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Jack Szostak's Lecture Part 1:
The Origins of Life

Teaching Tools were prepared by Kelsey Hass and Jack Szostak.

Contents

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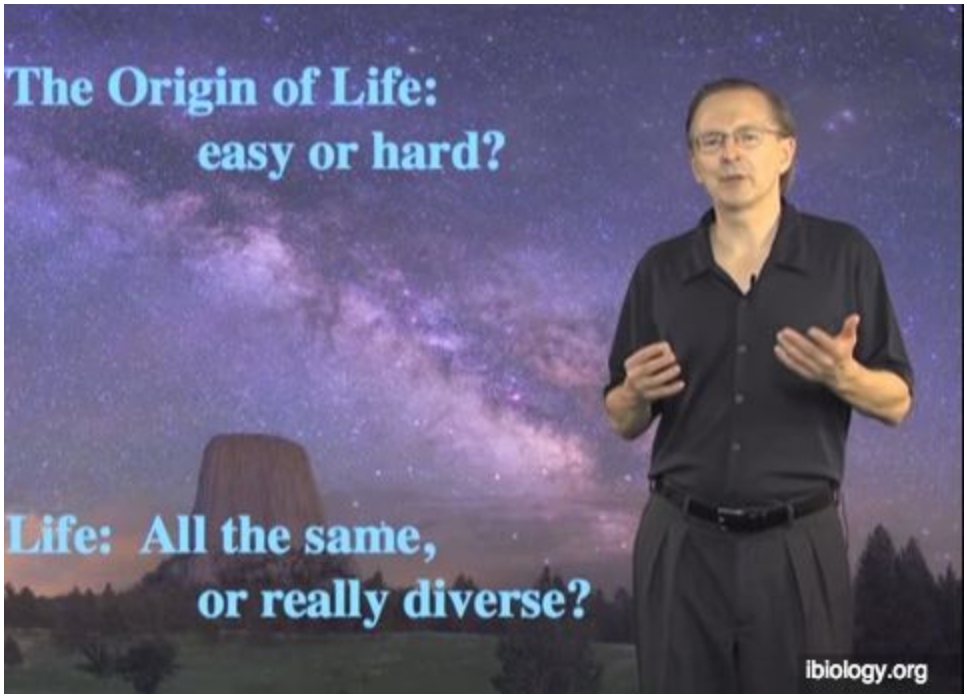
1. Keywords and Terms

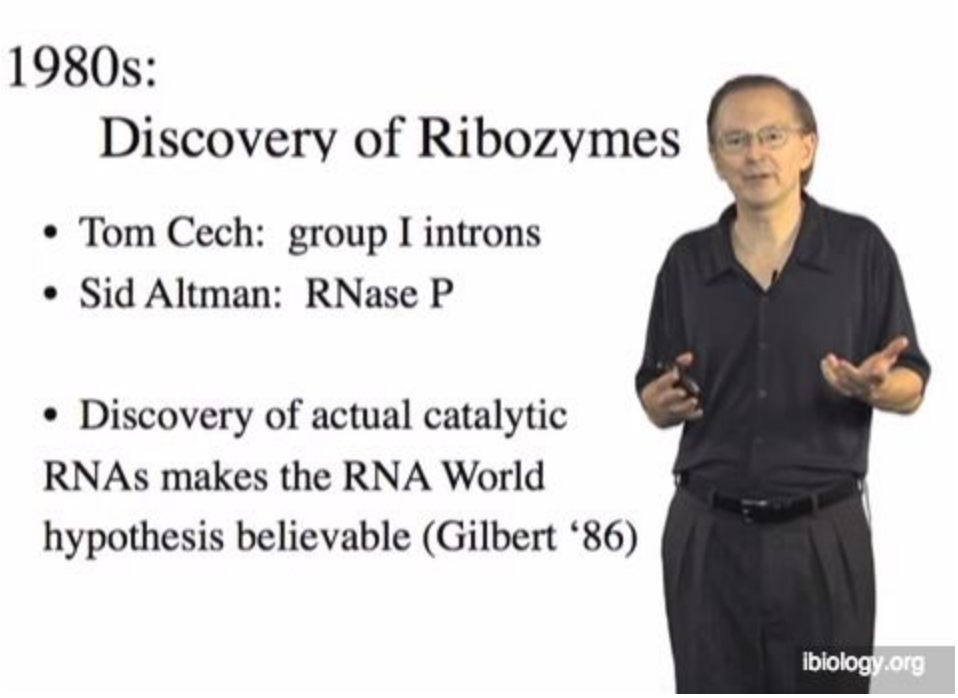
RNA world hypothesis, prebiotic chemistry, fatty acids, nucleotide triphosphates, protocell

