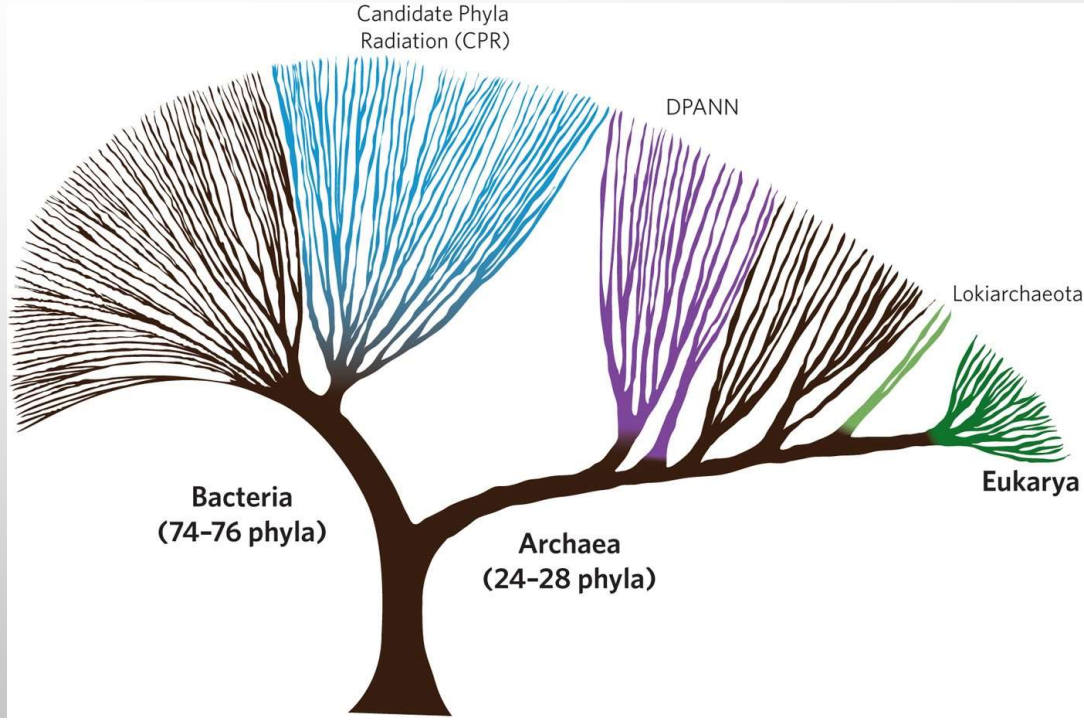
MOLECULAR INVENTIONS AND THE TREE OF LIFE

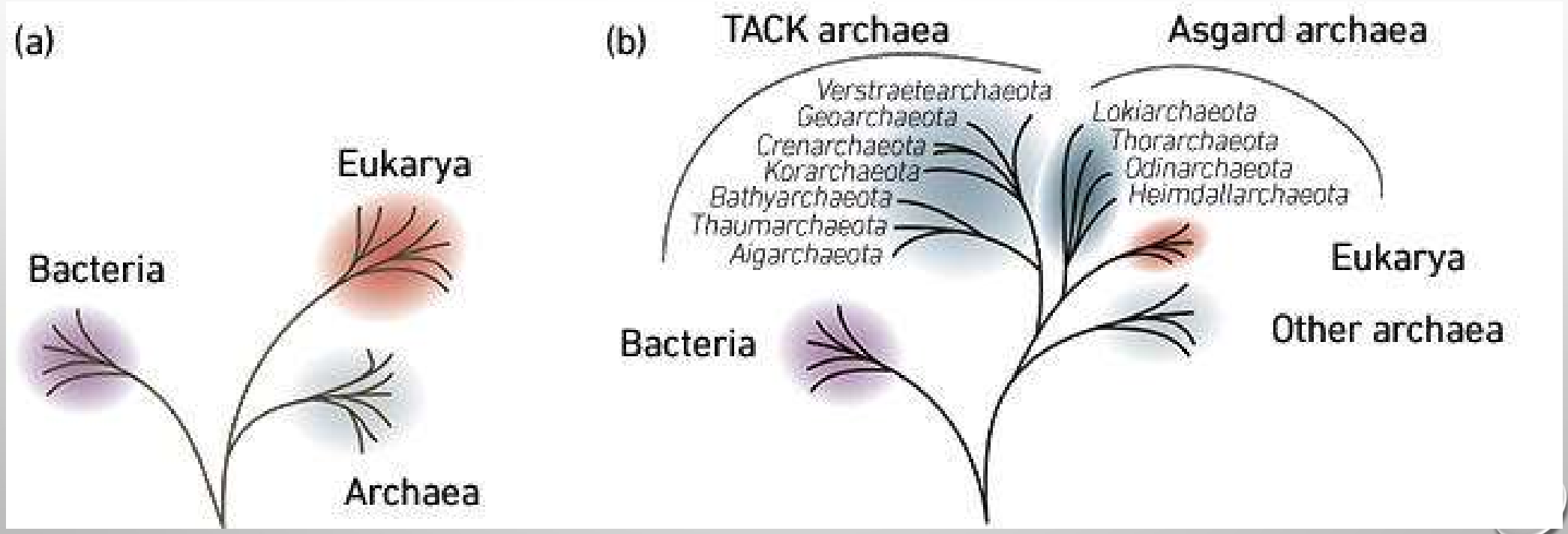
SPRING 2024



THE EUKARYOTES' ANCESTOR: AN EMERGING PICTURE

- THE ASGARD ARCHAEA EVOLVED MORE THAN 2 BILLION YEARS AGO
- ALTOGETHER, THE ASGARD ARCHAEAL ANCESTOR OF EUKARYOTES HAD, COMPARED WITH OTHER ARCHAEA, A RELATIVELY LARGE GENOME THAT RESULTED MAINLY FROM MORE NUMEROUS GENE DUPLICATIONS
- IT IS TEMPTING TO SPECULATE THAT THE INCREASED GENE DUPLICATION RATES OBSERVED ARE AN ANCESTRAL FEATURE AND THAT IT REMAINED THE PREDOMINANT MODE OF GENOME EVOLUTION DURING THE EARLY STAGES OF EUKARYOGENESIS
- ALTHOUGH ASGARD ARCHAEA PROBABLY HAD A THERMOPHILIC ANCESTRY, THE LINEAGE FROM WHICH EUKARYOTES EVOLVED WAS ADAPTED TO MESOPHILIC CONDITIONS. THIS FINDING IS COMPATIBLE WITH A GENERALLY ASSUMED MESOPHILIC ANCESTRY OF EUKARYOTES
- COMPLEX PATHWAYS INVOLVED IN PROTEIN TARGETING AND MEMBRANE TRAFFICKING AND IN GENOME MAINTENANCE AND EXPRESSION IN EUKARYOTES WERE PRESENT IN THE ASGARD ARCHAEAL ANCESTOR
- POTENTIAL FOR MEMBRANE DEFORMATION, CYTOSKELETON PROTEINS AND THE ABILITY TO DO PHAGOCYTOSIS WOULD BE INSTRUMENTAL IN THE EVOLUTION OF EUKARYOTES

