 10-100μm diameter in eukaryotes (extremes: ~0.3μm-1m long extensions in some neurons)

➔ **Why have organisms settled on these average cell sizes?**



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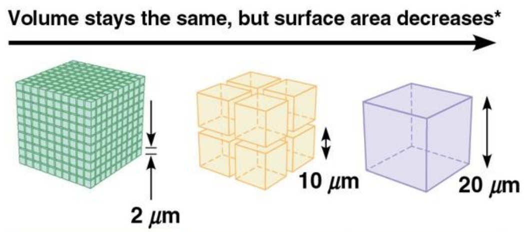

Number of cells	1000	8	1
Length of one side	2 µm	10 µm	20 µm
Total volume	8000 μm^3	8000 μm^3	8000 μm^3
Total surface area	24,000 μm^2	4800 μm^2	2400 μm^2
Surface area to volume ratio	3.0	0.6	0.3

*For a cube having a side with length s, volume = s^3 and surface area = $6s^2$.

Answer: **surface: volume ratio & diffusion limits**

- **surface:volume ratio matters** because it determines exchange rates between intracellular and extracellular
- **diffusion limits matter** because the time scale of diffusion, τ, relates to distance

$$\tau \approx x^2/D$$

x=distance; D=diffusion coefficient where

$$D \approx \frac{1}{MW}$$

MW = molecular weight in dalton

perspective: typical protein diffusion coefficient of ~10µm^2/s ➔ takes 10ms to diffuse across a bacterial cell (~1µm), 10s to diffuse across a mammalian cell (~20µm), and 10^6 s (= ~2weeks!) to diffuse down a 1 cm axon in a neve cell (➔ active transport mechanisms for cargos!; Mol and Cell Bio Collection)

compare: in cells, most chemical reactions take place at the millisecond time scale… so yes…diffusion limits are significant if you want a cell to run smoothly.

From Cells to Organisms

while **prokaryotes were self-sufficient at their given size**, another "breakthrough" was needed to advance towards eukaryotes and multicellular structures …. **what would that be?**

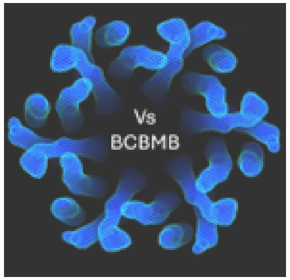

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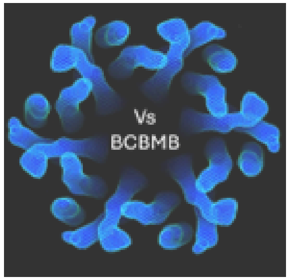
TABLE 1–1 Molecular Components of an *E. coli* Cell

	Percentage of total weight of +1cell	Approximate number of different
Water	70	1
Proteins	15	3,000
Nucleic acids		
DNA	1	1
RNA	6	>3,000
Polysaccharides	3	5
Lipids	2	20
Monomeric subunits and intermediates	2	500
Inorganic ions	1	20

Answer: comes from looking at the chemical complexity of a simple bacterial cell ….

→ **how does that compare to an organism like us?**
(if you worked through the "Molecular and Cell Biology" Collection …you should know the answer).



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Answer: the difference seems surprisingly small …. most significant differences:

- genome size (4**M**bp vs 3**G**bp) and
- 3,000-4,000 proteins (prokaryote) vs ~10,000 protein types expressed in human cells (out of ~20,000 gene repertoire, not accounting for alternate splicing, posttranslational modifications)

→ while this **increase in chemical complexity seems "small"**, building **and** maintaining this complexity not only requires lots of energy (recall: ~150 lbs of ATP **per day**)…but also needs mechanisms to generate **and** to use this energy more efficiently than in bacteria that are optimized for speed of cell growth
(= many bacterial processes are less energy efficient than counterparts in eukaryotes + timing of cellular processes is more flexible in prokaryotes than in eukaryotes)

The “Nuclear Bomb” Of Chemical Evolution

with the requirement for more efficient energy production and usage in mind …
what could have met this need?

