Possible origin of life between mica sheets

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ABSTRACT

The mica hypothesis is a new hypothesis about how life might have originated. The mica hypothesis provides simple solutions to many basic questions about the origins of life. In the mica hypothesis, the spaces between mica sheets functioned as the earliest cells. These 'cells' between mica sheets are filled with potassium ions, and they provide an environment in which: polymer entropy is low; cyclic wetting and drying can occur; molecules can evolve in isolated spaces and also migrate and ligate to form larger molecules. The mica hypothesis also proposes that mechanical energy (work) is a major energy source that could have been used on many length scales to form covalent bonds, to alter polymer conformations, and to bleb daughter cells off protocells. The mica hypothesis is consistent with many other origins hypotheses, including the RNA, lipid, and metabolic 'worlds'. Therefore the mica hypothesis has the potential to unify origins hypotheses, such that different molecular components and systems could simultaneously evolve in the spaces between mica sheets.

1. Introduction

Life imitates mica in many ways. Life as we know it is organized into compartments – cells – whose contents are high in potassium and filled with nano-structured surfaces that interact among themselves. Mica (Fig. 1) is a mineral source of potassium-rich compartments with nano-structured surfaces that interact among themselves. Muscovite mica is proposed, in the mica hypothesis (Hansma, 2007, 2009), to be the place where life originated.

The mica hypothesis brings new ideas about the origins of life on mineral surfaces. In the mica hypothesis, life originated *in* a mineral: between the sheets of mica (Fig. 2). In the mica hypothesis, mica provides a multitude of nano- and micro-environments in which extensive pre-cellular and proto-cellular evolution was possible, sheltered within the compartments between mica sheets. These tiny micaceous environments consist of vast numbers of separate spaces connected to one another at the edges of the sheets and by percolation of water in changing patterns in the spaces between sheets. These separate spaces allow separate evolution, while the connections between spaces allow new molecular combinations and increases in complexity.

The mica hypothesis proposes a new energy source for the origins of life – mechanical energy, *i.e.*, work (Figs. 3 and 4). Simple mechanical energy could have been a significant energy source for the origins of life. Mechanical energy came from the up-and-down movements of mica sheets in response to temperature changes and water flows. This mechanical energy of moving mica might have been used to form covalent bonds (Fig. 4), to change polymer conformations (Fig. 3), and to bleb daughter cells off of protocells, in the earliest form of cell division (Fig. 2B).

The mica hypothesis proposes that potassium ions (K⁺) between clay mineral sheets are the original source of intracellular potassium. More specifically, the mica hypothesis proposes that the K⁺ between *mica* sheets is the origin of intracellular K⁺. The mica hypothesis proposes that the spaces between mica sheets functioned as prebiotic cells before living cells were enclosed by membranes. Fluid percolates into and out of the spaces between mica sheets (Fig. 6). This provided alternating wet and dry cycles in the spaces between mica sheets.

The mica hypothesis proposes that confinement between mineral sheets is a form of entropy reduction used in the origins of life (Fig. 5). Confinement between mica sheets could also increase the specificity of solid phase synthesis. The mica hypothesis proposes that this confinement between crystalline mineral surfaces resulted in chiral biopolymers.

Darwinian evolution occurs, according to the mica hypothesis, because molecules between mica sheets have the potential to react and evolve, while molecules that leave the mica sheets are lost. The mica environment selects for larger molecules over smaller ones. The mica environment selects for polymers that interact with mica's crystal lattice and rejects polymers that lack any complementarity with the mica surface.

The mica hypothesis also complements much previous research on the origins of life, because the spaces between mica sheets provide a unique environment suitable for the RNA world (Figs. 7 and 8), prebiotic origins of life in lipid vesicles, and primitive metabolism (Chen et al., 2004; Gesteland et al., 2006; Gilbert, 1986; Segre and

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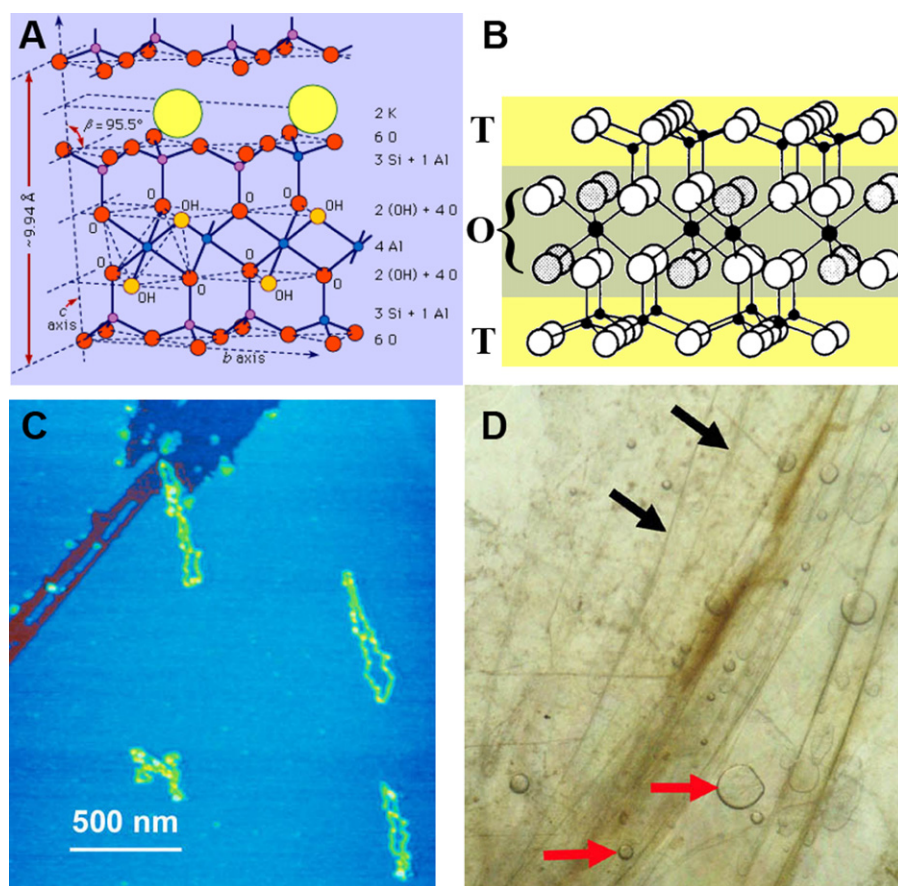 E-mail addresses: hhansma@physics.ucsb.edu, helen.hansma@gmail.com