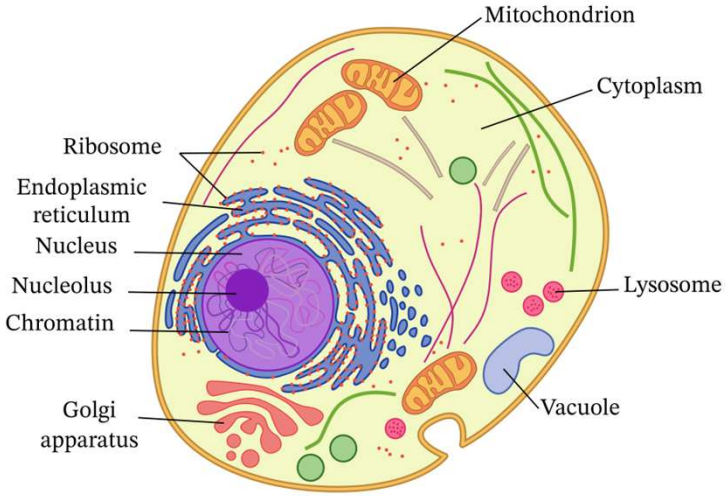
THE EMERGING TREE OF LIFE

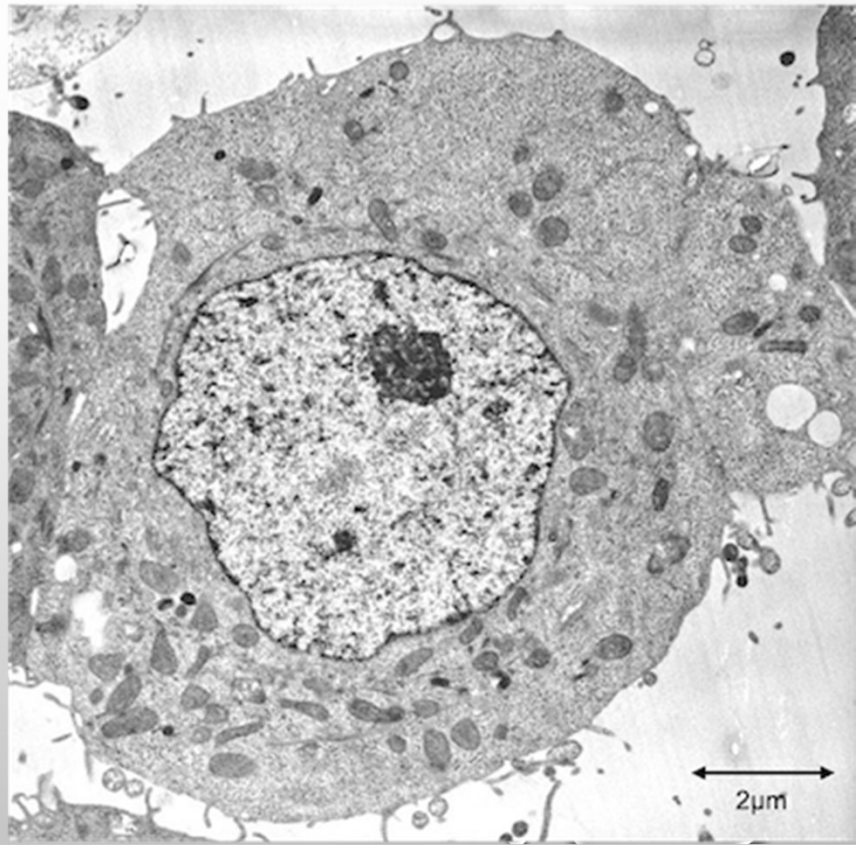


WHAT IS A EUKARYOTE?

- MEMBRANE-BOUND NUCLEUS CONTAINING THE DNA GENOME
- MITOCHONDRIA
- CELL-WIDE SYSTEM OF MEMBRANES
  - ENDOPLASMIC RETICULUM: AN INTERCONNECTED NETWORK OF FLATTENED, MEMBRANE-ENCLOSED SACS THAT FUNCTION IN THE SYNTHESIS, PROPER FOLDING AND TRANSPORT OF PROTEINS (AND LIPIDS)
  - GOLGI APPARATUS → IT PACKAGES PROTEINS INTO MEMBRANE-BOUND VESICLES INSIDE THE CELL BEFORE THE VESICLES ARE SENT TO THEIR DESTINATION
- CYTOSKELETON → A COMPLEX, DYNAMIC NETWORK OF INTERLINKING PROTEIN FILAMENTS AND MICROTUBULES CAPABLE OF RAPID GROWTH OR DISASSEMBLY DEPENDING ON THE CELL'S REQUIREMENTS
- SEXUAL REPRODUCTION

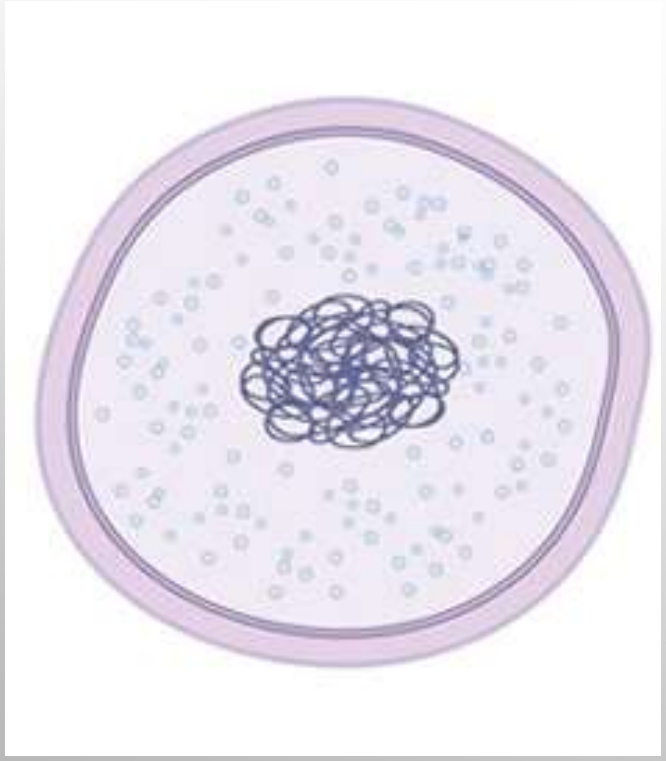
THE EUKARYOTIC CELL

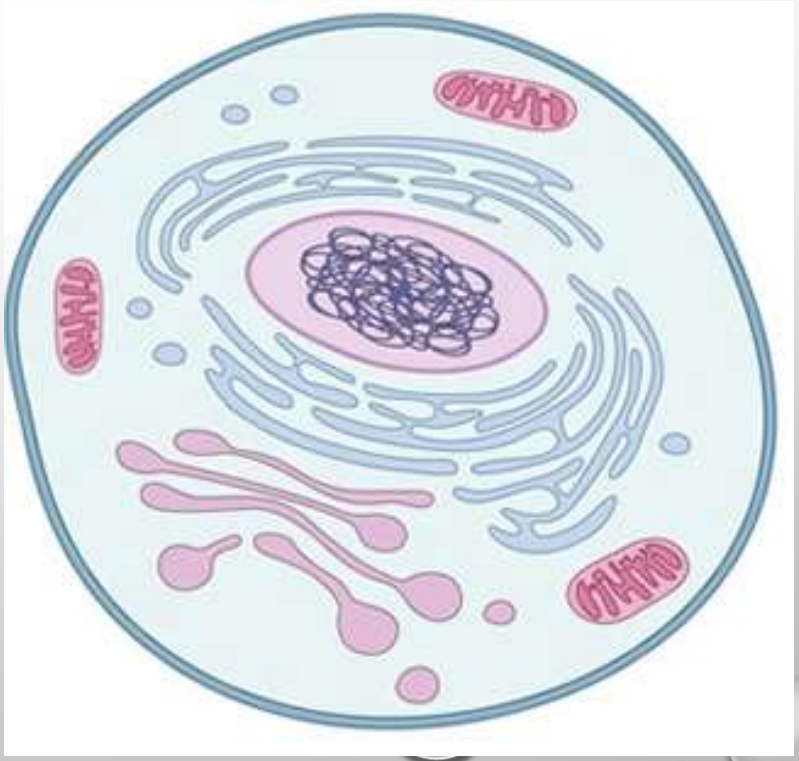




Eukaryotic cells are 100 to 10,000 times larger than Bacterial and archaeal cells