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ironically: an accident …some catalyst released the first molecular oxygen.

in an anaerobic world - oxygen was an extremely potent toxin at first, turning everything upside down - **can you explain this?**

The "Nuclear Bomb" Of Chemical Evolution

in an anaerobic world - oxygen was an extremely potent toxin at first, turning everything upside down - **can you explain this?**

Short answer: oxygen turns ferrous iron Fe(II) into insoluble ferric iron Fe(III) (slides 14 & 15) → disrupted redox chemistry of life

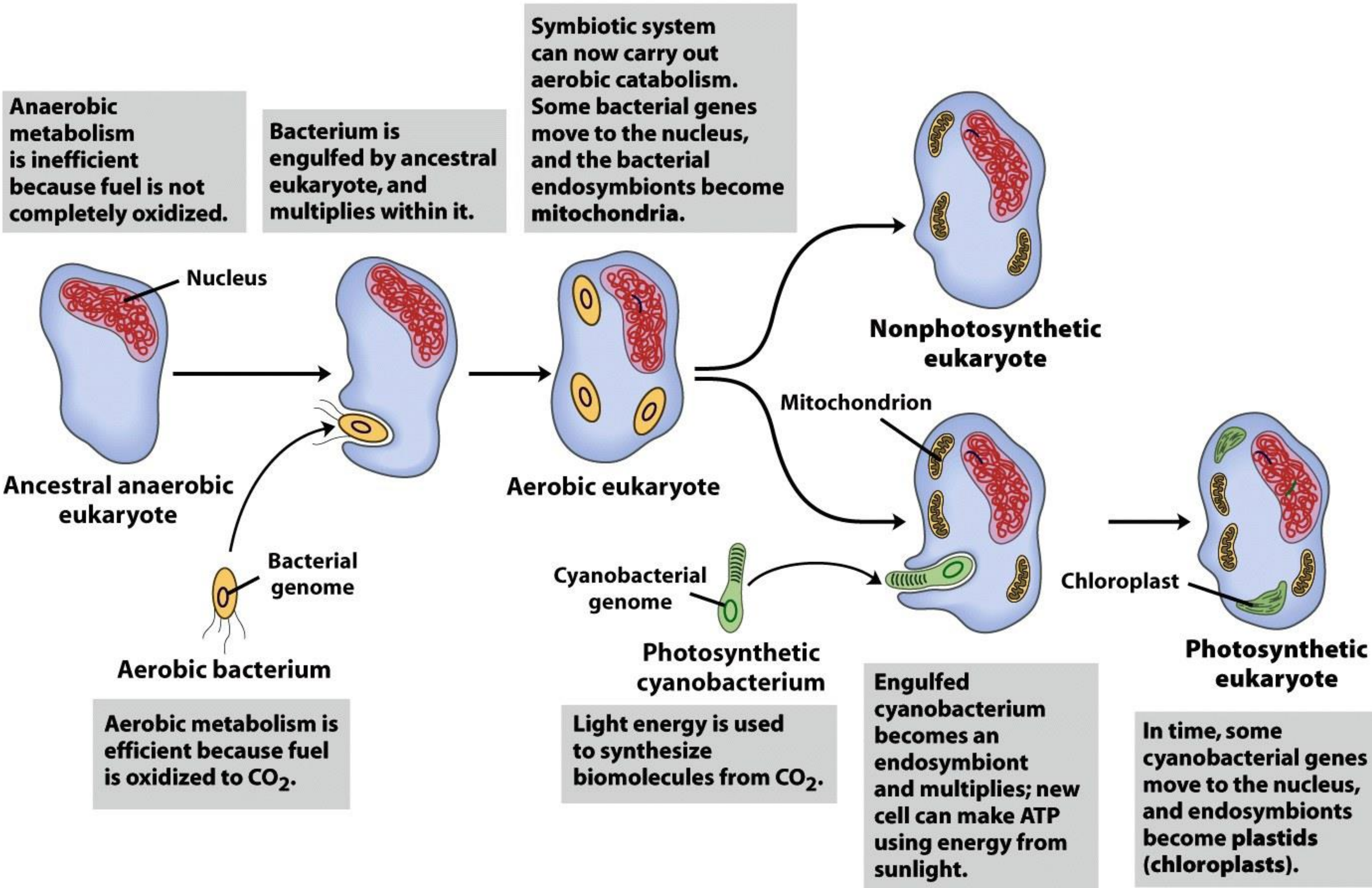
BUT … there was one great thing about oxygen:

complete oxidation of sugars to CO_2 and H_2O yields **MUCH** more energy than anaerobic processes (30-38 ATP/glucose molecule *vs*. just 2 if glucose is broken down anaerobically). Even **more important: oxygen enabled use of an entirely untapped energy resource: fatty acids**. While their hydrocarbon chains cannot be broken down to generate energy under anaerobic conditions, complete oxidation of the most common fatty acid (oleic acid, 18 carbons, 1 double bond) yields 120-122 ATP molecules. **THAT IS A GAMECHANGER!**