Fig. 1. Mica and Clay. A. Crystal structure of mica, with atoms, coordinates, and layer spacing, for the doubled unit formula: $K_2Al_4(Si_6Al_2)O_{20}(OH)_4$ (http://www.britannica.com/EBchecked/topic/379747/mica, 2009). Tetrahedral and Octahedral layers are as in B. **B.** Crystal structure of 2:1 layer type clay mineral, where small black circles in the top and bottom T layers are Si, open circles are O, shaded circles are usually OH and large black circles in the middle O layer are metals such as Al, Mg, Fe, etc. (Sposito et al., 1999) T=Tetrahedral layers; O=Octahedral layer. **C.** Molecules on mica, imaged with an AFM (Digital Instruments Multi-Mode) in air. The 4 yellow molecules are circular DNA coated with a single-stranded binding protein. The 2-nm-high mica step edges are due to damage during the splitting of mica, creating the diagonal defects on the upper left (purple) in the otherwise atomically flat mica surface (blue). **D.** Mica from an abandoned mica quarry, with water between some layers, showing step edges (e.g., black arrows at top), air bubbles in water (e.g., red arrows at bottom), and brown bands of organic matter and dirt. Step edges are hundreds of mica sheets high, because these step edges are visible at low magnification. Photographed through a dissecting microscope (Edmund Scientific) with a Pentax Optio S40 camera. Image width ~5 mm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Lancet, 2000; Smith et al., 2009; Szostak et al., 2001; Wachtershauser, 2007). Therefore, 'between mica sheets' is a location likely to be favorable for many of the prebiotic chemistry experiments that have been done and much of the theoretical work on the origins of life.

2. Mica and mica chemistry

Micas are sheet alumino-silicates arranged in 'books' of nm-thick mineral sheets. The monoclinic crystal sheets have perfect basal cleavage. Muscovite, a common dioctahedral mica, has the chemical structure, $KAl_2(Si_3Al)O_{10}(OH)_2$. Each mica sheet has a TOT (tetrahedral–octahedral–tetrahedral) layer structure, with the 'O' layer (aluminum oxide) sandwiched between 2 'T' layers of silicon oxide (Fig. 1A, B). The silicon oxide 'T' layers have random substitutions of Al for Si, which give mica its negative surface charge (Pashley and Israelachvili, 1984). In unsplit muscovite, K^+ holds adjacent sheets together by bridging recessed hydroxyls and oxygen anions on adjacent sheets, in a hexagonal grid with a periodicity of 0.5 nm (Pauling, 1930). These recessed oxygens occur at the centers of a hexagonal silicon-oxide network that forms the mineral surfaces. The recessed oxygens are bonded to aluminum, in the 'O' layer, slightly below the 'T' layer of the mica surface. Bound K^+ can exchange with other mono- or multi-valent cations in binding to these recessed oxygens. This can be seen in

Fig. 1A, in which large yellow K^+ bridge small yellow oxygens in adjacent mica sheets. The surface of freshly cleaved mica is hydrophilic; but the surface gradually becomes hydrophobic upon exposure to air, as organic contaminants bind to it.

Muscovite mica has an illite clay mineral structure and does not shrink or swell with drying and wetting. Although mica has the same crystal structure as illite clay minerals, mica differs from clay minerals in having many fewer random cation substitutions in its crystals, with the result that mica's crystalline sheets are far larger than the sheets of clay crystals (Sposito et al., 1999). These large mica sheets can have surface areas of many square centimeters, and there are large and stable spaces between them. This provides a larger and more stable environment than clays for life's emergence. The large mica sheets are also needed for transducing forces large enough to make covalent bonds.

Mica is old. Some micas are estimated to be over 4 billion years old (Hazen et al., 2008). Micas such as biotite have been found in regions containing the earliest evidence for the origins of life, ca. 3.8 billion years ago.