FROM HERE TO THERE





CLUES FROM EXTANT EUKARYOTES

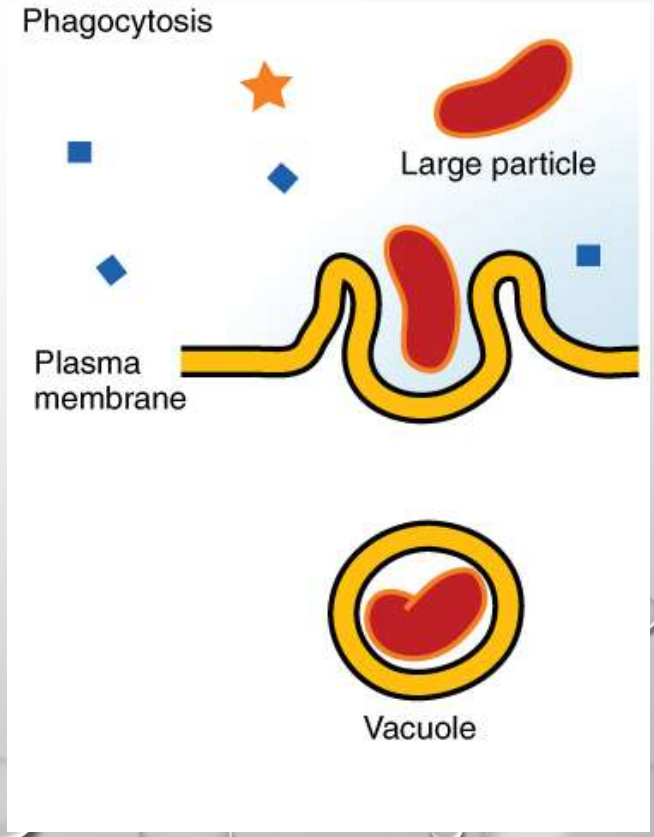
- ALL EUKARYOTES CONTAIN MITOCHONDRIA
- ALTHOUGH THERE ARE EUKARYOTES THAT DO NOT HAVE THEM, IT WAS A SECONDARY LOSS, NOT AN ORIGINAL FEATURE
- MITOCHONDRIA ARE APPROXIMATELY THE SAME SIZE AS BACTERIAL CELLS
- HAVE THEIR OWN DNA, WHICH IS ORGANIZED IN A CIRCULAR CHROMOSOME, AND THEIR GENOMES CONTAIN GENES THAT ARE VERY SIMILAR TO GENES FOUND IN BACTERIAL GENOMES
- MITOCHONDRIAL RIBOSOMES ARE SIMILAR TO BACTERIA
- REPRODUCE BY BINARY FISSION, THE PROCESS THAT BACTERIA AND ARCHAEA USE TO REPRODUCE
- IF THE MITOCHONDRIA ARE REMOVED FROM A EUKARYOTIC CELL, THE CELL HAS NO WAY TO PRODUCE NEW ONES → THE GENETIC INSTRUCTIONS TO MAKE NEW MITOCHONDRIA ARE NOT PRESENT IN THE EUKARYOTIC NUCLEAR GENOME; THEY ARE PRESENT IN THE MITOCHONDRIAL GENOME
- ALL MITOCHONDRIA ARE RELATED TO A SPECIFIC GROUP OF BACTERIA, THE ALPHAPROTEOBACTERIA

THE ANCESTRAL EUKARYOTE

- MOST LIKELY EVOLVED FROM AN ARCHAEON IN THE ASGARD GROUP
- THE ANCESTRAL EUKARYOTE ALMOST CERTAINLY HAD MITOCHONDRIA
- THE FIRST COMMON ANCESTOR OF MITOCHONDRIA WAS A 'PRE-MITOCHONDRIAL ALPHAPROTEOBACTERIUM' ACQUIRED BY THE ASGARD ARCHAEON AS AN ENDOSYMBIONT
- THE GROUP MOST SIMILAR TO MITOCHONDRIA BELONGS IN A GROUP OF FREE-LIVING MARINE BACTERIA ESTIMATED TO MAKE UP BETWEEN A QUARTER AND A HALF OF ALL BACTERIAL AND ARCHAEAL CELLS IN THE OCEAN
- DRASTIC REDUCTION FROM THE ENDOSYMBIONT TO A FULLY INTEGRATED ORGANELLE TOOK PLACE OVER TIME
- THE MITOCHONDRION OF THE ANCESTRAL EUKARYOTE WAS A FULLY INTEGRATED ORGANELLE, CAPABLE OF AEROBIC RESPIRATION
- WITH THAT, THE EMERGING EUKARYOTE WOULD HAVE GAINED AN ENORMOUS ENERGY ADVANTAGE

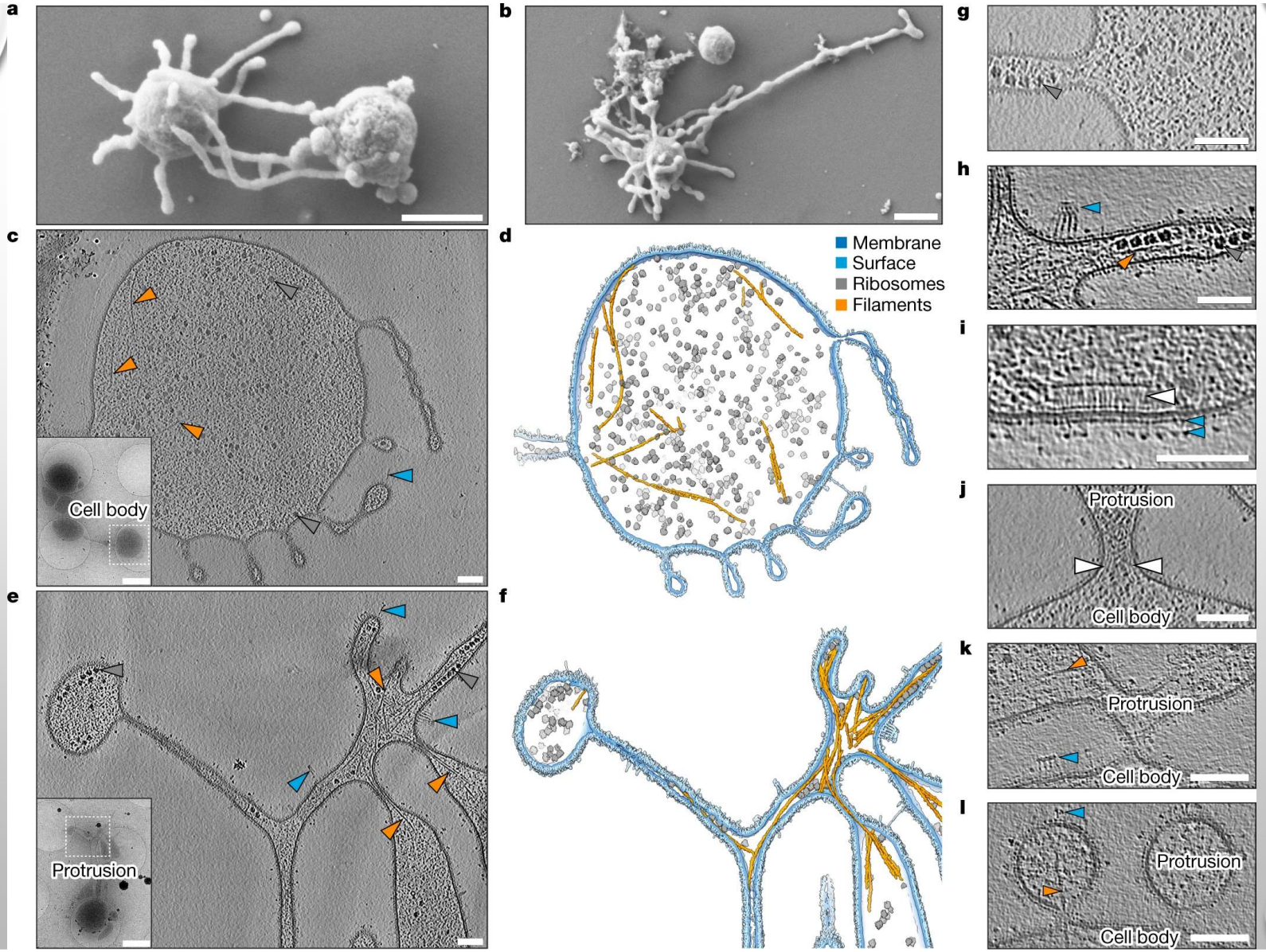
HOW WAS THE ENDOSYMBIONT ACQUIRED?