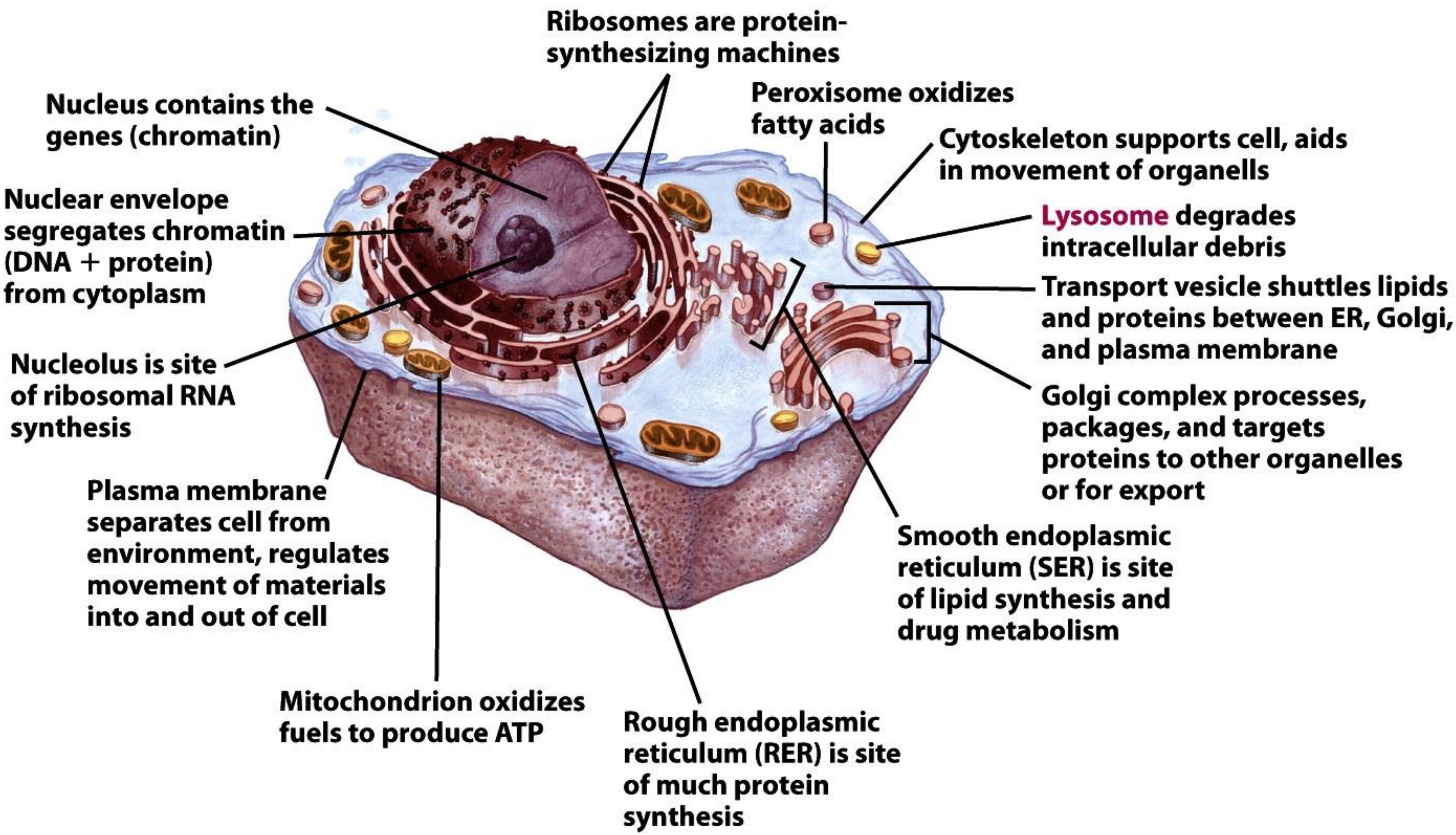
→ Also important to keep in mind: oxygen concentrations did not jump overnight to what they are today = oxygen concentrations rose slowly enough to give life enough time to adapt.