2.1. Ions and ion exchange on mica

Muscovite is the hypothetical mica of choice for the mica hypothesis, because it is bridged by K^+. Lepidolite is a pink mica
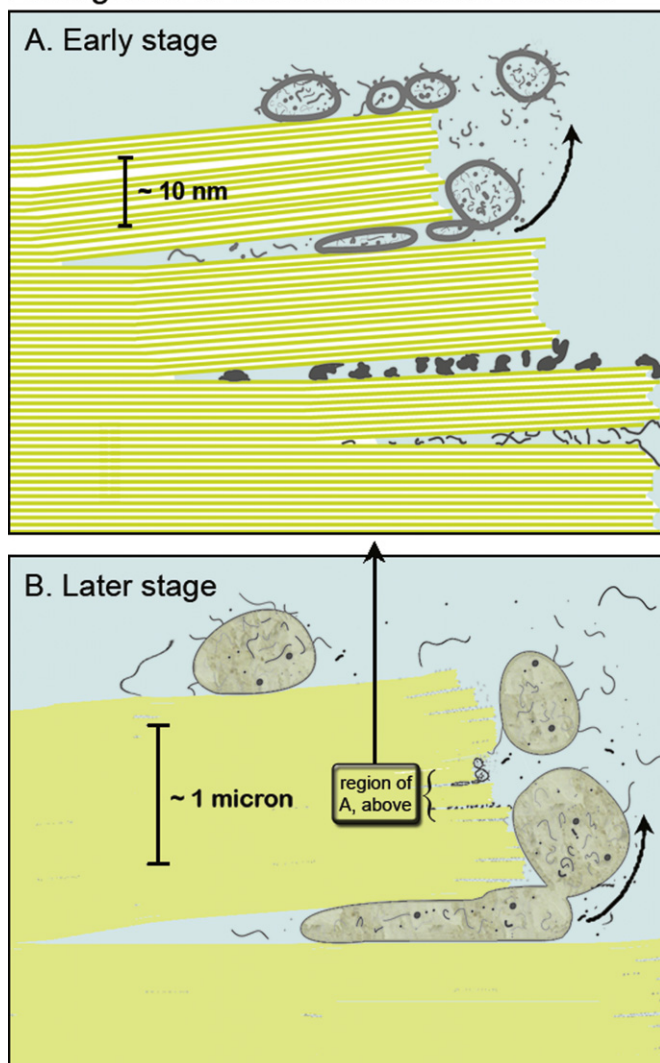
Emergence of life between mica sheets?

Fig. 2. Diagrams of the mica hypothesis for life's origins. **A.** At an early stage in the 'Mica World,' various molecules and lipid vesicles are seen. **B.** At a later stage, protocells are the largest structures. Curly bracket ({) indicates the small region of **B** that is shown in **A**. As compared with the vesicles in **A**, the protocells in **B** are much larger and are filled with a proto-cytoplasm that is more concentrated than the aqueous fluids inside the vesicles in **A**. Green lines are individual mica sheets; white spaces between the green lines contain potassium ions that bridge adjacent mica sheets. A stack of 10 mica sheets is $\sim$10 nm thick. Gray curving linear structures represent linear organic polymers; gray blobs represent larger aggregates of organic material; blue represents aqueous fluid surrounding mica, between separated mica sheets, and inside vesicles in **A**. Up-and-down movements of mica sheets are hypothesized to bleb off vesicles and protocells. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

bridged by Li$^+$, and biotite is a black mica bridged primarily by ions of Mg and Fe. Muscovite mica sheets are transparent to visible light but opaque to UV light. 'Books' of muscovite sheets have different hues of red, brown or green that are due to slight substitutions of elements such as Mg, Ca and Fe.

Ion-exchange occurs on the surfaces of mica sheets but not in the anhydrous spaces between mica sheets (Gaines, 1957). Ammonium ions (NH_4^+) exchange readily with K$^+$ on mica sheets. This is useful for the origins of life because of the role of ammonia in the synthesis of nitrogen-containing organic molecules. In some non-K micas, NH_4^+ is the main cation between the mica sheets (Tischendorf et al., 2007).

There is an extensive literature on the interactions of inorganic cations with the mica surface (Ducker and Pashley, 1989;
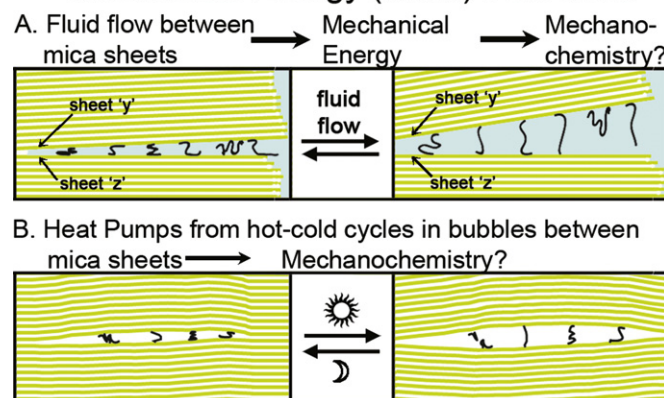
Mechanical Energy (Work) from Mica

Fig. 3. Work done by moving mica sheets is potentially capable of mechanochemistry. **A.** Mica sheets move up and down in response to water movements. **B.** Bubble between mica sheets functions as a heat pump in which hot and cold cycles cause bubble to expand and contract, exerting forces within the bubble. **A** and **B** show work-induced changes in polymer extension, but work from moving mica could also operate over shorter or longer distances to form covalent bonds (Fig. 4) or bleb off vesicles (Fig. 2A) and protocells (Fig. 2B). Mechanochemistry from moving mica basically mashes and stretches whatever is between the mica sheets.

Polyalanine Polymerization by Mechanochemistry
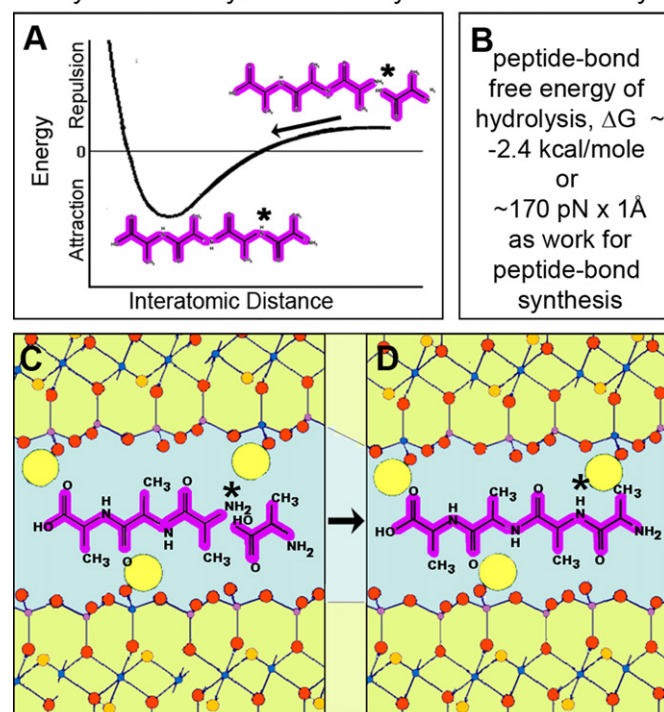