2. Lecture Notes



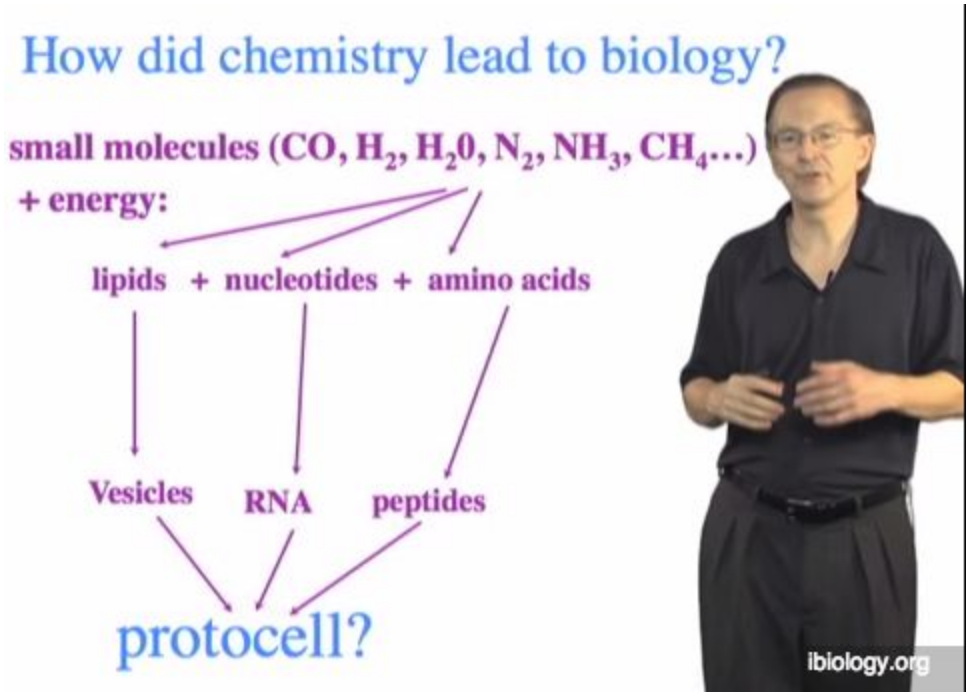
Time: 3:07

How did life originate on Earth in the harsh, extreme environments of the early planet? Was the chemistry to produce essential biomolecules relatively straightforward and simple, or was it complex and full of bottleneck steps? Is cellular life easy to evolve, or a rarity? How diverse are the biomolecular solutions that can support life? In this lecture, Jack Szostak introduces some fundamental experiments and hypotheses for the origins of life.