- ONE OF THE PRIMARY TRAITS SEPARATING EUKARYOTES FROM BACTERIA AND ARCHAEA IS THE ABILITY OF EUKARYOTES TO INTERNALIZE ENTIRE PREY CELLS VIA PHAGOCYTOSIS
- BUT PHAGOCYTOSIS IS A VERY COMPLEX MECHANISM
- WAS THE ARCHAEAL HOST CELL ALREADY CAPABLE OF PHAGOCYTOSIS, EVEN AT A RUDIMENTARY LEVEL, AND DID THIS ABILITY MEDIATE THE ACQUISITION OF THE ANCESTRAL MITOCHONDRION?



ENTANGLE-ENGULF-ENDOGENIZE MODEL

- THE ANCESTRAL MITOCHONDRION WAS NOT INTERNALIZED BY THE ARCHAEAL HOST VIA PHAGOCYTOSIS BUT THROUGH INTERACTIONS BETWEEN THE SYMBIONT AND EXTRACELLULAR STRUCTURES PROJECTED OUT BY THE HOST CELL INTO THE SURROUNDING ENVIRONMENT
- A COMBINATION OF PROTRUSION FORMATION (ENTANGLING) AND BLEBBING (BULGING OF THE MEMBRANE, CHARACTERIZED BY A SPHERICAL, "BLISTER-LIKE", BULKY MORPHOLOGY) MAY HAVE BEEN INVOLVED IN SURROUNDING AND ULTIMATELY ENCAPSULATING THE ANCESTRAL MITOCHONDRION
- THIS EARLIER FORM OF “PHAGOCYTOSIS”, EVEN IF INEFFICIENT COMPARED TO THAT OF MODERN EUKARYOTES, WOULD HAVE STILL OFFERED A SIGNIFICANT SELECTIVE ADVANTAGE AT A TIME IN EARTH HISTORY WHEN NO OTHER CELLS WERE CAPABLE OF ENGULFING ANOTHER
- THE FUNDAMENTAL ACTIN-BASED MACHINERY UNDERLYING PHAGOCYTOSIS, PRESENT AND CONSERVED ACROSS THE EUKARYOTIC TREE, WAS ULTIMATELY INHERITED FROM THE EUKARYOTIC HOST CELL, AN ASGARD ARCHAEON, AND REFINED LATER IN EVOLUTION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Cell body
Protrusion
Protrusion
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Cell body

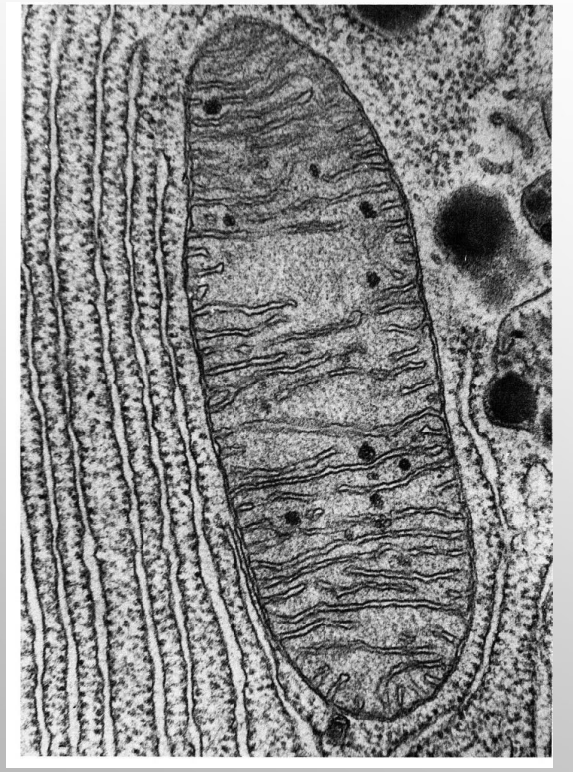
A COMPETING HYPOTHESIS

- SOME RESEARCHERS QUESTION THE PHYLOGENETIC POSITION OF THE MITOCHONDRIAL ANCESTOR
- THEY CLAIM THAT THE MITOCHONDRIAL ANCESTOR IS MORE CLOSELY RELATED TO RICKETTSIA AND OTHER SIMILAR ALPHAPROTEOBACTERIA
- THIS GROUP INCLUDES PATHOGENS THAT CAUSE ROCKY MOUNTAIN SPOTTED FEVER, EPIDEMIC TYPHUS, AND OTHER DISEASES
- THE INITIAL ASSOCIATION BETWEEN THE ASGARD ARCHAEON AND THE MITOCHONDRIAL ANCESTOR MAY HAVE BEEN A CASE OF PARASITIC INVASION OF THE ARCHAEON BY THE BACTERIUM

BECOMING AN ORGANELLE

- DESPITE UNCERTAINTY OVER THE NATURE OF THE INITIAL ENDOSYMBIOSIS, MITOCHONDRIA WERE ULTIMATELY RETAINED IN LARGE PART BECAUSE OF THEIR CAPACITY TO EFFICIENTLY GENERATE ATP THROUGH AEROBIC RESPIRATION
- THE CAPABILITY TO GENERATE ATP BY FULLY OXIDIZING ORGANIC 'FOOD', CARBOHYDRATES, AMINO ACIDS AND LIPIDS, THROUGH AEROBIC RESPIRATION MAY HAVE BEEN A NEW PHYSIOLOGICAL PROPERTY BROUGHT TO AN ANAEROBIC HOST BY THE MITOCHONDRIAL ENDOSYMBIONT
- THE TRANSFORMATION OF A BACTERIUM INTO AN ORGANELLE WAS A PROCESS OF INTEGRATION WITH THE HOST AS THE ENDOSYMBIONT LOST ITS AUTONOMY AND EVENTUALLY BECAME SPECIALIZED AS AN AEROBICALLY RESPIRING ATP-PRODUCING ORGANELLE WITH ADDITIONAL ROLES IN A MULTITUDE OF METABOLIC AND BIOSYNTHETIC PATHWAYS

