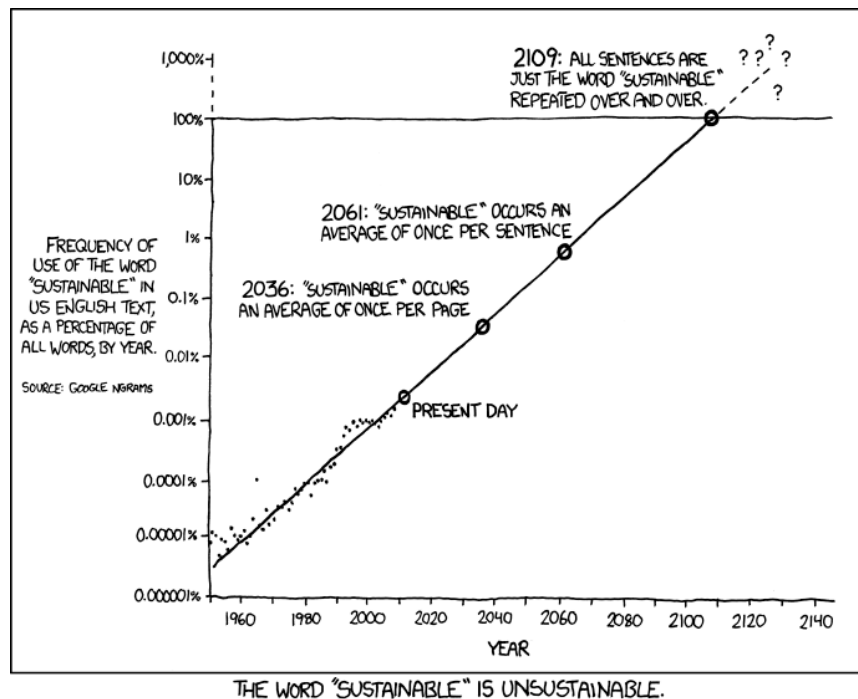


Microbes (Lecture Notes)

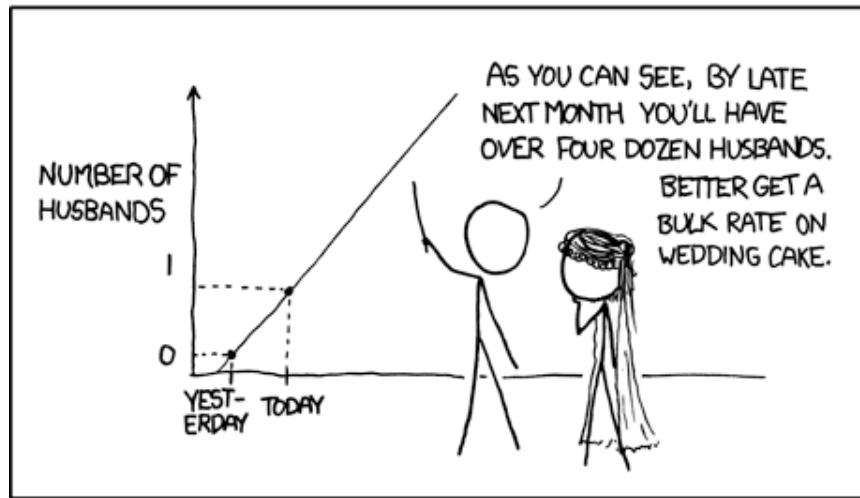
≡ Week	
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▼ Microbial Systematics

- < Learning about finding changes that comes along evolutionary paths from Ancient Microbes to Recent Microbes.
- < Sometimes the changes are transient
- < Time scale plays a vital role in Evolution Characterisation .
- < By seeing at the changes we 'Estimate' time of divergence [Ancestor → Descendants]
- < But in this estimation a problem arises - 'Extrapolation'



MY HOBBY: EXTRAPOLATING



< But evolution doesn't occur random because of 'Hesitance of Organism' . Organism shows reluctance in randomisation.

< **Estimation of time is given by clock {(evolutionary) bio-molecular clock}**

- This bio-molecular clocks gives us the estimation of time about evolution.

< The divergence occurs according to obstacles those organisms overcame in the course of evolutionary time history.

< Information of the obstacles are in the organism.

< In the process of overcoming obstacles the organisms gains some sort of Bio-molecular Signatures , which are basically signals and can be observed better in microbes as Microbes are in more number.

< Living Ancestors are better for mapping the phylogenetic tree & changes occurred .

< Shape observation/ Growing in lab doesn't guarantee an ancestor.