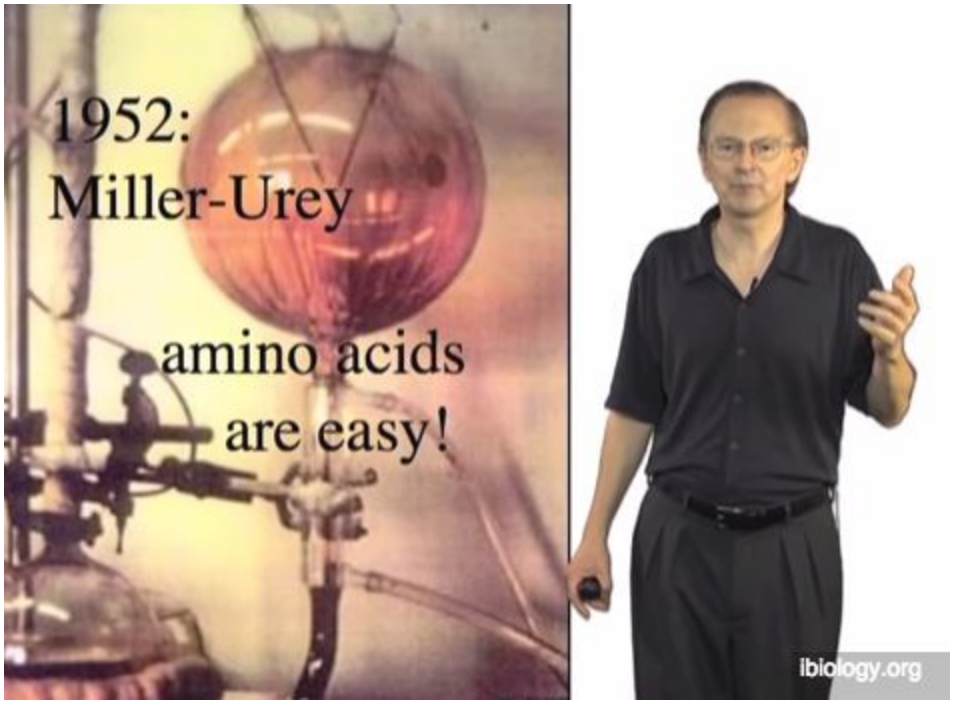
Time: 13:22

The discovery that RNA species could catalyze reactions and carry information led to the hypothesis that life emerged as a single cell carrying RNA polymer(s). As evolution continued, specialized storage molecules, transport systems, and compartments of modern cells would emerge.



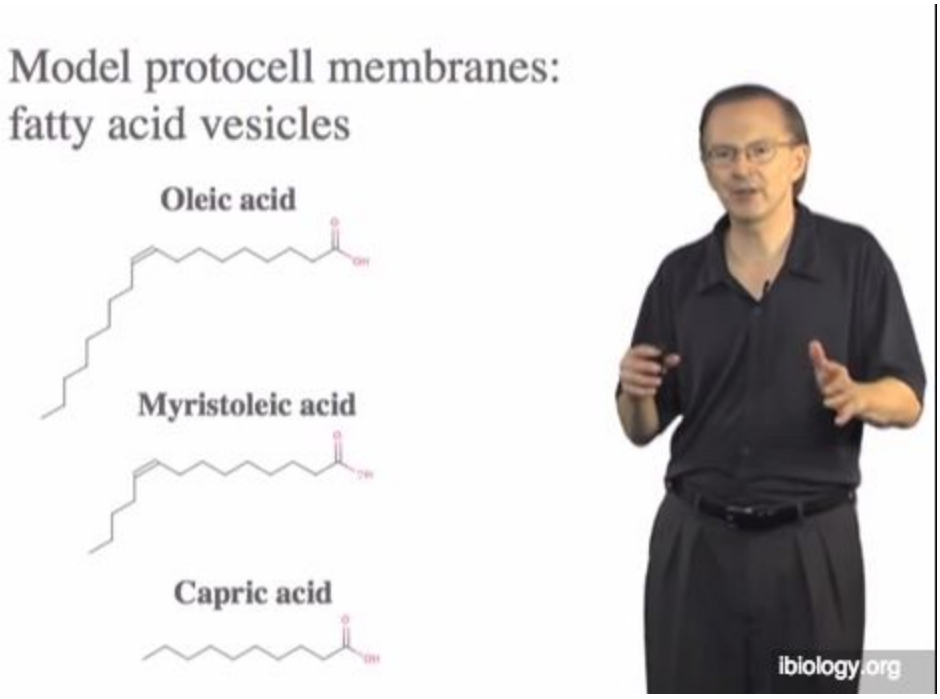
Time: 21:00

How did chemistry lead to biology? One model is that simple prebiotic molecules spontaneously combined in different ways with energy input from extreme environmental conditions. The products of these combinations were primitive precursors that could further combine to generate fundamental biomolecules.