switching to aerobic metabolism enabled individual life units (aka cells) to get more and more complex and to sustain highly convoluted structures
(b/c you can make as many proteins, metabolites as you want and "on demand" at "no" cost).
let's have a look what level of complexity this is……

Endosymbiont Hypothesis For Cellular Evolution

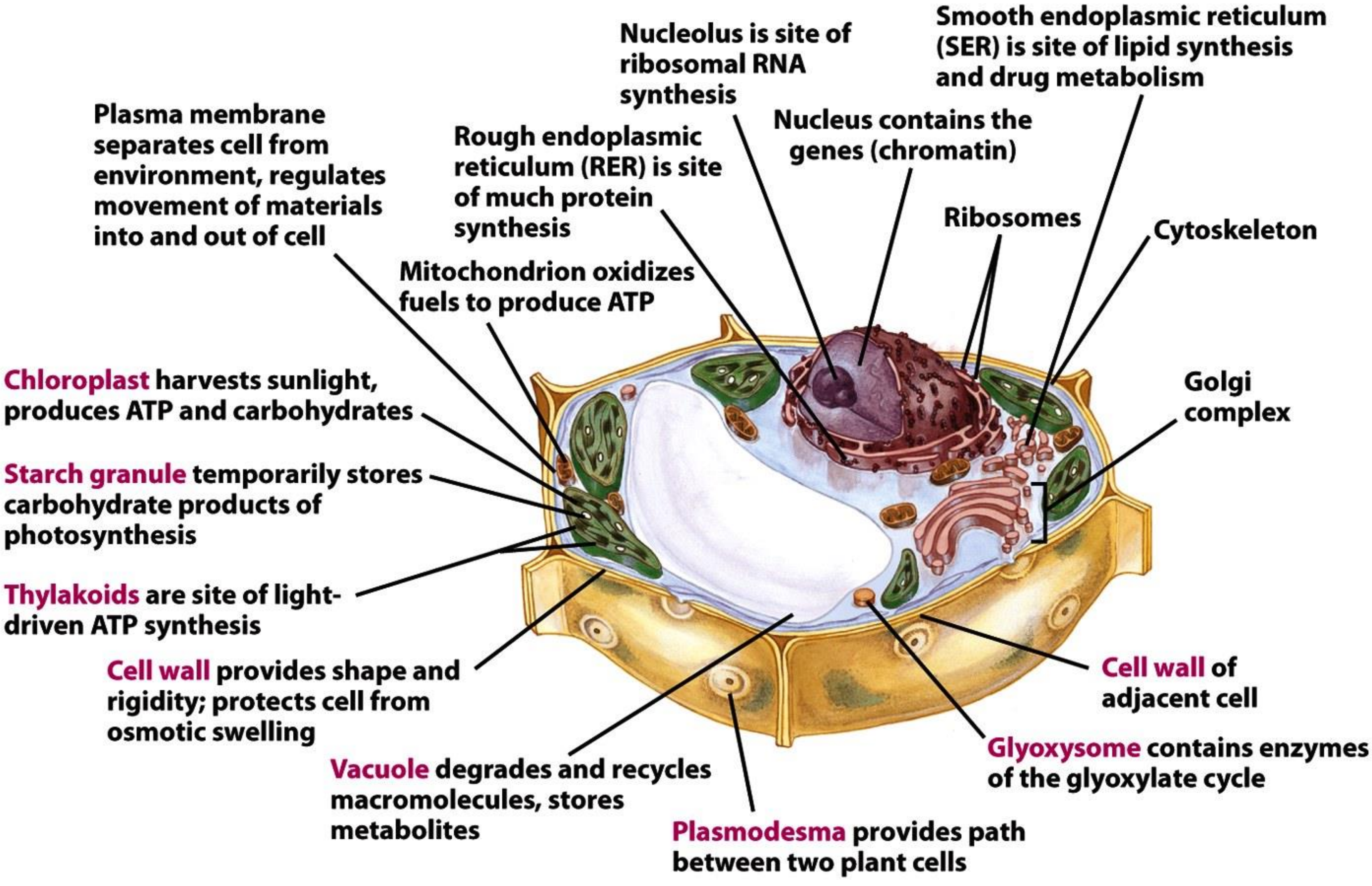


Animal cell



Ribosomes are protein-synthesizing machines
Nucleus contains the genes (chromatin)
Peroxisome oxidizes fatty acids
Cytoskeleton supports cell, aids in movement of organells
Nuclear envelope segregates chromatin (DNA + protein) from cytoplasm
Lysosome degrades intracellular debris
Transport vesicle shuttles lipids and proteins between ER, Golgi, and plasma membrane
Nucleolus is site of ribosomal RNA synthesis
Golgi complex processes, packages, and targets proteins to other organelles or for export
Plasma membrane separates cell from environment, regulates movement of materials into and out of cell
Smooth endoplasmic reticulum (SER) is site of lipid synthesis and drug metabolism
Mitochondrion oxidizes fuels to produce ATP
Rough endoplasmic reticulum (RER) is site of much protein synthesis