Fig. 4. Diagram of the hypothesis that mica's up-and-down movements could power mechanochemistry, such as the polymerization of alanine, by mashing molecules together: **A**, by pushing molecules into the attractive regime of the energy profile, in this schematic free energy diagram. **B**, work per molecule to make or break a peptide bond in water, estimated from the data in (Martin, 1998) **C**, Tri-alanine and alanine, before mechanochemical polymerization and, **D**, tetra-alanine, after mica sheets have mashed molecules together in a mechanochemical reaction. Asterisks ($\ast$) indicate site of bond formation. Atoms in mica are identified in Fig. 1A. Mica structure adapted from (http://www.britannica.com/EBchecked/topic/379747/mica, 2009).

McGuiggan and Pashley, 1988; Pashley, 1981, 1984; Pashley and Israelachvili, 1984; Xu and Salmeron, 1998b). Ions differ in their affinity for the mica surface. K$^+$ on the mica surface can be exchanged for Ca^{++}, Mg^{++}, or H$^+$, but not Na$^+$ (Xu and Salmeron,

Mica provides huge entropy reduction
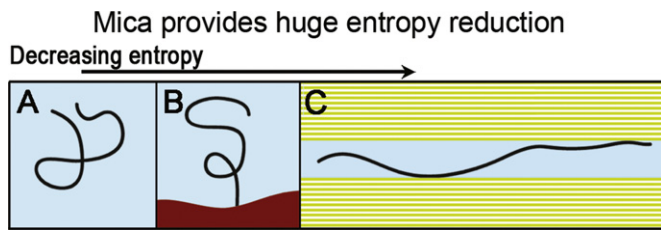
Decreasing entropy

Fig. 5. Entropy is much higher for polymers in solution (**A**), or attached to a surface (**B**), as compared with polymers confined between sheets (**C**). Both the internal motions and the translational motions of the polymer are constrained by the increasing confinement from **A** to **C**.

1998a). Ion-exchanged mica was prepared by soaking the mica in solutions of Ca, Mg or Na salts or in water (for H^+-mica). After rinsing with water, Ca^{++} and Mg^{++} remained on the mica, but Na^+ washed off, leaving H^+-mica. These same cations – K^+, Mg^{++}, and Ca^{++} – bridge biopolymers in living cells. Like mica sheets, the biopolymers in cells are primarily anionic.

Also relevant to the mica hypothesis is the extensive body of research on forces between mica sheets in liquids, using the Surface Forces Apparatus (Israelachvili and McGuiggan, 1988). In 1 mM K^+ solutions, forces between mica sheets oscillate as the sheets approach each other, over the last ~2 nm before contact. The periodicity of these oscillations, ~2.5 Å, is approximately the

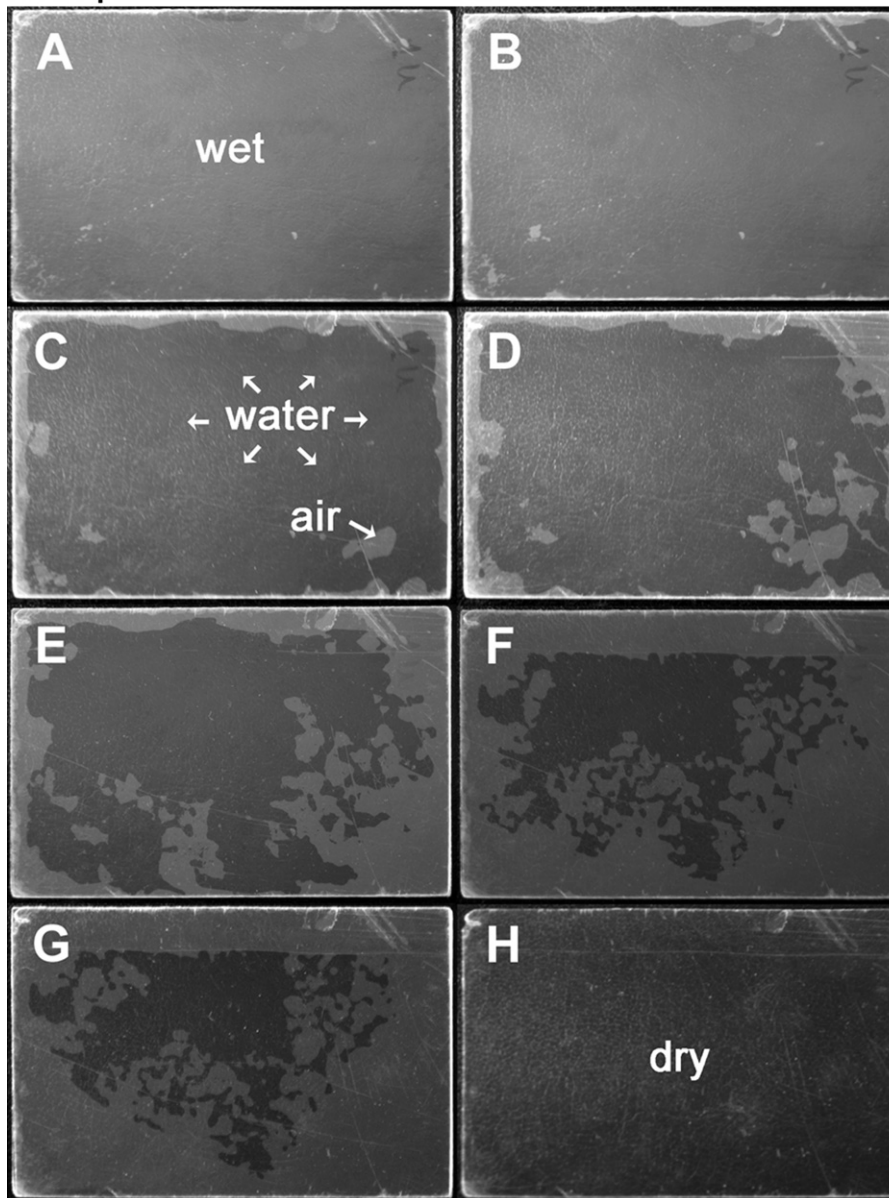## Evaporation of water between mica sheets