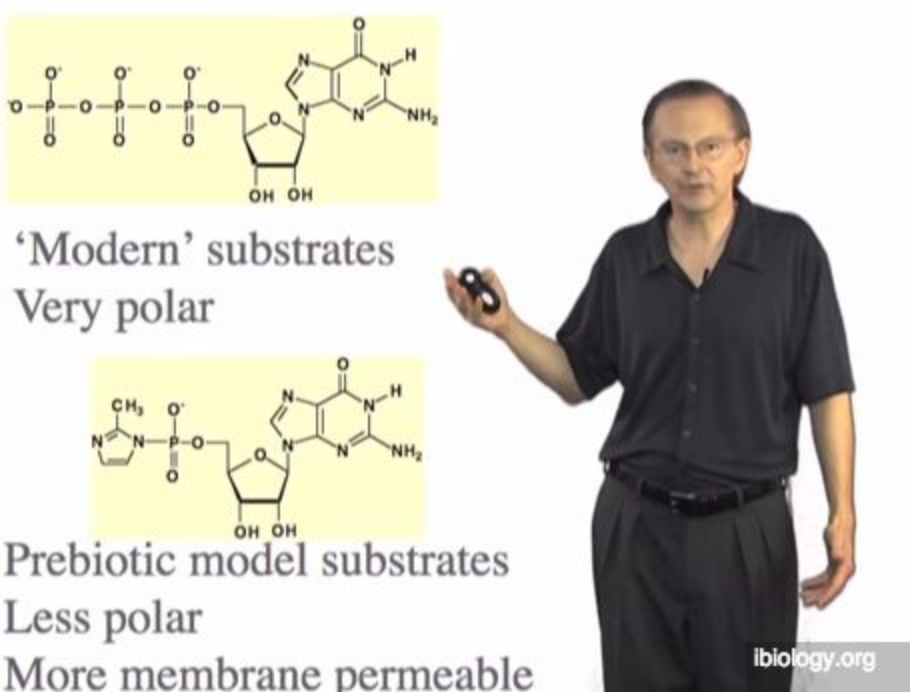
Time: 27:56

An important proof-of-principle experiment was the Miller-Urey experiment. Here, exposing reducing gases to an electric spark discharge generated many different amino acids. This suggested other biomolecules may be generated from high energy prebiotic building blocks under extreme environmental conditions.



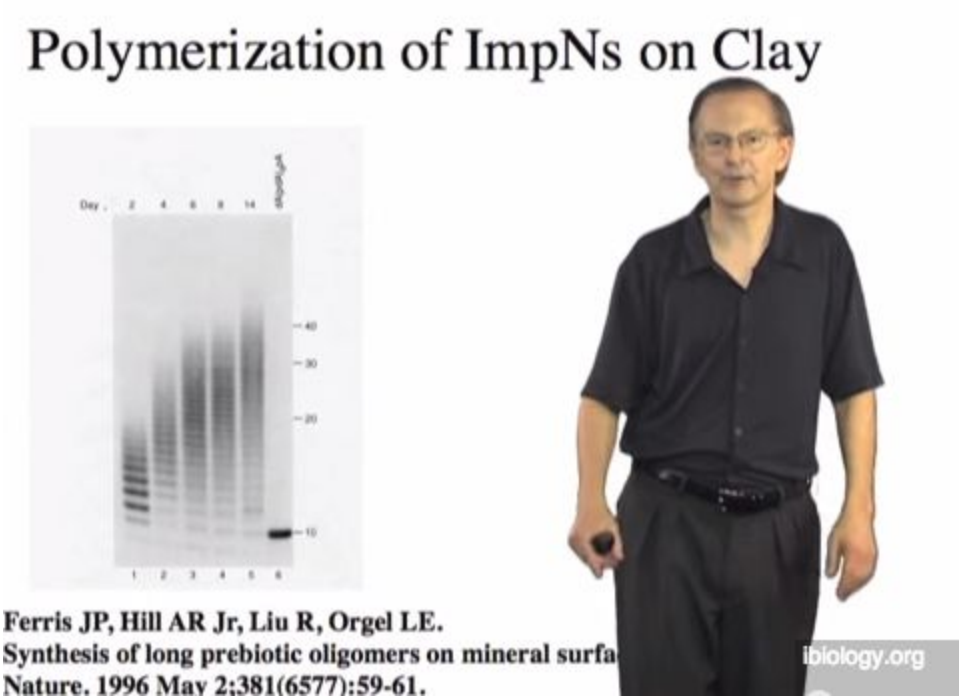
Time: 33:42

Short chain fatty acids are plausible molecules to form protocell membranes. Fatty acid membranes are permeable and allow diffusion of prebiotic molecules. Simply shaking these lipids in water, salt and buffer can form a heterogeneous mixture of different-sized single- or multilamellar vesicles.