Plant cell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Nucleus contains the genes (chromatin)
Ribosomes
Cytoskeleton
Mitochondrion oxidizes fuels to produce ATP
Chloroplast harvests sunlight, produces ATP and carbohydrates
Golgi complex
Starch granule temporarily stores carbohydrate products of photosynthesis
Thylakoids are site of light-driven ATP synthesis
Cell wall provides shape and rigidity; protects cell from osmotic swelling
Cell wall of adjacent cell
Glyoxysome contains enzymes of the glyoxylate cycle
Vacuole degrades and recycles macromolecules, stores metabolites
Plasmodesma provides path between two plant cells

How Did Science Arrive At The Cartoons That Are Familiar To Everybody?

In memoriam Dr. Marc Paypaert, Yale Univ CCMI

a typical electron micrograph showing thin section of a cell

→ demonstrates existence of distinct intracellular compartments

the black dots are "gold labels" attached to antibodies that specifically recognize cellular components (like a protein you are interested in localizing inside the cell)

now let's put this into visual perspective … **close your eyes and imagine a cell … what do you see?**

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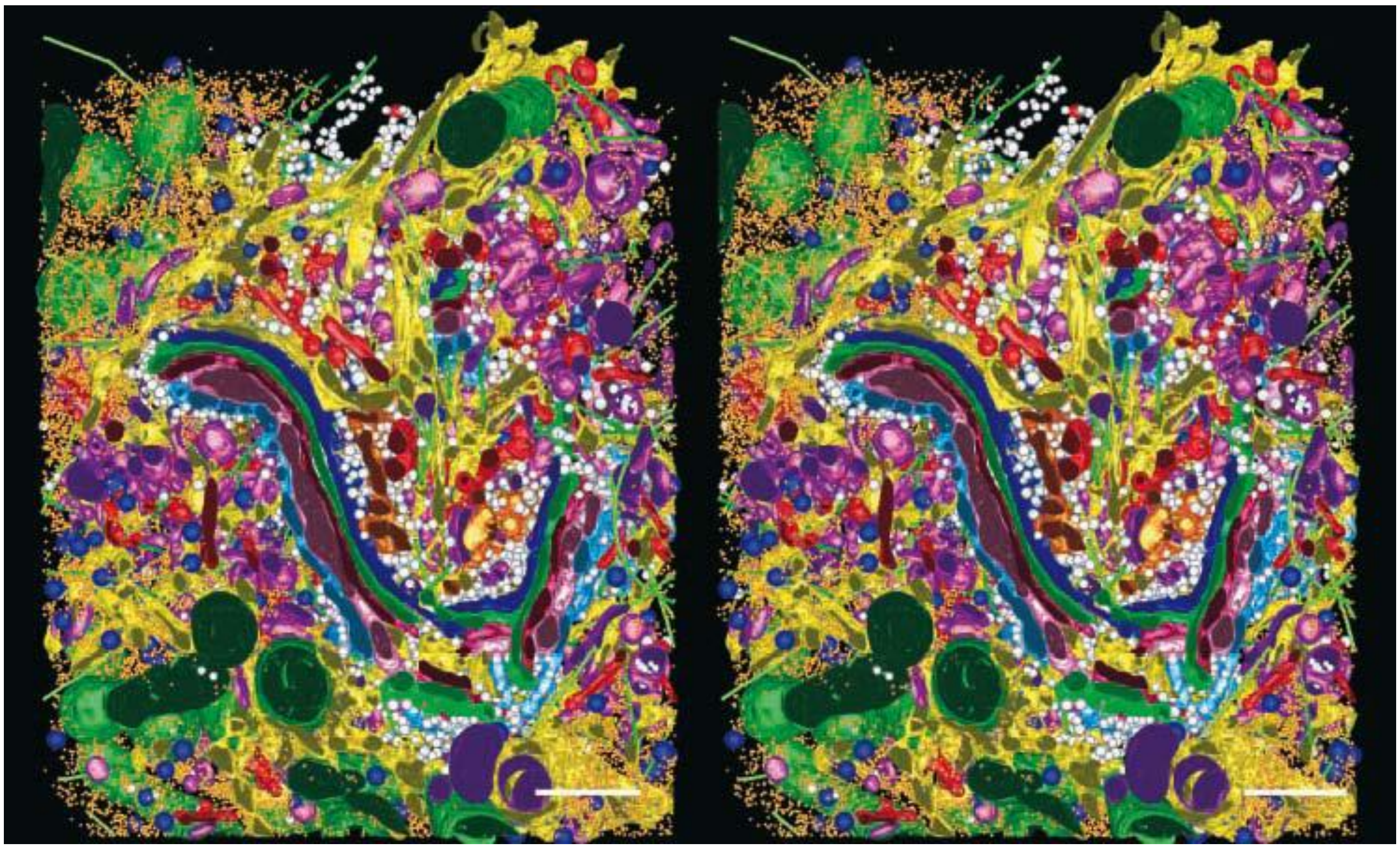
many people see a "big bag with molecules and organelles floating around" - a mental picture that is easily reinforced by representations like this:

https://www.youtube.com/watch?v=wJyUtbn0O5Y

the video is beautiful and super useful to capture general ideas about cellular function – but how accurate is it?

Cross-eye Stereoview
of a slab of cytoplasm of cultured HIT-T15 cells derived by tomography from three 400nm thick serial sections (volume: 3.1x3.2x1.2 µm^3)

To get the stereo effect: look at image from ~30cm distance, cross your eyes and adjust eyes + tilt head left/right as needed until you achieve the 3D impression.



Yellow; endoplasmic reticulum; Blue: membrane bound ribosomes; Orange: free ribosomes; Dark Green: mitochondria ; White: clathrin free vesicles; Purple: clathrin free compartments (e.g Golgi)

Cellular Environment Is Crowded And Busy

looking at an "un-sanitized" view of a cell can serve as a "wakeup" call because such pictures reveal that cells are **EXTREMELY** crowded (a bad LA rush hour is kindergarten in comparison, not even Bangkok comes close)

there is another "**disturbing fact**" about cellular environments … and that has to do with the **protein concentration inside cells … what do you think it is?**

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what is the physical state of this?

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what is the physical state of this?

It's a gel … !

the implication here is that cellular environments are far from "ideal mixtures" (if thermodynamics "speaks to you") - in fact, a significant amount of water inside cells is structured because every molecule and ion is surrounded by a hydration shell.

in the past, this was ignored. However, more recently biophysicists, biochemists and even (cell) biologists have begun to realize that acknowledging reality is important for understanding how things work in cellular environments.

One Final Step: Starting With A Single, Structurally Complex, Eukaryotic Cell – Why Did Nature Choose To Go Even Further By Building Multicellular Organisms?

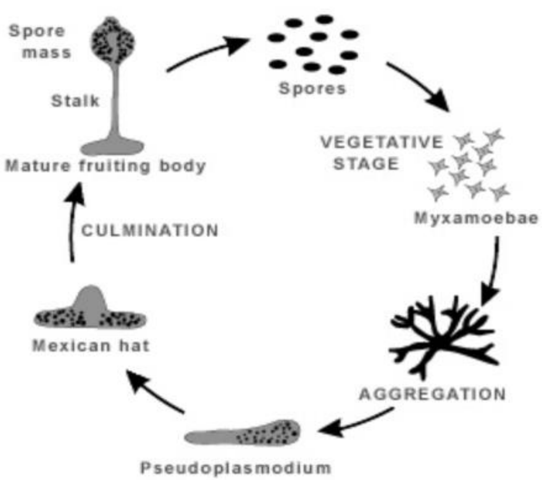
Answer: **separation of labor = more flexibility and adaptability** (eg bacteria cannot protect themselves from environmental hazards). Sounds perfectly reasonable, but what is the cost? = what new requirements does that create?