MITOCHONDRIA

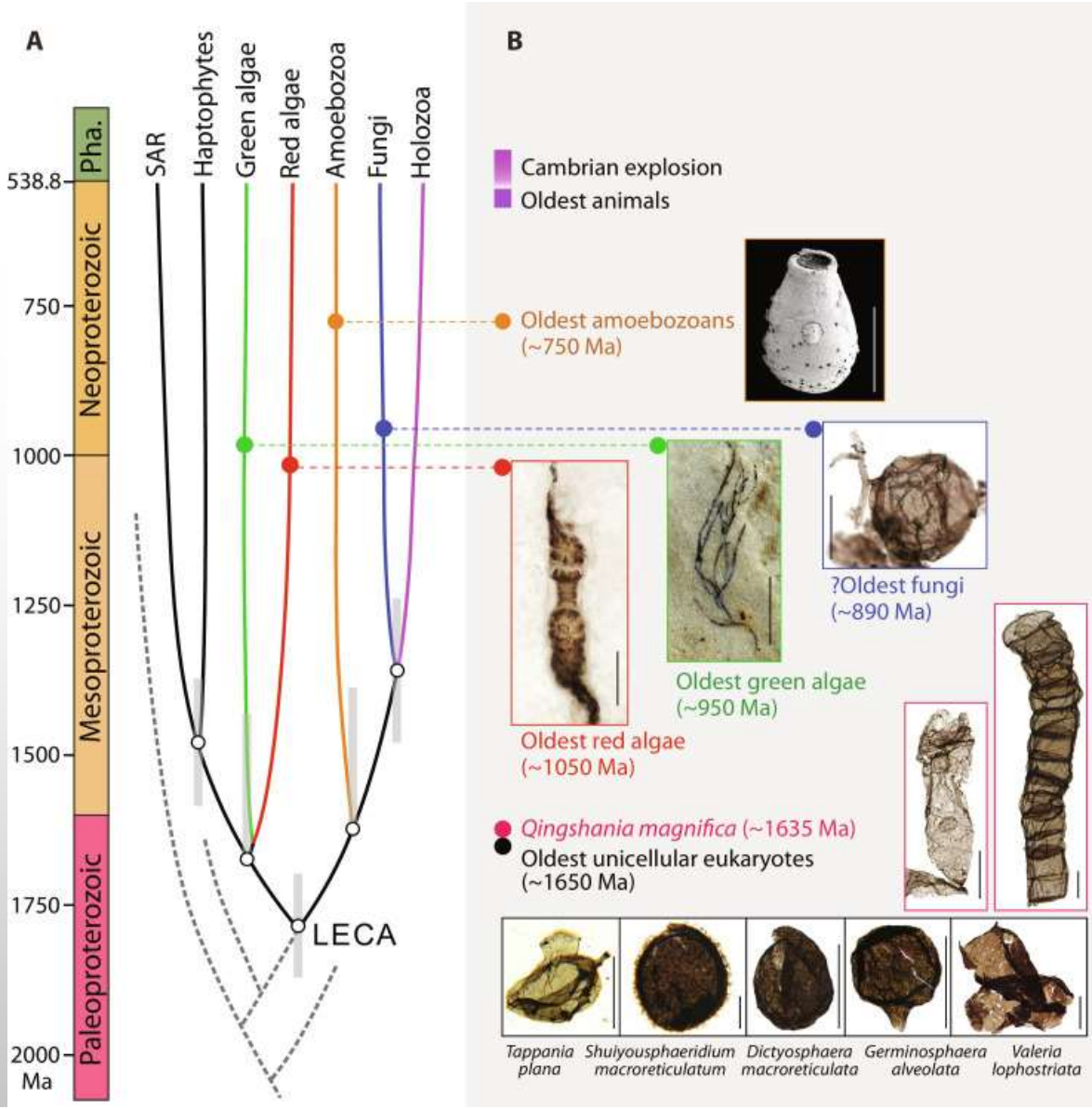


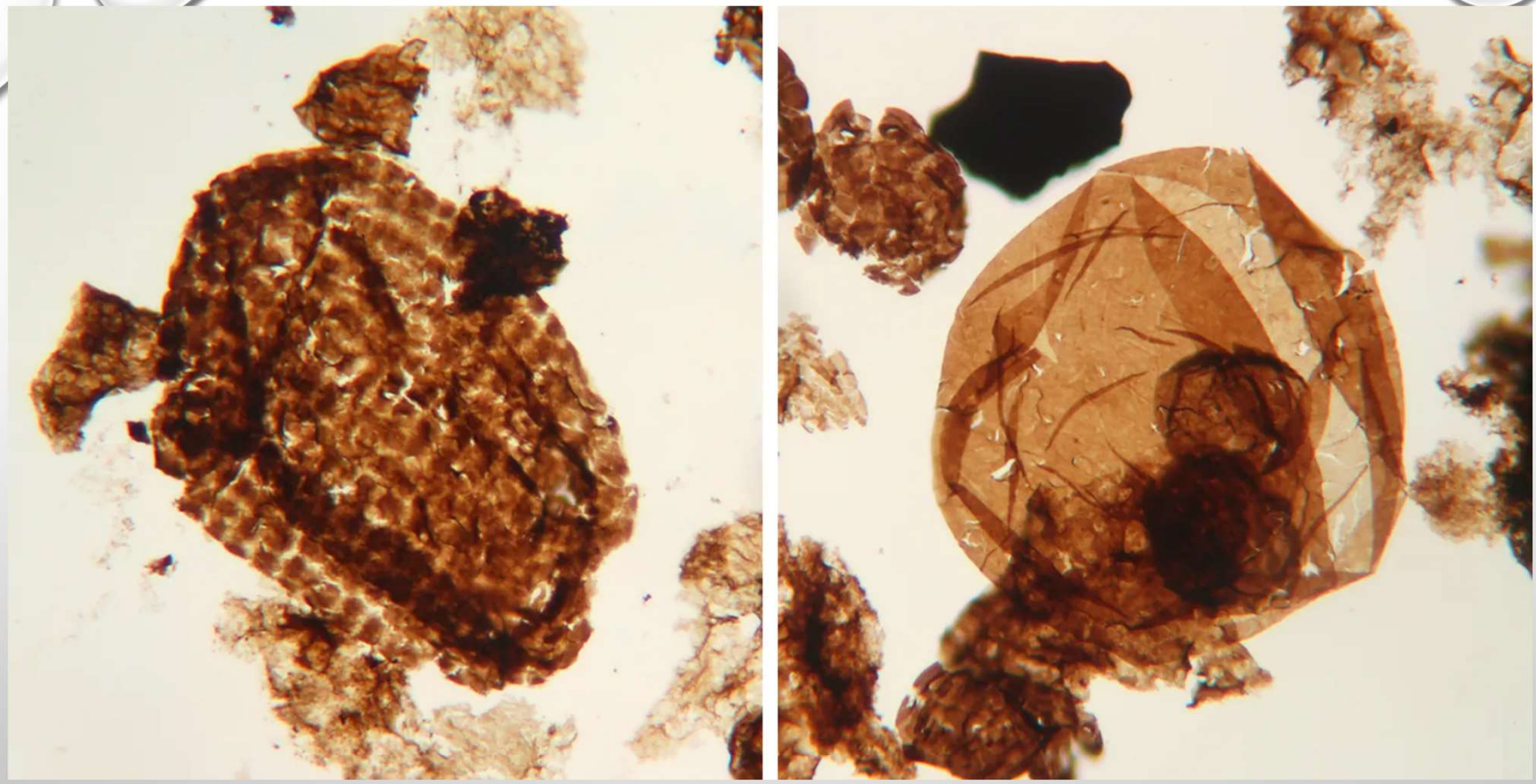
- MAIN FUNCTION: PRODUCE ATP, THE ENERGY CURRENCY OF THE CELL
- FATTY ACID SYNTHESIS
- UPTAKE, STORAGE AND RELEASE OF CALCIUM (INVOLVED IN HORMONE RELEASE AND NEUROTRANSMITTER ACTION, AMONG OTHER THINGS)
- REGULATION OF CELLULAR METABOLISM
- CELLULAR PROLIFERATION IN CANCERS AND PROGRAMMED CELL DEATH
- NUMBER OF MITOCHONDRIA IN A CELL CAN VARY WIDELY BY ORGANISM, TISSUE AND CELL TYPE
- IN ANIMALS, THE MITOCHONDRIAL GENOME IS TYPICALLY A SINGLE CIRCULAR CHROMOSOME APPROXIMATELY 16 KB LONG AND HAS 37 GENES

THIS IS THE NEXT MOLECULAR INVENTION: THE MARRIAGE OF TWO LINES, WITH EUKARYOTES AS THE DESCENDANTS

- WHEN DID THIS HAPPEN?