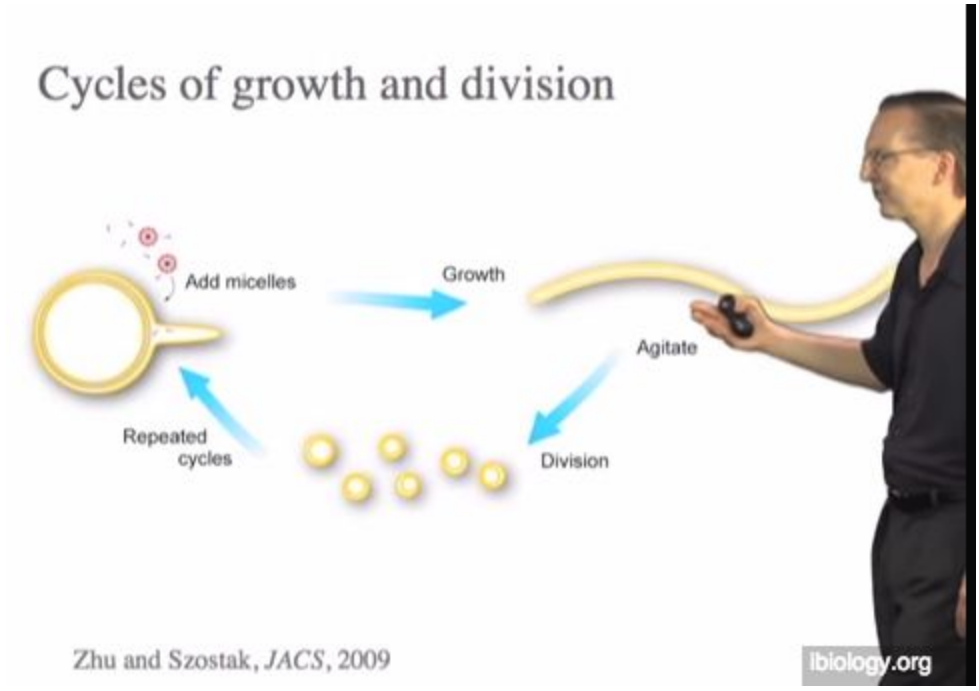
Time: 38:22

Prebiotic nucleotide triphosphates have different chemical and structural properties compared to modern nucleotide triphosphates, as summarized on this slide.



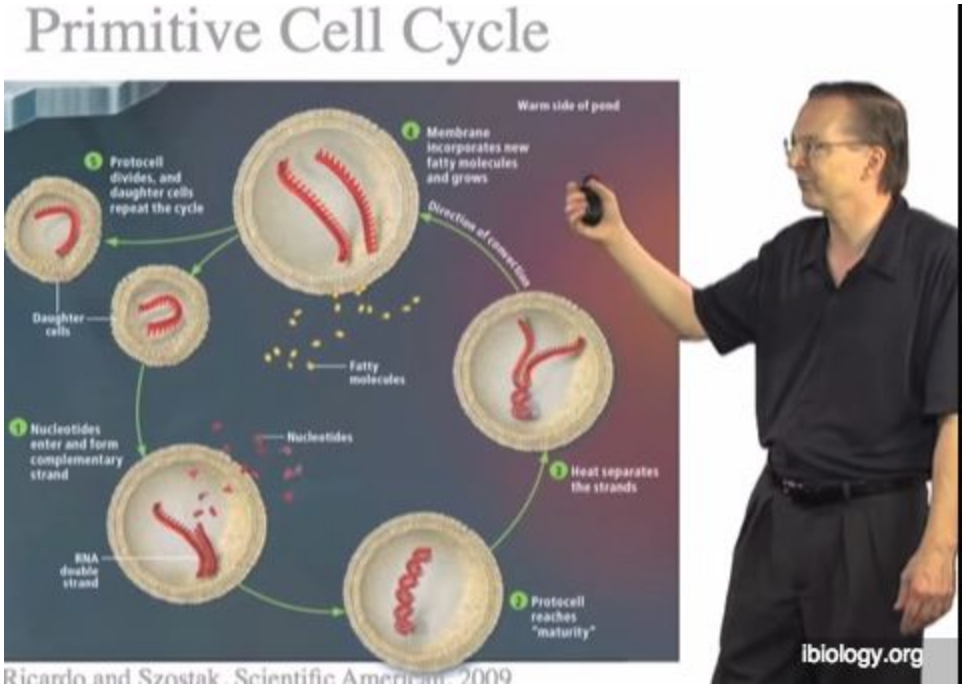
Time: 41:59

How could the first genetic material be synthesized in early Earth environments? This example experiment showed that specific prebiotic nucleotides (ImpNs) could spontaneously polymerize to form long-chain polymers when exposed to a clay surface.



Time: 50:23

The current model for growth and division of protocell membranes is summarized to the left. Vesicles can grow in the presence of fatty acid micelle food, forming filamentous structures that shear into daughter cells.



Time: 51:17

The primitive cell cycle model is summarized to the left. Here, a protocell contains genetic material molecules that can spontaneously self-replicate with rapid influx of precursor nucleotides. Genetic material can be passed down to daughter cells as the membrane grows in size and splits. The ideal environment for replicating protocells would be in geothermal convection currents; cold temperatures favor replication chemistry while high temperatures favor strand separation and division.