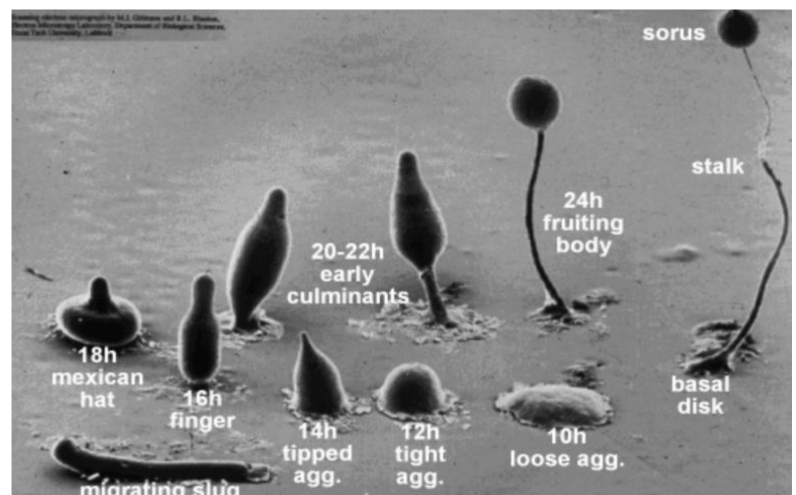
(1) cells need to develop strategies to "stick" to each other (to form organs/tissues)
(2) each cell needs to maintain ability to perform housekeeping functions
(3) as new cellular functions emerge, they need to be implemented through specialization (e.g. stem cells --> terminally differentiated cells) and need to be controlled (regulation)
(4) you need communication/signaling to achieve homeostatic control at the level of organs/tissues and the entire organism
(5) creating more types of cells also requires implementation of redundancy, to allow for fine tuning by using appropriate subsets of closely related molecular assemblies/catalysts (e.g. various types of transporters for things like glucose, or isoforms of enzymes that respond differently to a given metabolic cue)
(6) You need to increase "genomic stability/reproducibility" because errors in any part will affect the entire organism

a primitive implementation of some of these requirements is already present in bacteria: **quorum sensing** (=the ability to sense the population density in the environment), **and the formation of biofilms** (where bacteria glue themselves to each other, which is a big problem in some clinical applications because these films are hard to destroy). Regardless, these ancient “homeostatic units” still involve ensembles of basically identical cells.

an organism that bridges both worlds (unicellular and multicellular) is the slime mold *Dictyostelium discoideum*. It's life cycle served as a model to study signaling, migration and differentiation in the early days of developmental biology.

Today, "Dicty" has been replaced by other model organisms that are easier to manipulate (fruitflies, nematodes, zebrafish). While these organisms lack the switch between uni- to multicellular, they provide "easy" access to study the complex processes that occur during embryonic development