THE EUKARYOTIC FOSSIL RECORD

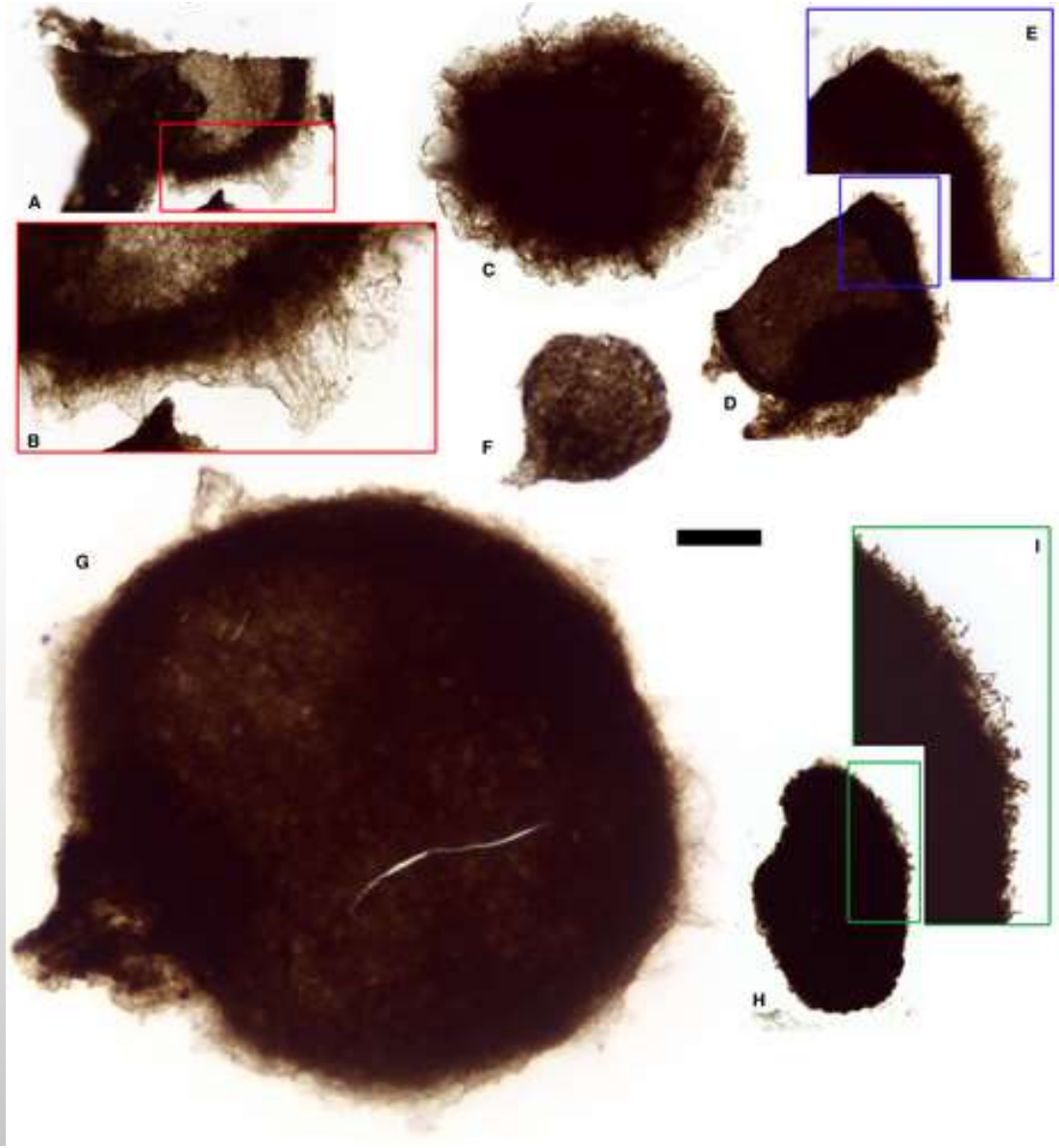




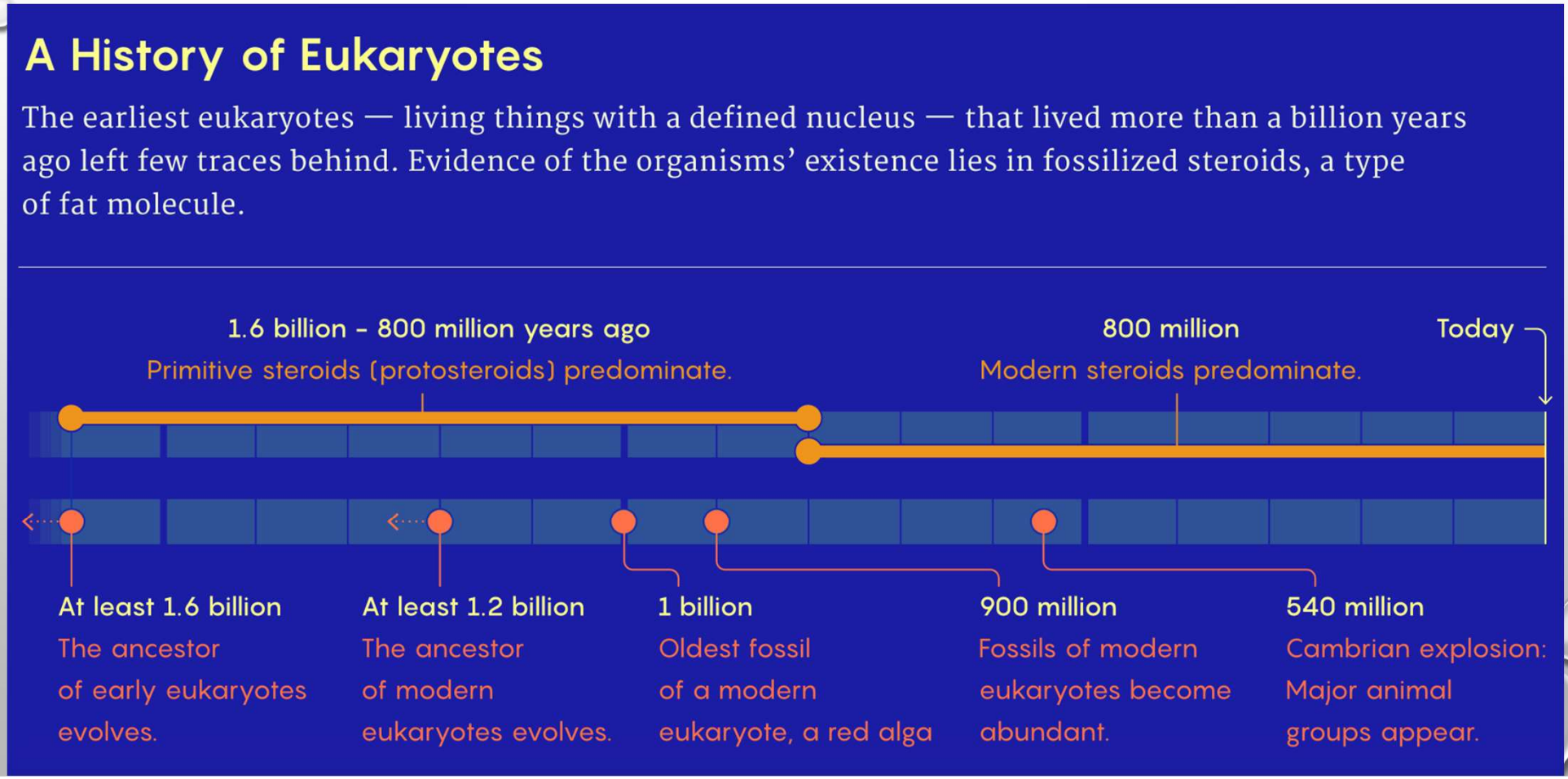
Rare microscopic fossils of ancient life provide time stamps for when eukaryotes evolved. *Satka favosa* (left) and *Valeria lophostriata* date to 1.6 billion years ago

THE NEWEST DATA

- DECEMBER 2023
- UNIVERSITY OF CALIFORNIA SANTA BARBARA
- NORTHERN AUSTRALIA
- 1.64 BILLION YEARS OLD
- EUKARYOTES SYNTHESIZE STEROIDS, A COMPONENT OF THE CELL MEMBRANE
- FOSSILIZED STEROIDS PROVIDE A MOLECULAR MARKER FOR THE PRESENCE OF EUKARYOTES