Fig. 6. Mica provides a good environment for cycles of wetting and drying, as shown in this time series of water drying between pieces of mica. Two dry mica pieces, 50 mm × 75 mm (Ted Pella, Inc.), were wetted with tap water and pressed together to form a mica–water–mica sandwich with the water reaching to the edges of the mica sheets (**A**). Photographs were taken (Canon SD1100 in ambient light) at intervals after adding water: **B**, 15 min; **C**, 30 min; **D**, 1 h; **E**, 3 h; **F**, 6.5 h; **G**,7.5 h; **H**, 21 h. By 21 h, water had evaporated completely from the space between the mica sheets. Mica-water sandwiches in air, like the one in A, have water layers that are ~0.2 ± 0.1 μm thick (mean ± std. dev.; n=7; H. Hansma, unpublished).

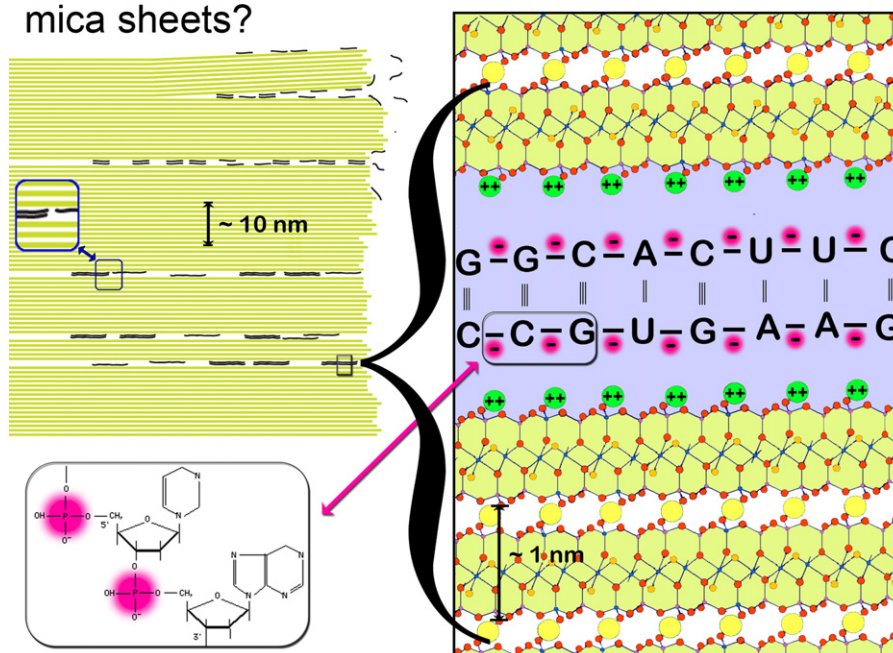## Polymerization and replication between mica sheets?

Fig. 7. The environment between mica sheets might be favorable for RNA polymerization and replication between mica sheets. Diagram shows pairs of mica sheets providing an environment for RNA polymerization, base-pairing, and replication. See Fig. 1 caption for identifying atomic composition of mica in the right panel. The sheets represented in this figure as mica might also be micron-sized illite clay sheets. The periodicity of mica and the spacing of nucleotides in single-stranded RNA and DNA are all 0.5 nm.

diameter of a water molecule and is hypothesized to be due to the layering of water near the mica surface.

2.2. Mica chemistry

The surfaces of mica sheets are generally regarded as being chemically inert, due to a lack of surface hydroxyls (Kessel and Granick, 1991; Xiao et al., 1995). Silane reagents do not appear to form covalent bonds with mica; in contrast, silane reagents do form covalent bonds with silicon oxide and glass surfaces, via their surface hydroxyls. Covalently modifying mica with silane reagents requires that the mica be pretreated by plasma discharge (Parker et al., 1990). Plasma discharge produces unstable mica surfaces and, at high power in an oxygen plasma, surfaces with greatly increased roughness (Liu et al., 1997). Silylation of untreated mica appears to create a self-assembled surface layer of silanes with intermolecular crosslinks between silane reagent molecules but not with the mica surface. There is some question about these results, because spectroscopy cannot easily distinguish between intermolecular silane cross-linking and silane-mica bonds (Xiao et al., 1995).

Mica's interactions with water have also been studied extensively, especially at coverages of a sub-monolayer to a few layers of water (Ewing, 2006; Feibelman, 2010; Verdaguer et al., 2006; Xu et al., 1998). Water grows epitaxially on the mica crystal lattice in patches one or 2 water layers thick, at relative humidities below 90%. The first water layer differs from the second and successive water layers. The first water layer is fully hydrogen-bonded, either to mica or to itself, while the second and successive water layers have hydroxyl groups pointing out. The first water layer is thus hydrophobic relative to the second water layer and successive water layers.