#Example: Stromatolites are living fossils and the oldest living lifeforms on our planet. The name derives from the Greek, stroma , meaning "mattress", and lithos, meaning "rock". Stromatolite literally means "layered rock". The existence of these ancient rocks extends three-quarters of the way back to the origins of the Solar System. (Ancient Form of Cyanobacteria)



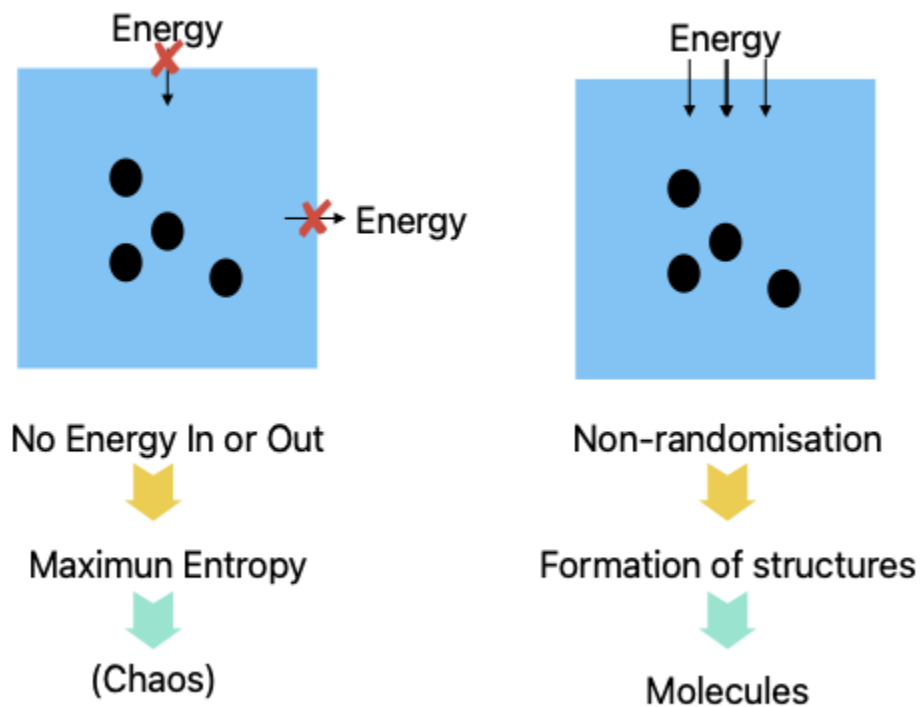
Stromatolites are living fossils and the oldest living lifeforms on our planet (Credit: MaXPdia /Getty Images)

< Evolutionary Process (Mutation-Change in Nucleotide Sequence → Adaptations)

< Horizontal Gene Transfer , Gene Duplication , Gene loss shouldn't be thought as evolution. So these things need to be taken into account.

< Bio films [Example - Bad Odour from shoes] and the fragments of genes that secrete the polysaccharide (due to which foul odour forms) can be taken up by some other microorganism (to which that piece of gene is useful) : 'Horizontal Gene Transfer'

▼ Why early molecules formed in the earth at the very first place?



< When protein folds : entropy of protein ↓ decreases and the entropy of surroundings increase ↑

▼ Molecular Clocks - Chronometers

It's crucial to choose the molecule of interest. We've to know **where** to look for **the signals** (i.e. the signals for changing or adapting with environment), or the bio-molecular clock.

One such fascinating bio-molecular clock is SSU rRNA. The important facts about it are -

i) it is present in all Domains of life but has different name

such as in prokaryotes it is named as 16S rRNA and in eukaryotes it is named as 18S rRNA

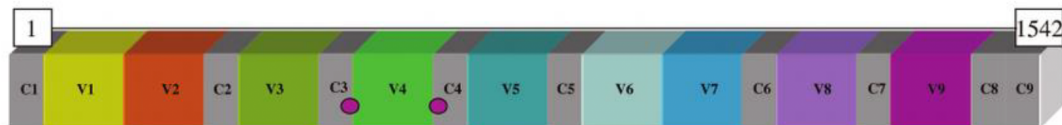
ii) Has mosaic arrangement of constant and variable region.