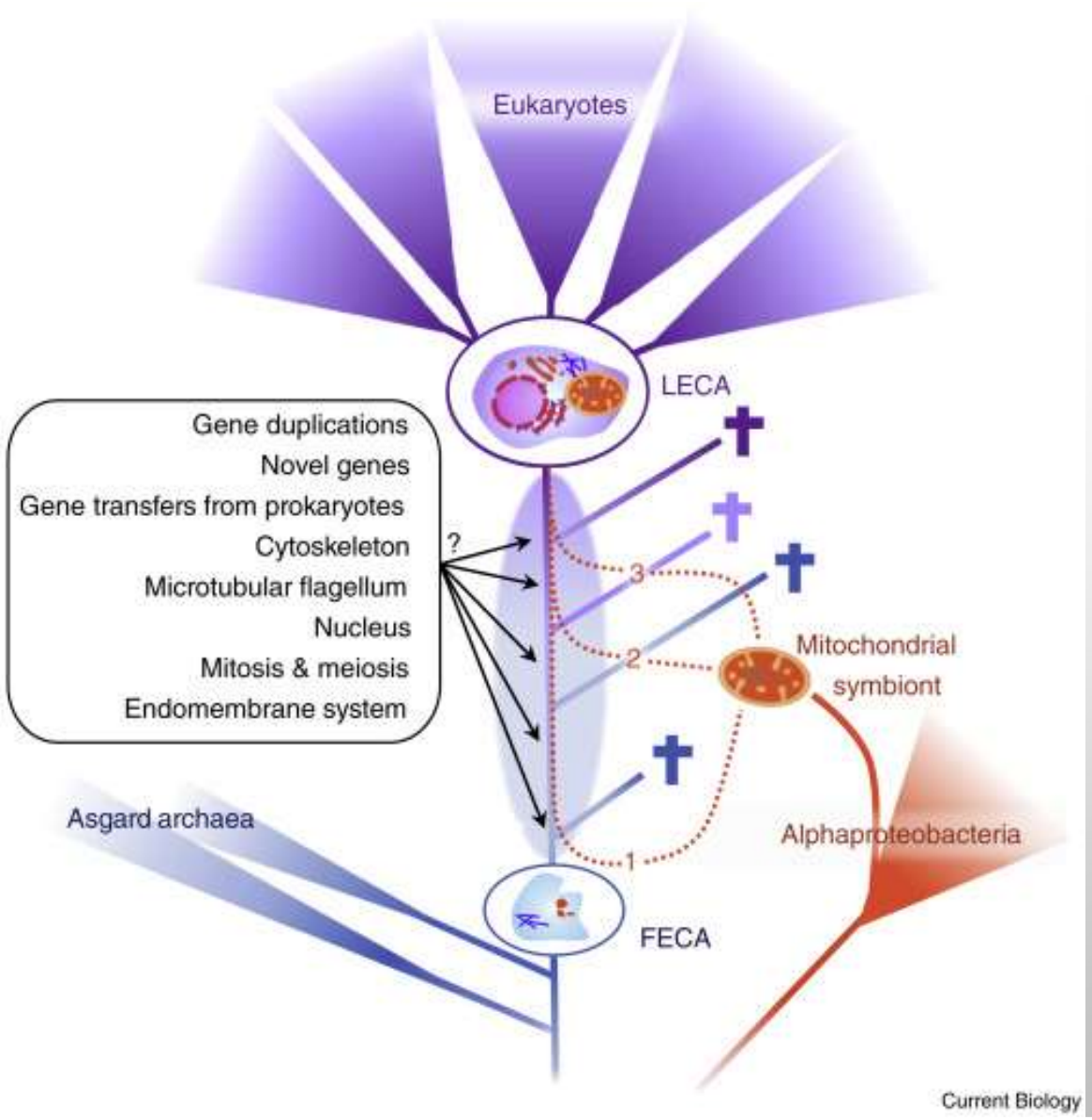


STEROIDS AS MARKERS OF EUKARYOTIC EVOLUTION



PHYLOGENETIC ANALYSES

- MOLECULAR CLOCK ANALYSES OFFER BROAD ESTIMATES FOR THE TIMING OF THE EMERGENCE OF EUKARYOTES
- THE EUKARYOTIC TOTAL GROUP (MARKED BY THE APPEARANCE OF THE FIRST EUKARYOTIC COMMON ANCESTOR) PROBABLY APPEARED SOMETIME BETWEEN 3000 AND 2300 MA
- CROWN GROUP EUKARYOTES (HERALDED BY THE LAST EUKARYOTIC COMMON ANCESTOR) PROBABLY APPEARED BETWEEN 1900 AND 1000 MA

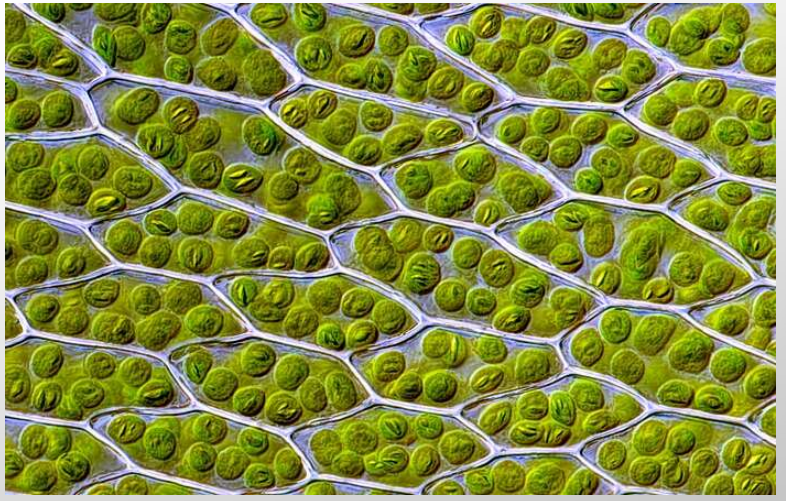

Eukaryotes
LECA
Gene duplications
Novel genes
Gene transfers from prokaryotes
Cytoskeleton
Microtubular flagellum
Nucleus
Mitosis & meiosis
Endomembrane system
?
3
2
1
Mitochondrial
symbiont
Asgard archaea
Alphaproteobacteria
FECA
Current Biology

MARRIAGE OF LINEAGES CONTINUES

- SOMEWHERE BETWEEN 1 AND 2 BILLION YEARS AGO, A FREE-LIVING CYANOBACTERIUM ENTERED AN EARLY EUKARYOTIC CELL
- THE NEW CELLULAR RESIDENT QUICKLY BECAME AN ADVANTAGE, PROVIDING FOOD FOR THE EUKARYOTIC HOST
- OVER TIME, THE CYANOBACTERIUM WAS ASSIMILATED, AND MANY OF ITS GENES WERE LOST OR TRANSFERRED TO THE NUCLEUS OF THE HOST (SIMILARLY TO WHAT HAPPENED TO THE ALPHAPROTEOBACTERIUM THAT BECAME THE MITOCHONDRIA)
- SO THE **CHLOROPLAST** CAME TO BE
- FROM GENOMES THAT PROBABLY ORIGINALLY CONTAINED OVER 3000 GENES ONLY ABOUT 130 GENES REMAIN IN THE CHLOROPLASTS OF CONTEMPORARY PLANTS