2.3. Mica's affinity for biomolecules

The mica surface is a good environment for living cells and for all the major classes of biological macromolecules – proteins, nucleic acids, carbohydrates, and lipids (Jena and Horber, 2002). In fact, two major fields of research build heavily on mica's affinity for biomolecules. These are the research areas that use the atomic force microscope (AFM) and the surface forces apparatus (SFA) (Binnig et al., 1986; Israelachvili and McGuiggan, 1988; Rugar and Hansma, 1990). The SFA moves mica surfaces toward and away from each other and measures properties of the material between the mica sheets. The AFM feels sample surfaces and records topographic profiles of the surface at a resolution that readily resolves DNA and RNA molecules on a mica surface. The AFM can even image biomolecules 'swimming' or waving their 'arms' on the mica surface under aqueous solution, as in Fig. 9 (Argaman et al., 1997; Bezanilla et al., 1994; Chen et al., 1998; Kasas et al., 1997).

To image DNA 'swimming' on the mica surface under aqueous solution, we first rinsed the mica with 1 mM $NiCl_2$. This exchanged the surface K^+ for surface Ni^{+}. A lot of DNA action can be observed in Fig. 9. One DNA molecule in Fig. 9C is tethered, and about half the molecule flips to a different position on the mica in the second image of Fig. 9C. There are 3 or 4 DNA molecules moving in Fig. 9B and in the mica area of Fig. 9A that is shown in Fig. 9B. One molecule, immediately below the white blobs of *Escherichia coli* RNA polymerase, is seen only in Fig. 9A and not in Fig. 9B. Another molecule enters at the right in the first panel of Fig. 9B and moves in stages across the mica; this molecule's motion is hindered by the other molecule on the right side of Fig. 9B, in the second and third frame, and then it moves towards the left side of the mica in the last 3 frames. The molecule on the left side of Fig. 9B has a more elongated conformation in
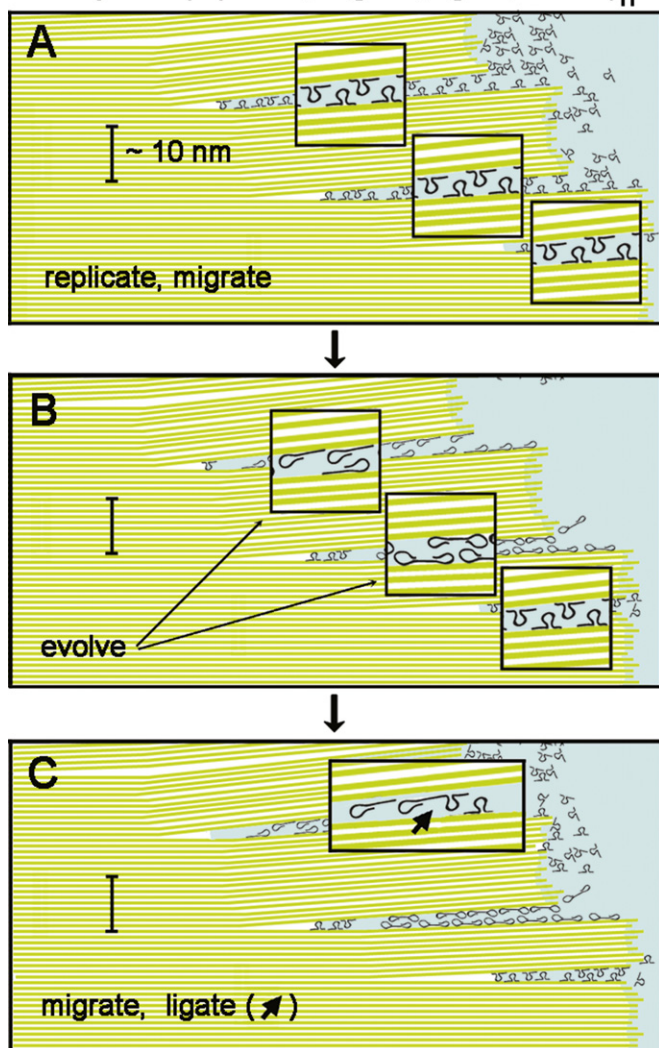
Ribozymes: (replicate, migrate, ligate, evolve)$_n$

Fig. 8. The environment between mica sheets might be good for ribozyme replication, migration, ligation and evolution in an RNA World. Diagram shows mica sheets providing an environment for: **A**, ribozyme replication and migration to other niches between mica sheets; **B**, ribozymes evolving in two of the niches into different ribozymes and replicating within these niches; **C**, ribozymes migrating to another occupied niche and ligating with a ribozyme in this niche to form a new longer ribozyme. Mica provides massive redundancy in the many spaces between its sheets.

Fig. 9. DNA swimming on mica. Time-lapse movie of double-stranded DNA molecules moving on mica. White blobs are molecules of *E. coli* RNA Polymerase. Images were taken at successive intervals of ∼1 min. **A.** This image shows the DNA molecules at the beginning of the movie. **B** and **C** show selected successive frames from the movie. Blue arrows indicate which regions of the field in A are seen in B and C. Experimental details are in Bezanilla et al. (1994). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 9A and then remains in a somewhat compact conformation for the last 4 frames of Fig. 9B.

DNA binds weakly to mica in Mg^{++} or Ca^{++} and strongly in Ni^{++}, Co^{++} or Zn^{++} (Hansma and Laney, 1996). Cation-assisted binding of DNA to mica correlates with the enthalpy of hydration and the radius of the cation (Fig. 10). Ni^{++} also affects the sequence-dependent conformations of DNA on mica (Sitko et al., 2003). Oligomeric nucleic acids as small as 25 mers bind readily to mica (Hansma et al., 1996). RNA is likely to have similar interactions with divalent cations on mica; but this old work was done with DNA, which is not cleaved by the RNases that are ubiquitous in today's world. Research with RNA on mica includes tecto-RNA structures and tobacco mosaic virus RNA (Chworos et al., 2004; Drygin et al., 1998).