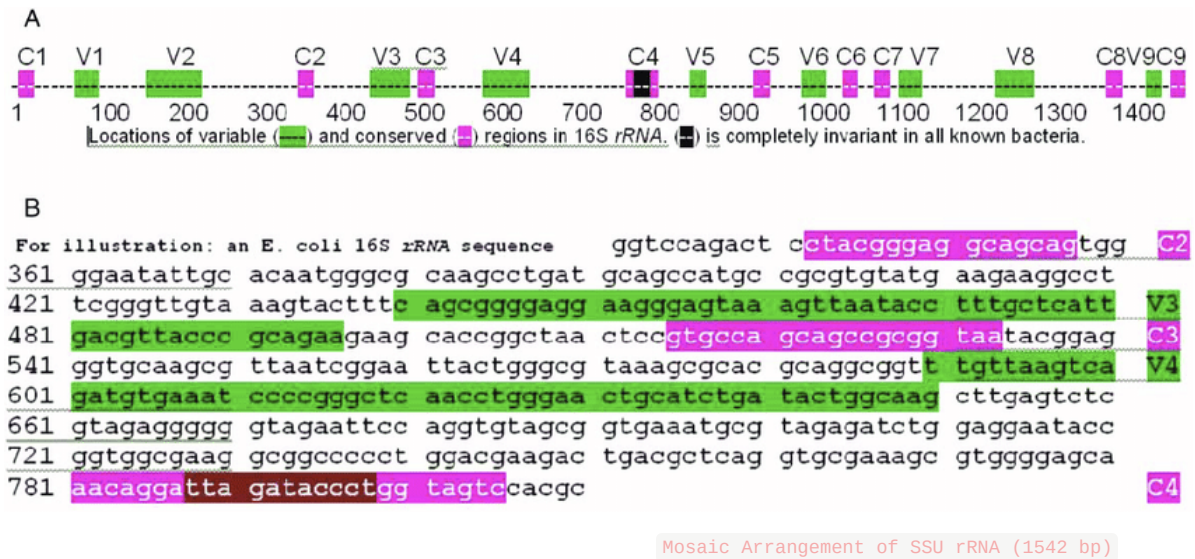
And again why SSU rRNA and we are not considering other bio-molecules as the Molecular Clocks or Chronometers? Answer is simple , because it is present in all domains of the life. And here is another point , within a specific population it doesn't change much(Functionally Constant). It also changes slowly.

Small subunit (=SSU) rRNA is called differently for the different domains. For Bacteria and Archaea (Prokaryotes), it is called 16S rRNA. For Eukarya (Eukaryotes), it is called 18S rRNA. Please note that **SSU rRNA of mitochondrion and plastid (chloroplast) is also called 16S rRNA**. All genes coding SSU (16S or 18S) are homologous, meaning that they share a common ancestor. Therefore, you should be able to (roughly) see how they are phylogenetically related by calculating sequence similarities.

Evolutionary Analysis: Theoretical Aspects

- The most widely used molecular clocks are small subunit ribosomal RNA (SSU rRNA)
 - Found in all domains of life
 - 16S rRNA in prokaryotes and 18S rRNA in eukaryotes
 - Functionally constant
 - Sufficiently conserved (change slowly)
 - random sequence changes are a more accurate measure of time (evolution)
 - Sufficient length
 - Multiple copies (*E. coli*- 7 copies of rRNA)





Professor gave an analogy : Suppose you have managed your friend to get a very important and rare book of your course for just half an hour . You know you cannot read the whole book by just 30 mins . So what you do is simply copying the data , and Holla! you've your copy of that same data to understand and read for the rest of your lifetime. Now you can see some regions of your xerox is hazy and some portion is legible. By look at those different regions you can say why this one is hazy and that one is legible.

You get the point?

PCR - copying the same data for a thousands of time to read and understand it better. And by looking at the different regions(constant regions and variable regions you can get the data/signal that why this one is constant and this one is 'hazy' variable)

▼ Why not 23s rRNA or 5s RNA?

