What is the Origin of Life... a paradigm question for CMB...
or what is the origins of the Primordial Cell...

Was it a chemical evolution or an astrobiological event?

CURRENT PARADIGM...
most experimental evidence favors a chemical evolutionary origin of life...
"simple chemical self-assembly has lead to complex self-replicating systems"

Earth forms 4.5 billion years ago,

between 4.5 to 4.0 bya - asteroids bombard & sterilize planet's surface

then by 4.0 bya - first fossil evidence of microscopic life

Initial chemical event may have been evolution of CARBON BASED MOLECULES

Ancient atmosphere (was reducing) with single carbon gases... CO, CO₂, & CH₄

Is origin of Research Life Experimentally Testable?
4 experimental approaches used in today's Origin of Life Research

1st approach: Search for bioorganic precursor molecules of life...

A) formed from a chemically reactive soup... in early oceans of Earth
   1953 - Miller & Urey ---&gt; abiotic making of organics in lab experiments
   &gt; H2O, NH3, CH4, & H2 make HCN & formaldehyde: then amino acids, nucleotides,& sugars
   *link to timeline of experimental organic syntheses & origins of life*

B) 1979 - Deep dwelling (ocean) hydrothermal vents... (deep sea volcanic plumes)
   &gt; vents are full of organically rich molecules --&gt; life [ tube worms & bacteria ]
   Speculation: life may have originated in vents regions...

C) 1990's - astrobiological origins for biomolecules... Great 20th Century Discoveries
   &gt; Space Debris... space dust, meteorites, asteroids
   may have deposited organics on newly formed planet Earth.
   &gt; Comets are mostly ice crystals on cores of silicates & carbon
     contain about 10% CO, CO2, CH4, CH3OH, and NH3
   &gt; Asteroids contain molecules as... kerogen [a PAH], nucleobases, quinones,
     COOH's, amines & amides = some 70 amino acids, with 8 of common 20.

D) 2007 &gt; repeat of Miller-Urey famous experiments
2nd experimental approach: **MODEL MOLECULAR REPLICATIVE SYSTEMS**

**Evolution of an RNA world...** (which came 1st DNA or RNA)

in 1989 **Sidney Altman** and **Thomas Cech** showed that **RNA molecules RIBOZYMES** had **CATALYTIC ACTIVITY**

i.e., these RNA's catalyze hydrolysis & condensation rxs of phosphodiester bonds.

If RNA's can be a template and also **catalyze polymerization** of like molecules, i.e., **replicate itself**, then RNA molecules may have been the 1st **SELF-REPLICATING** living entity.  

No self-replicating **RNA molecules** exists naturally today, but lab experimentation may establish that it was feasible, and that **RNA molecules** can be selected for via Darwinian evolutionary mechanisms (**molecular natural selection**).
SELF-REPLICATING MOLECULES...
are an experimental bridges between molecules & living organisms...
the origin of stable self-replicating molecules represents a fundamental obstacle
to our understanding of the events in the origin of life.

Ribozymes/an RNA World... Gerald Joyce, et al (@ Scripps, LaJolla)
Joyce & Wright used a test tube of ribozymes that can reproduce indefinitely,
some with mutations, which improved rate of replication... Scripps Report
and studied ribozyme molecular selection.
"...with a starting ribozyme molecule, with barely detectable DNA-cleavage activity,
after 63 "generations" of in vitro selection for catalysis, showed a number
variants of ribozymes, that cleave single-stranded DNA with high efficiency and
specificity. These ribozymes had accumulated an average of 27 mutations
relative to the wild type ribozymes and had improved their ability to cleave DNA
by 10^6-fold"...

In Jan 2009 Tracey Lincoln & Gerald Joyce demonstrated a pair of RNA ribozymes each of which
could make copies of the other by joining together two shorter RNA strands. In 30 hours, they
found a population of RNA molecules could grow 100 million times bigger. Unfortunately,
success in their experiments required the presence of preexisting RNA pieces that were far too
long and complex to have accumulated spontaneously. Still, the results suggest that RNA has
the raw catalytic power to catalyze its own replication... Lincoln and Joyce kept their RNA
molecules in beakers... to demo the evolution of artificial replicating RNA molecules they
should be packed into cells...
Jack Szostak (Mass. General Lab) - REPLICA... Molecular Replication Systems...

He investigates how fatty acids (lipids) might have trapped RNA producing...

“evolving” new ribozymes.
Szostak started with trillions of random RNA sequences, selected ones that had catalytic properties, & made copies of those. At each round of copying some of the new RNA strands underwent mutations that turned them into more efficient catalysts. He was able to produce ribozymes that can catalyze the copying of relatively short strands of other RNAs. He then vesicleized his replicase RNA molecular complex that had the ability to make a copy of itself and direct other RNA molecules to replicate themselves…

His "vesicles" can add new fatty acids & grow and leaky enough to bring in new nucleotides, making a first "proto..."*

BUT - No self-replicating artificial RNA molecules exists naturally today,

However, lab experimentation may be able to establish that it was feasible, and that RNA molecules can be selected for via Darwinian evolutionary mechanism (natural selection).
3rd approach:

**PROTOBIONTS... chemically made artificial cells**

Sidney W. Fox  University of Miami (1912 - 1998)
Director of the NASA supported Institute for Molecular Evolution at UM.
His laboratory conducted analyses of the first moon rock samples...
- produced **proteinoids** from amino acids... dropped on hot lava rock, sand or clay.
- definition of **Protobiont** - an aggregate of abiotic made, chemically reactive molecules
- Internally... **chemically different** from their environment, & are metabolically active.