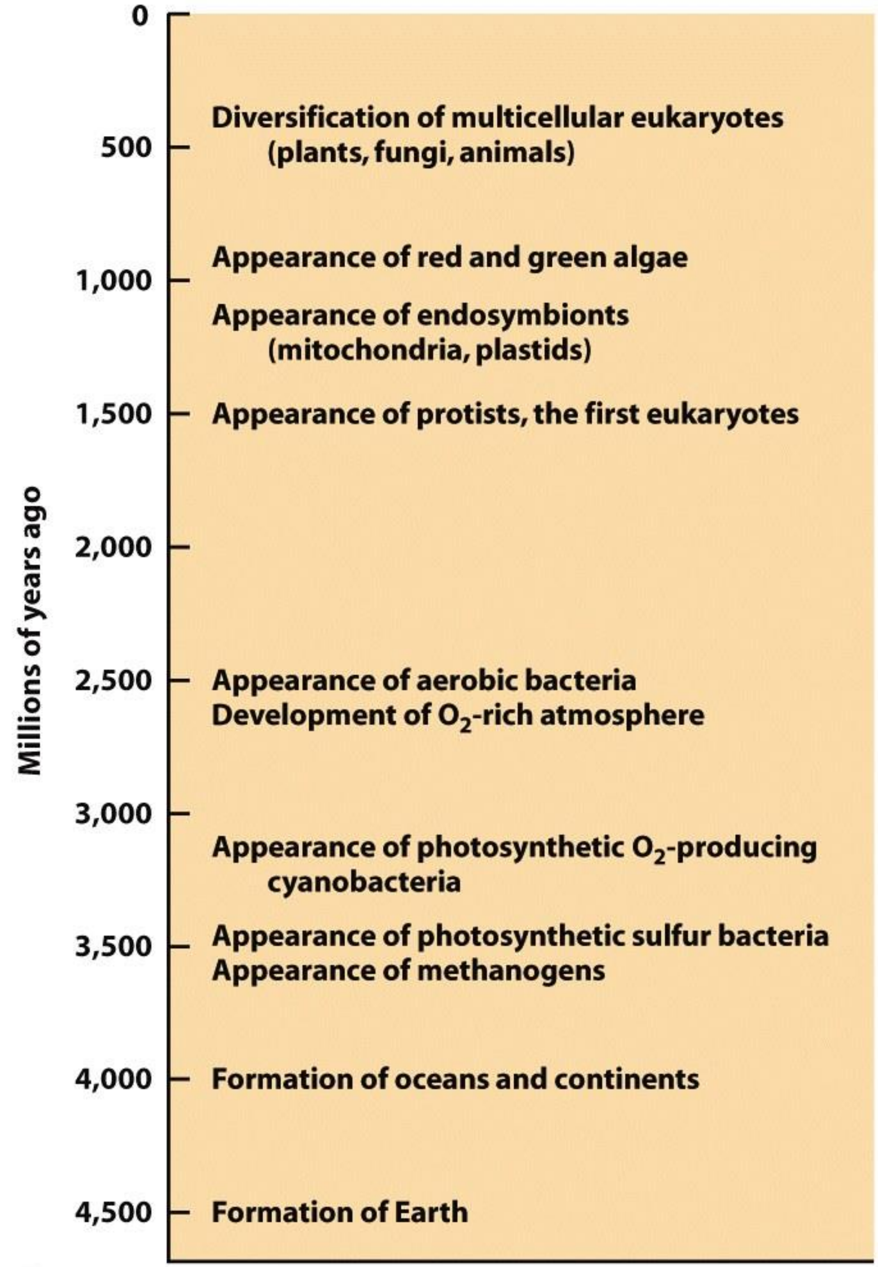




From "Nothing" To Life

Overall Timeline

note the long lag phase for complex multicellular organisms to arise, and the acceleration of lifeform diversification since.



From "Nothing" to Life – The "Engineering" View - Recap

start with **Earth as open system,** able to support brute force, unbiased (organic) chemistry ➔ simple organic compounds → complex organic compounds (made from more than one compound class)
this system **gets stuck** because everything gets diluted to the point where chemistry cannot be controlled to create a stable environment

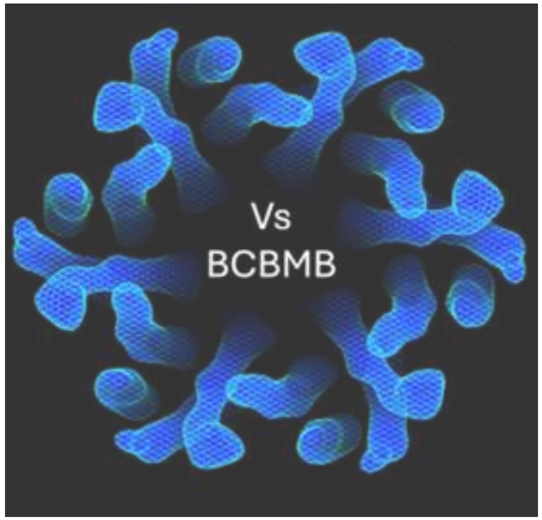
key **breakthrough**: emergence of phospholipids that spontaneously create stable enveloped compartment.
this system, in principle, allows for acceleration and control of chemistry but **gets stuck** because despite "imperfections/leakiness of early membranes", the newly created compartments practically behaved as "closed systems" regarding life's complex chemical needs.

key **breakthrough**: emergence of RNA
as a nucleic acid, RNA's chemistry of formation allowed for spontaneous synthesis of short chains that not only carried information about themselves (= potential to become "selfish") but also were able to function as primitive catalysts, assisting with the crude synthesis of the first peptides → initiates the emergence of proteins, some of which can serve as selective pores to allow for specific transport of matter across the bilayer boundaries → sets the stage for creating the primitive, but functional cells.
This system **gets stuck** because energy supplies become limiting for building cells with internal compartments and, further on, multicellular organisms

key **breakthrough**: emergence of "photosynthesis" = oxygenation event.
despite the extreme toxicity of molecular oxygen to early organisms, the slow rate of oxygen accumulation allowed adaptations that harnessed the potential to generate unlimited energy by complete oxidation of fuels to CO_2 and H_2O (fatty acids in particular)

…life gets "unstuck" and is free to evolve ….

notably though …life has begun to devolve as technological/medical progress eliminates the selection pressures that caused life to "improve its skill set"



Thank You for Working Through This
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