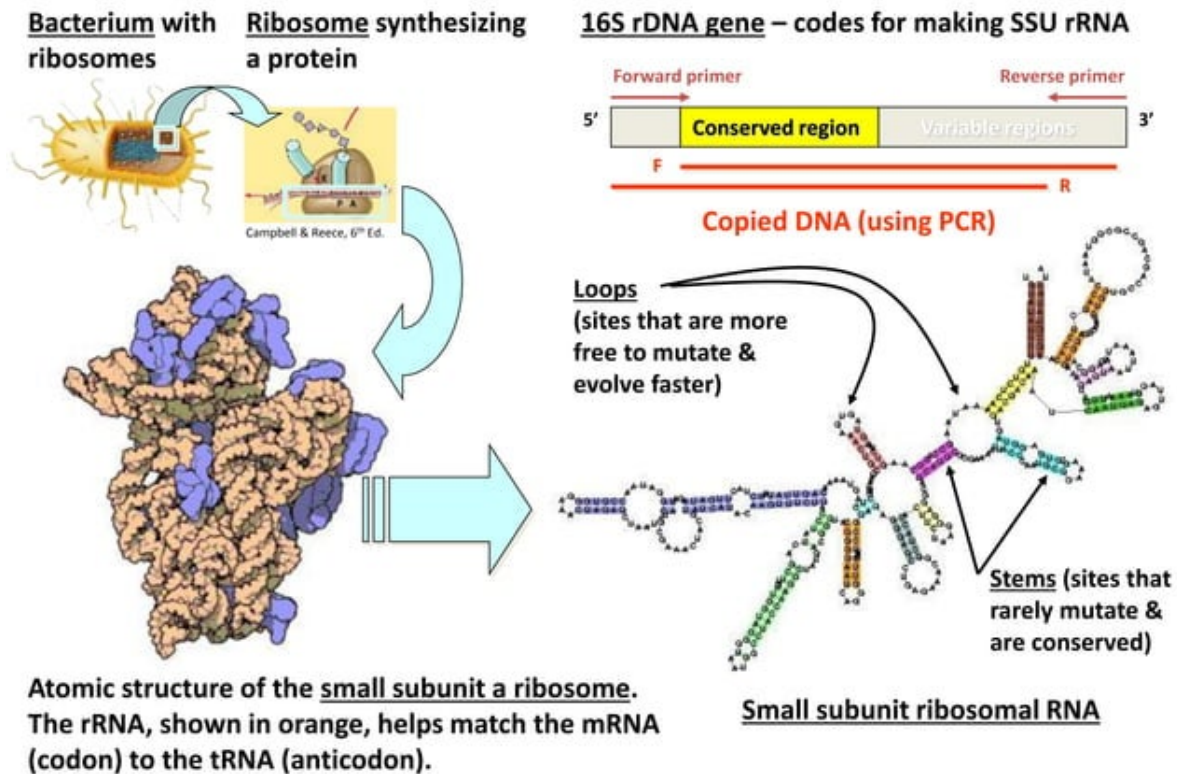
Because we want our process to be cheap , cost effective , sustainable.

In case of 5S rRNA , this will not have randomisation data. Because it is small.

Ribosomal RNAs in Prokaryotes:

NAME	SIZE (NUCLEOTIDES)	LOCATION
5S	120	Large subunit of ribosome
16S	1500	Small subunit of ribosome
23S	2900	Large subunit of ribosome

Use of primers to copy the 16S rDNA gene in bacteria



The names like hairpin structure is given for visual analogy.

There is some other things also , one of them is shape . Shape is related to surface area and that is further related to bio volume($L*B*H$)

Sequenced data gives experimental proof of shape. How?

In case of e.coli , it has oval shape by most of the time. Some times it shows rod shaped species.

So, by looking at only shapes leads to ambiguity. So alone shape cannot attributes for the chronology. And this doesn't reflect evolution in respect to biology.

Further to prove that a rod shaped cell is e.coli and not bacillus , we need sequenced data.

Alone sequenced data can also give wrong direction. Like 18S rRNA of chimp and Human are similar , this implies that chimp and human both evolved back in same time , and that is not true for sure.

In a bacterial cell, mutation cannot happen very easily. It has some mechanism to fix any error in its genetic material. Then how variable regions

▼ Recap

‘There are thousands of microbes in this classroom and most of them is bacteria’ , to prove this statement we need some systemic approach . And that is where microbial systematics comes into play. So what is the approach? First thing will be identifying bio-molecules . And by looking through their circuits we can ‘see’ when the diversification happened, what controls that diversification etc. Now we have to know where to look in this circuit , because millions of nucleotides(bio-molecules) are there . So we choose that one bio-molecule which is present in all domains of life ,

Cell shapes vary within a population eg. E.coli under stress become oval shaped , rod shaped and even E.coli shaped. So cell shapes can’t alone attributes for chronology.

We gather information that is there in 16s rRNA and we look at the sequence conservation level , which are nearly universal and which are hypervariables .

▼ More on Molecular-Clocks

Carl Woese first used SSU rRNA for phylogenetic studies. He established the presence of three domains of life: *Bacteria*, *Archaea*, and *Eukarya*. Provided a unified phylogenetic framework for *Bacteria*.

Sequenced data has signatures for different species.

So, **Comparative RNA sequencing** has three steps :

PCR(Photocopy) , Visualisation of the data , Calibration & Authentication

Mitochondrial DNA is used to classify higher eukaryotes.

Because of 16s rRNA sequenced data common ancestor concept is debated.

▼ Suppose in a region of SSU rRNA G,C is more than other base . What is the evolutionary signal?

GC ratio is an fantastic parameter to look into the chronology of a bacteria. G-C is more means , stability in the circuit is more thus the mutation would be slower. This symbolise that some organisms evolved in systems which are very dynamic , like hydrothermal vents.

<https://thebiomedicalseientist.net/science/big-story-petri-dish>