3. Review Questions

1. What is the RNA world hypothesis?
2. What biological building block was spontaneously generated in the Miller-Urey experiment?

    a. Nucleic acids
    b. Fatty acids
    c. Amino acids
    d. Sugars

e. Proteins

3. What biomolecule is hypothesized to compose primitive protocell membranes?

4. What are the chemical property/properties of prebiotic nucleotides?

    a. Less polar
    b. High membrane permeability
    c. Reactive nucleophilic group
    d. A and B only
    e. A-C

5. What primitive environmental material is hypothesized to deliver RNA into vesicles and allow RNA self-replication?

4. Answers to Review Questions

1. The RNA world hypothesis states that RNA may have been the primitive cell's genetic molecule, since RNA can store genetic information in a nucleotide base code and catalyze reactions ranging from RNA self-replication to chemical/ribozyme functions.

2. Answer: c.

3. Short chain fatty acids are hypothesized to compose the protocell membrane.

4. Answer: e.

5. Clay is hypothesized to deliver RNA into vesicles.

5. Discussion Questions

1. What insights from the modern world/modern ecosystems lead us to believe that life could emerge even in harsh, extreme environments?

2. What experimental evidence or results might indicate that life is "easy" to emerge in harsh environments? What evidence or results might indicate that life is "hard" to emerge in harsh environments?

3. Explain the protocell. How could these cells undergo Darwinian evolution?

6. Answers to Discussion Questions

1. A variety of harsh, extreme environments support microbial life. For example, microbes have been identified in hydrothermal deep sea vents (with high temperature and pressure) and Yellowstone hot springs, photosynthetic cyanobacteria have been found in acidic rocks (pH 1), and microbes have been found in acidic water bodies in Spain (pH 1.7-2.5). It is therefore plausible that early life could have emerged from similar extreme environments.

2. Early life is hypothesized to have “simple” chemical reactions that support protocell functions (eg growth, division, genetic information replication). Using modern biomolecules, we can deduce the likely prebiotic starting materials that would have been abundant in prebiotic environments. Whether life was “easy” or “hard” to emerge depends on the specific chemistry required to generate important biomolecules from these precursors. If the chemical pathways are relatively straightforward and rapid, and precursors can undergo simple chemical reactions in the lab to generate larger biomolecules, then life can be seen as “easy” to emerge. If the chemical pathways rarely occur or contain bottleneck chemical steps (steps that limit the rate or quantity of biomolecule synthesis), then life can be seen as “hard” to emerge.

3. The protocell is a model primitive cell that contains a fatty acid membrane enclosing a few molecules of RNA (or other long-chain nucleic acid derivative). Vesicle membranes likely contained short chain fatty acids, as these lipids increase membrane permeability. The protocell is hypothesized to grow by engulfing fatty acids from the environment, morphing into a filamentous structure and breaking apart into new tiny protocell vesicles. Long-chain nucleic acids were likely spontaneously generated and replicated following Watson-Crick base-pairing rules on clay, ice crystals or other surfaces. Long-chain nucleic acids are composed of prebiotic nucleotide triphosphate derivatives that diffused through the protocell membrane from the environment.

   After spontaneous generation, these cells could undergo Darwinian evolution because each protocell isolated specific RNA molecules inside its membrane compartment. Any additional mutation made in the RNA that conferred an advantage would only affect that protocell. This lucky protocell would then have a selective

advantage for its environment, allowing it to replicate more efficiently and increase its descendants in the population.

7. Explain or Teach These Concepts to a Friend

1. Teach the primitive cell cycle to a friend. Explain/draw a protocell (including its membrane composition and vesicle content), and describe/draw how it i hypothesized to self-replicate.
2. If you were a scientist in this field, what scientific question about the origins of life and/or protocell life cycle model would you tackle? What experiments would you conduct?

   Note: This may require students to do a bit of literature research. One advised starting point: look up Jack Szostak on PubMed, and read other papers cited in research or review articles written by members of his lab. Students should be encouraged to choose questions/experiments not yet conducted in published research articles.

8. Paper for Journal Club

Discussion Paper

Adamala, K. and Szostak, J.W. “Nonenzymatic Template-Directed RNA Synthesis Inside Model Protocells.” 2013. Science. 342: 1098-1100.

Discussion Questions

Note: Students are not limited to answers provided below; answers serve as one example for the discussion section. Students are encouraged to pursue other avenues of discussion for each question.