Some Examples of **Protobionts**

- **coacervates** made of polypeptides, polysaccharides & nucleic acids and lipids
  – form liposomes that are enzymatically active

- **Proteinoid microspheres** - are selectively permeable & have membrane potentials

- **liposomes** made from Lipids - are microscopic spherical vesicles that form when phospholipids are hydrated; can engulf smaller proteinoids making more active ones

  **It’s a big jump form protobionts to what a eukaryote of today is ???**
Could basic physiochemical properties acting in elementary artificial protocells have given rise to essential cellular behaviors?

Evidence to date includes:

- fatty acids have been found in meteorites & have been made under a variety of prebiotic conditions & self-assemble.
- artificial protocells may be made in the lab by encapsulating a self-replicating genome inside a chemically simple self-replicating membrane vesicle.
- artificial vesicles encapsulating active genome replicators do generate an osmotic pressure, which causes a vesicle to "steal" membrane fragments from other vesicles with less active genome pieces (figure*). Such "genomic fitness" may have evolved into cellular fitness.
- as fatty acid vesicles grow larger micelles, a transmembrane pH gradient can be generated, due to faster flip-flop of protonated FA's on outer leaflet. Acidification of a vesicle's interior stores energy in form of a pH gradient, a primary metabolic system of living cells.
4th experimental approach
Synthetic Biology & Protocell Research...

a. bottom-up approach: one can't truly understand what one can't build

goals: to assemble all the components to synthetically form life
to understand why & how matter can self-organize... and become living
an artificial man-made cell??????

Synthetic Biology is constructing fully functional cells from scratch...
the engineering of new genetic circuits, entire genomes, or organisms
to make complex biological machines
taking genetic elements to the level of engineering a cell and
altering gene content & arrangements to make novel designer genes

i.e., artificial creation of DNA molecules, genes, viri, & cells
that mimic, or surpass, natural systems.
Some examples of what has been done in Synthetic Biology so far:

1. **Synthetic Polio Virus**: July 2002: Molecular Origin of Life Research?

   E. Wimmer from the University of New York at Stony Brook used the poliovirus' widely known genetic sequence to synthesize the virus from shelf chemicals. They followed a recipe they downloaded from the internet and used gene sequences from a mail-order supplier. The artificially constructed virus appears identical to its natural counterpart; when injected it into mice the animals were paralyzed and died.

2. **Phi X-174** virus synthesized - November 2003:

   Craig Venter and colleagues created an artificial version of Phi X-174 by piecing together synthetic DNA ordered from a biotechnology company. They used a technique called polymerase cycle assembly (PCA) to link the strands of DNA together.
3. The 1918 Spanish Flu Virus is Reconstructed - October 2005:

Jeffery K. Taubenberger, a molecular pathologist at the Armed Forces Institute of Pathology and his colleagues were able to piece together the virus's genes from two unusual sources:

1) lung tissue removed at autopsy from a 21-year-old soldier and
2) the frozen body of an Inuit woman who died of influenza in November 1918 and was buried in the Alaskan permafrost.

These sources provided intact pieces of viral RNA that could be analysed and sequenced. The virus's has eight "RNA gene segments" and by gene sequencing and PCR they reassembled the virus. Two of the 8 genes: Hemagglutinin-A type [H5] and Neuraminidase type 1 [N1] are protein surface coatings.

There are at least 16 different HA antigens, which binds the virus to the host cell. Neuraminidase is an antigenic glycoprotein enzyme found on the surface of the flu virus. Nine neuraminidase subtypes are known, which aid in the efficiency of virus release from infected cell.
b. synthetic biology top-down-up approach:
   looking for a minimalist essential genome required to make a cell...

   J. Craig Venter, a principle investigator (P.I.) of the Human Genome Project is attempting to make a synthetic new type of bacterium using DNA manufactured in the lab; using the sequenced genes of a bacterium Mycoplasma genitalium, a gram-positive parasitic bacterium, whose primary infection site may be the human urogenital tract that causes non-gonococcal urethritis. It's circular chromosome has 580,073 base pairs, the smallest known genome of any free-living organism determined. M.g. has a total of only 525 genes (482 encoding for proteins; & 43 RNA genes).

> Venter's researchers began systematically removing genes [so called knock-out cells] to determine how many genes are essential for life. In 1999, they published the narrowed the needs of M. genitalium to between 265 & 350 genes.

> How many genes does it take to make an organism? What is the minimum genes a cell needs? The scientists at The Institute for Genomic Research (TIGR) who determined the Mycoplasma genitalium sequence followed this work by systematically destroying its genes (by mutating them with insertions) to see which ones are essential to life and which are dispensable. Of the 480 protein-encoding genes, they conclude that only 265–350 of them are essential to life.

> next step: to artificially assemble these 300+ genes & create SYNTHETIC CELL
Some (unexplained) Events in Chemical Evolution of Eukaryotes

the evolution of the eukarya was single most important step in evolution of multicellular life forms & was a key step that lead to plant & animal life.

1. cell membrane encapsulates genetic DNA... development of nucleus greatest evolutionary invention - it internalized the genome
2. loss of a rigid cell wall...
   cells developed ability of phagocytosis - allowed engulfing of foods also allowed cells to clump together --> multicellularity --> tissues
3. evolve a selectively permeable membrane...
   protects cell, allows uptake nutrients & exchange with environment
4. evolve a cytoskeleton...
   provides framework- allowed cell to grow larger, move, & permitted metabolism; eukarya are 10x larger that bacteria
5. evolve aerobic respiration... more efficient energy transformation
6. develop various organelles... (maybe by endosymbiosis)...
   a sub-cell part that catalyzes a specific metabolic function
7. development of sexual cell cycles... (transposons - moveable genes)...
   a method to shuffle genes along chromosomes favored cellular evolution.