CHLOROPLASTS

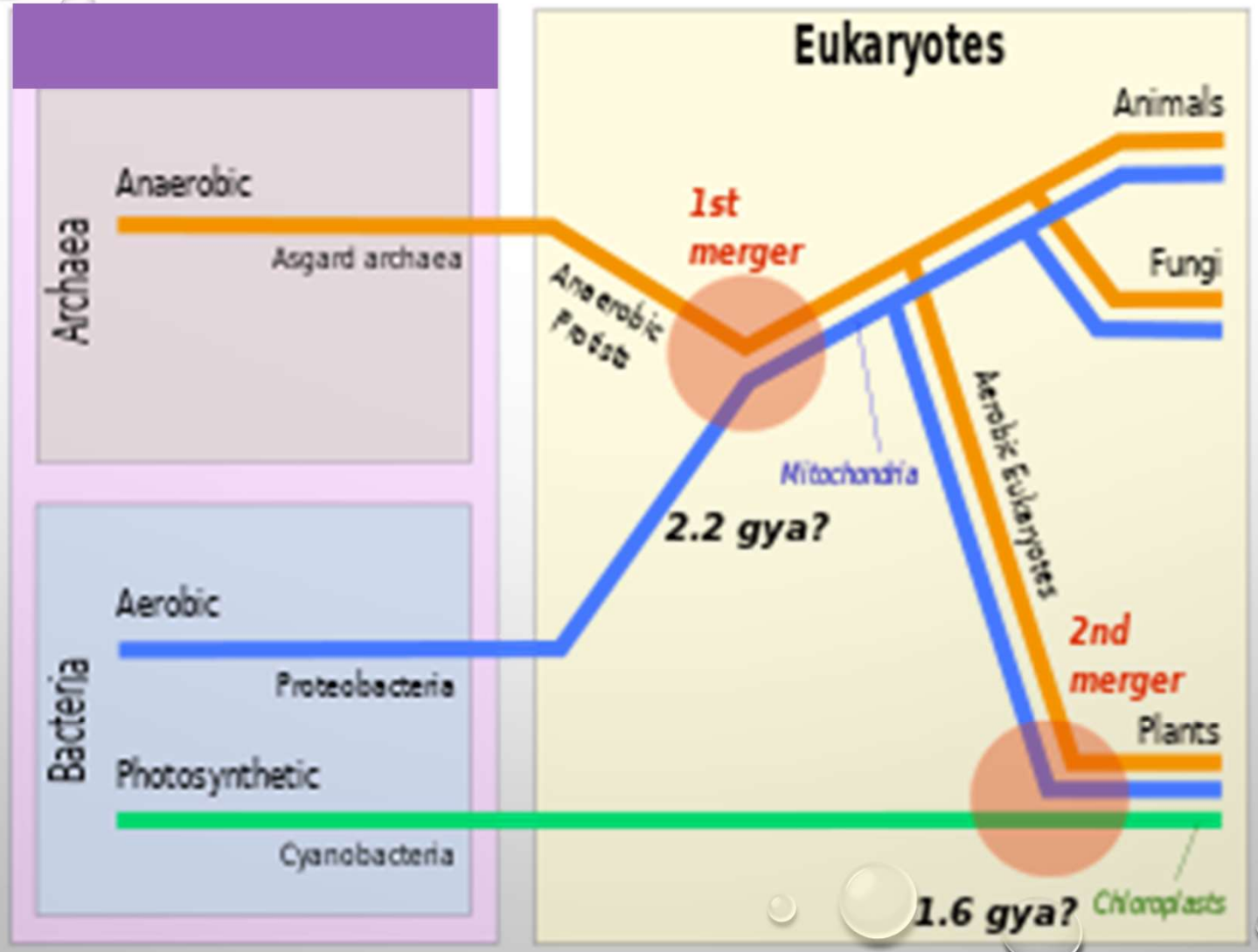
- MADE UP OF STACKS OF MEMBRANES
- THESE MEMBRANES ARE FULLY PACKED WITH THE APPARATUS FOR PHOTOSYNTHESIS
- THE REST OF THE ORGANELLE IS PACKED ALMOST FULLY WITH ONE DOMINANT PROTEIN WHOSE MAIN FUNCTION IS TO FIX CO_2
- NUMBER OF CHLOROPLASTS PER CELL VARIES SIGNIFICANTLY BETWEEN ORGANISMS AND EVEN WITHIN A GIVEN SPECIES CAN CHANGE SIGNIFICANTLY DEPENDING UPON GROWTH CONDITIONS
- THE CHLOROPLAST GENOME ENCODES CA. 130 GENES



- ALL CHLOROPLASTS CAN BE TRACED BACK TO A SINGLE ENDOSYMBIOTIC EVENT
- THIS ENDOSYMBIOSIS AND THE RESULTING EXPLOSION OF PHOTOSYNTHETIC ALGAE HELPED TRANSFORM THE BIOSPHERE FROM LOW TO HIGH OXYGEN
- EVERY RULE HAS ITS EXCEPTION
- MEET *PAULINELLA*, A PHOTOSYNTHETIC AMOEBA
- IS THE ONLY CASE OF PRIMARY ENDOSYMBIOSIS THAT LED TO THE ESTABLISHMENT OF A PHOTOSYNTHETIC ORGANELLE **OTHER THAN** THE EVENT THAT LED TO PLANTS 1.6 GA

EVOLUTION OF A SYMBIOSIS

- THE ENDOSYMBIOTIC EVENT HAPPENED ABOUT 90–140 MILLION YEARS AGO
- A WINDOW INTO EVOLUTION DYNAMICS: HOW A SYMBIONT BECOMES AN ORGANELLE
- THE ENDOSYMBIONT GENOME HAS GONE THROUGH A REDUCTION, AND IS NOW JUST ONE THIRD THE SIZE OF ITS CLOSEST FREE LIVING RELATIVES
- STILL 10-FOLD LARGER THAN MOST CHLOROPLAST GENOMES
- SOME OF THE GENES HAVE BEEN LOST, OTHERS HAVE MIGRATED TO THE AMOEBA'S NUCLEUS
- THIS CHANGED THE METABOLISM OF THE AMOEBA SO MUCH THAT IT COULD NO LONGER FEED ON MICROBES LIKE ITS ANCESTORS, AND IT BECAME COMPLETELY DEPENDENT ON ITS ENDOSYMBIONT
- WHICH IN TURN HAS LOST SO MANY GENES IT CAN NO LONGER SURVIVE OUTSIDE ITS HOST CELL


Archaea
Anaerobic
Asgard archaea
Bacteria
Aerobic
Proteobacteria
Photosynthetic
Cyanobacteria
Eukaryotes
Animals
Fungi
Plants
1st merger
Anaerobic Proto
Mitochondria
2.2 gya?
Aerobic Eukaryotes
2nd merger
1.6 gya?
Chloroplasts

THE TRIALS AND TRIBULATIONS OF BEING BIGGER

- FLEXIBLE CELL MEMBRANES THAT ALLOWS FOR EXPANSION (THINK PHAGOCYTOSIS)
- COUPLING THE PROTEIN SYNTHESIZING MACHINERY TO AN INTERNAL MEMBRANE SYSTEM THAT ALLOWS FOR ITS DISTRIBUTION AND TARGETING
- MEMBRANE DYNAMICS ALLOW FOR INCORPORATION, TRANSPORT, PACKAGING INTO VESICLES AND SECRETION, WHEN NEEDED
- A DYNAMIC CYTOSKELETON, WHICH WILL BE INSTRUMENTAL IN CELL DIVISION

GENOME COMPARISON

BACTERIA AND ARCHAEA

- SMALLEST GENOME: 1.3 MB (OCEANIC ALPHAPROTEOBACTERIA)
- LARGEST GENOME: 13 MB (SOIL BACTERIUM)
- CIRCULAR CHROMOSOMES

EUKARYOTES

- SMALLEST GENOME: 13.2 MB (OCEANIC GREEN ALGA)
- LARGEST GENOME: 150,000 MB (GRASS)
- LINEAR CHROMOSOMES

WHY LINEAR CHROMOSOMES

- THE FACTORS WHICH LED TO THE EVOLUTION OF LINEAR CHROMOSOMES IN EUKARYOTES ARE NOT WELL UNDERSTOOD
- LINEAR CHROMOSOMES MAY MAKE TRANSCRIPTION AND REPLICATION OF LARGE GENOMES EASIER
- MULTIPLE ORIGINS OF REPLICATION
- THE LINEAR ARRANGEMENT OF THE EUKARYOTIC CHROMOSOME ALLOWS MORE DNA TO BE PACKED BY TIGHTLY WINDING IT AROUND HISTONES AND AVOIDING TORQUE
- CHROMOSOMES COME IN PAIRS

THE END REPLICATION PROBLEM

- DNA REPLICATES IN A SPECIFIC DIRECTION → SYNTHESIS ALONG ONE OF THE STRANDS IS DISCONTINUOUS
- ONE STRAND GETS PROGRESSIVELY SHORTER!!!!
- IF CODING SEQUENCES ARE DEGRADED IN THIS PROCESS, POTENTIALLY VITAL GENETIC CODE WOULD BE LOST
- HENCE TELOMERES: REPETITIVE NON-CODING SEQUENCES THAT CAP THE ENDS OF CHROMOSOMES
- THEY DO GET SHORTENED WITH EVERY ROUND OF REPLICATION, BUT THEY PROVIDE A BUFFER ZONE BETWEEN THE ENDS AND CODING SEQUENCES
- ALSO, THE ENZYME TELOMERASE CAN REPLENISH TELOMERES BY ADDING REPETITIVE NUCLEOTIDE SEQUENCES TO THE ENDS OF THE DNA