1. What was the “roadblock” in non-enzymatic RNA replication chemistry that the authors addressed?

The authors’ model for prebiotic cells has two major components: fatty acid membranes and RNA molecules that self-replicate. The chemistry to accomplish RNA copying

requires a high concentration of magnesium ions, among other divalent (2+ charged) ions. However, in high concentrations of magnesium, the fatty acids become water-insoluble and aggregate into clumps (precipitate out). This destroys spontaneous arrangement of fatty acids into vesicles to form the prebiotic cell. The major roadblock addressed in this paper is resolving the incompatibility between fatty acid integrity as vesicles and successful RNA copying in high concentrations of magnesium.

Other avenues of discussion:

Why do you think magnesium ions are required for RNA replication chemistry?

[Magnesium ions are necessary for catalysis chemistry in linking free-floating nucleotide triphosphates to the growing RNA strand backbone.]

If you were given this “roadblock”, what experiments would you do to find a solution?

2. Talk through Figure 2. What did they test, and how? Describe the main conclusion(s). What could be some biases in their primer-extension assay? What properties of chelators are beneficial for a primitive protocell?

In Figure 2, the authors tested the rate of RNA synthesis in the presence of Mg^{2+} and/or the chelators identified in Figure 1 that protected fatty acid membranes.

The rate of RNA synthesis was tested by primer-extension assay. Here, RNA duplexes were pre-formed such that two strands, a template and primer, were stably base-paired into a duplex at room temperature.

The template strand contained an additional stretch of 4 cytosine residues, and the corresponding strand (lacking the C residues) served as a primer for spontaneous base addition of primitive guanosine derivative, 2MeImpG. The primer strand also contains a Cy3-fluorophore at the 5’end, which allows the authors to visualize the length of the primer strand over time by gel analysis. At different time points, aliquots of the reaction were taken. RNA was isolated by ethanol precipitation, and analyzed by gel electrophoresis. Gel bands were visualized by fluorescence imaging. The results would be similar to the gel in Figure 2C, which shows the amount of primer extension (all bands except the bottom row) and unused primer template (bands in the bottom-most row of the gel) with 50mM Mg^{2+} and 200mM citrate over time.

Gel band intensities were then quantified by a specific analysis program. The quantitation is useful to determine total primer intensity (P_0 in Figure 2A; all bands per one lane in 2C) and unextended primer intensities (P in Figure 2A; bottom-most band

per one lane in 2C). Graphing the ratio of unextended primer/total primer (y-axis Figure 2A) over time (x-axis Figure 2A) gives the rate of RNA synthesis (essentially, depletion of unextended primers over time). The slope of the graph represents the rate of RNA synthesis. The slope of the line for each condition in Figure 2A is plotted in Figure 2B.

Looking at Figures 2A and 2B, we see the 50mM Mg^{2+}/no chelator condition gives the smallest amount of unextended primer, and the maximal rate of RNA synthesis at 1.4 $hour^{-1}$. However, this concentration of Mg^{2+} is incompatible with membrane vesicles (see question 1 above or authors' supplementary text). Of the two chelators tested with 50mM Mg^{2+}, citrate allows the highest rate of RNA synthesis at 0.67 $hour^{-1}$. The rate of RNA catalysis with Mg^{2+} and EDTA is close to 0 $hour^{-1}$, showing EDTA is too efficient of a chelator (it immobilizes too many Mg^{2+} ions thereby depleting the stock available for RNA catalysis, resulting in large amounts of unextended primer over time).

The main conclusion of Figure 2 is that the membrane-protective chelator, citrate, allows a reasonable rate of non-enzymatic RNA template copying in 50mM Mg^{2+}, both in solution and with oleate vesicles.

One potential bias of the primer-extension assay is the choice of nucleotide base. The authors measured synthesis rates with only one base, the guanosine derivative 2MeImpG. However, primitive adenine and uracil bases are known to have slower synthesis rates in non-enzymatic RNA synthesis chemistry. It is unknown if citrate could be an effective chelator for all bases (A, U, C and G), or if the timing would be too slow with other bases (A, U) for sufficient RNA synthesis in protocells.

In model protocells, chelators would perform two roles: (1) protection against fatty acid membrane dissolution by high concentrations of Mg^{2+} ions, and (2) protection against Mg^{2+}-induced RNA degradation.

Other potential answer: Chelators must prevent increases in the melting temperature (Tm) of a RNA duplex. If the melting temperature is high, the duplex is stable. Spontaneous "unwinding" of the duplex to allow single-stranded template copying will occur less frequently. Therefore, with high melting temperatures, the protocell would not be able to quickly replicate its RNA.