Mica also binds other biomolecules, including proteins and carbohydrates (Chen and Hansma, 2000; Drake et al., 1989; Kirby et al., 1995; McIntire et al., 1995). Laminin is a cross-shaped
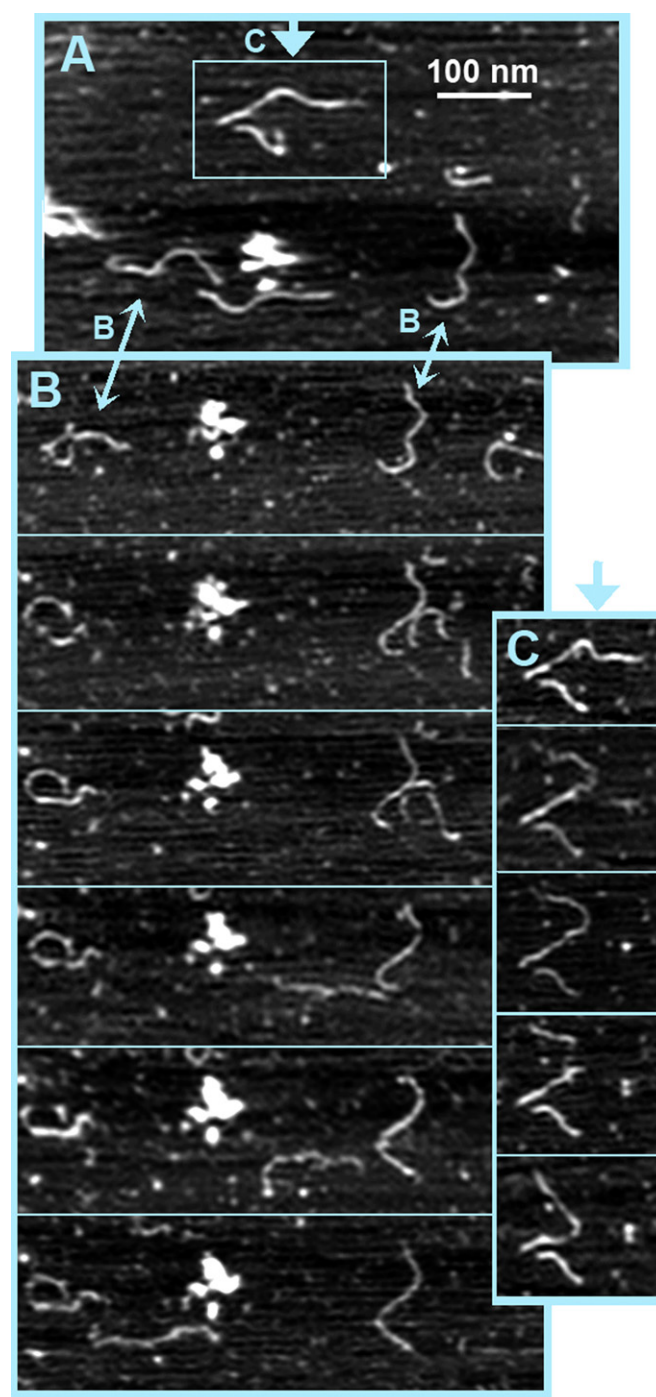
protein from the extracellular matrix that waves its arms under aqueous solution in the AFM (Chen et al., 1998).

Mica is a preferred substrate for depositing lipid layers with the Langmuir–Blodgett technique, including monolayers, vesicles, bilayers, and multilayers (Hansma et al., 1991; Richter and Brisson, 2005; Zasadzinski et al., 1994). Surfactant micelles above the critical micellar concentration assemble on mica as
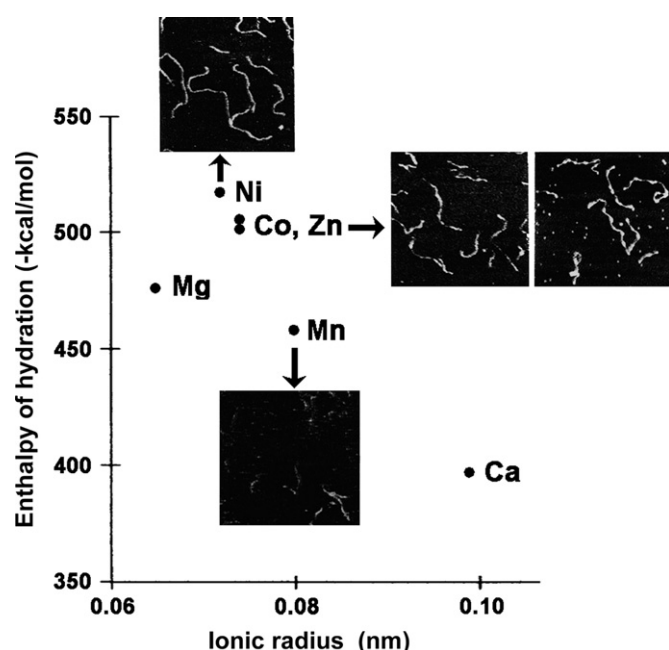
Fig. 10. Inorganic cations affect the binding of DNA to mica under aqueous solutions. AFM images show that DNA binds well to mica in solutions containing Ni^{*+}, Co^{*+}, or Zn^{*+}, which have high enthalpies of hydration relative to other inorganic cations of similar radii. In Mn^{*+}, DNA binds poorly to mica; Mg^{*+} and Ca^{*+} results are similar to Mn^{*+} (data not shown). DNA is a digest of ϕX-174 RF DNA. Images are 500 × 500 nm. Adapted from (Hansma and Laney, 1996).

meandering stripes, with a spacing indicating tubular micelles (Manne and Gaub, 1995). Lipids assemble on mica as two-dimensional crystals with molecular periodicities expected from the sizes of their head-groups (Weisenhorn et al., 1991).