3. Talk through Figure 3. What did they test, and how? What is the "liposome dialyzer", and what is the method's significance for the paper (focus on 3E)? Any critiques for this figure?

In Figure 3, the authors measured the amounts of primer extension (RNA synthesis) in different membrane vesicle compositions over time.

RNA synthesis was tested by primer extension assay, with a template region of 7 C residues (Figure 3AD) or template region of mixed G and C residues (Figure 3E). Primer strands are complementary to template strands but lack the specified template regions, promoting non-enzymatic RNA synthesis off of the 3' end of the primer. The authors formed vesicles with different fatty acid compositions (Figure 3B-D), and each vesicle encapsulated template-primer duplex RNA, Mg^{2+} ions and citrate. Vesicles were

incubated with 2MeImpG nucleotide base (Figure 3A-D), or a mixture of prebiotic G- and C-derivative bases (Figure 3E). At indicated times, the authors collected aliquots of vesicles, extracted and purified the RNA, and analyzed primer RNA length by polyacrylamide gel electrophoresis. Primer extension was visualized by fluorescence imaging of the 5' Cy3 fluorophore signal on the primer. Bands in the bottom row of the gel correspond to unextended primer, and bands at increasing lengths represent one base extended (per interval) on the primer strand.

As shown in Figures 3A-D, most of the primer is extended by 24-72 hours after one-time addition of excess nucleotide base. Figure 3A serves as a control reaction (in solution, no vesicles), while Figures 3B-D show non-enzymatic RNA synthesis in three different model protocell membrane compositions. 3D shows the most prebiotically plausible membrane composition, as it consists of shorter chain lipids that are naturally permeable to reagents like prebiotic nucleotide triphosphates.

Figure 3E analyzes two important conditions: the efficiency of synthesizing across a mixed G/C-base template region, and the efficiency of RNA catalysis with fresh monomer addition. Fresh monomers are added by incubating vesicles in a liposome dialyzer over the course of the reaction. In the lipid dialyzer, vesicles are constantly exposed to fresh sources of the prebiotic nucleotide triphosphates. This is significant because the lipid dialyzer more accurately mimics the environmental conditions that prebiotic protocells would have experienced. With fresh monomers, primer extension occurs as efficiently for vesicle-encapsulated RNA primers (lanes 1 and 2, Figure 3E) as for RNA primers in solution (no vesicles, lane 3, Figure 3E). Addition of fresh nucleotide bases results in a more intense fully-extended primer band (lane 3, Figure 3E) than one-time addition of nucleotide bases (lane 4, Figure 3E), implying fresh addition of nucleotide base can increase the amount of extended primer at a given time point.

One critique for this figure is that "mixed bases" only refers to G/C-base template. There is still no data offered on A and U base synthesis.

Other avenues of discussion: Is citrate a viable prebiotic molecule? Would it have a more likely prebiotic form (for example, the “prebiotic form” of guanosine nucleotide triphosphate is 2MeImpG), and would this prebiotic form act differently?

4. Comment on the time scale for RNA replication vs. RNA degradation in a fatty acid vesicle containing Mg^{2+} and citrate. Does this seem plausible for early life?

RNA replication with 50mM Mg^{2+} and 200mM citrate in solution or in oleate vesicles was ~0.7 $hour^{-1}$, while RNA degradation with 50mM Mg^{2+} and 200mM citrate in solution was ~0.004 $hour^{-1}$. With these numbers, spontaneous RNA synthesis occurs ~175 times faster than degradation when 2MeImpG nucleotide base is in excess. The time scale is certainly plausible for a protocell to non-enzymatically replicate its RNA.

5. Recall the model for the protocell life cycle (Jack Szostak iBio seminars). To what extent does this paper piece together critical elements of the model? What aspects of the model remain to be tested?

The model for the protocell life cycle involves spontaneous protocell vesicle membrane growth/division and spontaneous, non-enzymatic replication of vesicle-encapsulated RNA. This paper focuses on the latter, in particular resolving the incompatibility between high Mg^{2+} concentrations required for RNA replication chemistry and Mg^{2+}-catalyzed destabilization of the vesicle membrane. The Szostak lab has previously shown that vesicles can spontaneously grow and divide through a multilamellar vesicle intermediate; this paper shows RNA molecules can self-replicate in fatty acid vesicles with Mg^{2+} and a chelator. What remains to be tested is combining the two facets together: combining the replication chemistry with conditions that promote self-replicating vesicles to mimic the complete protocell life cycle.