3. Mica hypothesis and mineral origins-of-life research

Origin of life on mineral surfaces, or 'life on the rocks,' has been a popular hypothesis for over half a century (Bernal, 1951; Orgel, 1998). The minerals that have been considered include silicate minerals, such as clays and feldspars, and layered double hydroxides (LDHs) such as brucite.

3.1. Silicate minerals

3.1.1. Clays
Clays are the main minerals that have been used in research on the origins of life. Clays are layered alumino-silicate minerals. Clays served as a solid support and sometimes also as a catalyst for the polymerization of activated nucleotides and amino acids (Ferris et al., 1996). In these clay experiments, peptides and RNA were synthesized on clays to sizes of ~40–50 mers through daily 'feeding' with activated monomer and washing away of by-products. In contrast, reactions in solution produced only ~5–10 mers or less. Montmorillonite clay catalyzed the polymerization of activated nucleotides (Ferris and Ertem, 1993). Amino acid polymerization was done on an illite clay (mica has an illite mineral structure). The illite clay supported but did not appear to catalyze the reaction. Catalysis is not essential for the origins of life, because catalysts only speed up reactions, as opposed to facilitating reactions that do not occur otherwise. Time was readily available on the prebiotic earth.

The mean polymer length depends on the half times for polymerization and hydrolysis. The mean polymer length is longer if the rate of hydrolysis is slower, as proposed for the spaces between mica sheets where little water is present.

Lipids have been synthesized on clays from dilute methanol at high temperatures and pressures simulating possible environments where life originated (Williams et al., 2005). The swelling clays montmorillonite and saponite produced much larger quantities of aromatic hydrocarbons, as compared with illite clay. Vesicles have also been extruded from montmorillonite clay in research simulating the origins of life (Hanczyc et al., 2003).

Experiments with chemical blocking agents show that the surfaces of clay sheets are the reactive sites for nucleotide polymerization. The edges of the sheets are not needed for nucleotide polymerization (Ertem, 2004). Mica sheets have a ~10^6 larger ratio of surface area to edge area, compared with clay sheets, assuming a mica surface area of 1 cm^2 and a clay surface area of 1 μm^2.

Clays can function as redox reagents, in addition to being solid supports and sometimes catalysts. Clay redox and catalysis occur at substitution sites such as Fe (Coyne et al., 1991; Coyne and Summers, 1991). Fe substitutions are found both in the tetrahedral (T) surface layers of clay sheets and the octahedral (O) sandwich layer. Like clay, mica's Fe substitutions may be capable of redox and catalysis under some conditions.

Swelling smectite clays such as montmorillonite have generally been more useful for origins research, and for catalysis in general, than non-swelling illite clays such as mica (Pinnavaia, 1983). The crystalline structures and compositions of illites and smectites are quite similar; the main difference is in the cations that bridge the sheets. This difference in bridging cations causes the different behaviors in water, of swelling vs. not swelling. When the cations bridging the clay sheets are monovalent, such as Na^+ and K^+, smectite clay sheets are typically bridged by Na^+, and illite clay sheets are typically bridged by K^+. K^+ has a larger ionic radius than Na^+. The smaller Na^+ between smectite clay sheets is hydrated, while the larger K^+ between illite clay sheets is anhydrous. Hydrated Na^+ and anhydrous K^+ have similar radii, thus similarly filling the spaces between the sheets. Smectite clays are swelling clays, because they can take up additional water into the Na^+-bridging layer, which already has water. Smectite clay sheets can separate to distances of 10 nm or more when water enters the interlayer space (Williams et al., 2005). Illite clays and mica do not swell in water. Mica's resistance to swelling is an advantage for the origins of life. Mica provides a relatively stable environment through the cycles of wetting and drying that might have occurred where life evolved. The environment between mica sheets is similar in this respect to the environment in living cells, which also resist swelling.

A novel hypothesis proposes that minerals such as clays or mica formed the earliest version of the genetic code (Cairns-Smith, 2008). The mica hypothesis does not postulate that any genetic code is present in the mineral structure of mica.

3.1.2. Feldspar
Life in open micron-sized compartments in weathered feldspar is another version of 'life on the rocks'. These weathered feldspar compartments provide much less confinement than the nanoscale spaces between mica sheets. The weathered feldspar compartments are zeolite-like alumino-silicates that have lost some of their aluminum during the weathering of the feldspar (Parsons et al., 1998; Smith, 1998).

3.2. Layered double hydroxides (LDHs)

In another hypothesis for life emerging between mineral sheets, LDHs are the mineral of choice (Arrhenius et al., 1997).

LDHs, like clays, are layered minerals. LDHs include brucite, which is the solid mineral form of Mg(OH)$_2$, and other minerals such as magnesium aluminum oxides (Arrhenius et al., 1997; Sideris et al., 2008) Unlike clays, the sheets of LDHs have a positive surface charge, neutralized by anions between the mineral layers. They have been proposed as sites for the origins of life because of the affinity of cationic surfaces for anionic polymers. Many biological lipids and other biopolymers such as DNA and RNA are anionic. This is the opposite of the mica hypothesis, because the mica hypothesis says that anionic polymers evolved on an anionic mineral surface with mobile cationic bridges.

3.3. Life's origins on positively vs. negatively charged mineral surfaces

In life today, when biopolymers of one charge interact with minerals of the opposite charge, stable biomineralized structures form. For example, bones and teeth have anionic aspartate-rich protein polymers that provide the matrix for the Ca^{++}-rich cationic mineral phase (Weiner, 2008). Similarly, there are stable Si-rich structures in organisms such as diatoms and sponges. The predominant proteins in biomineralized Si are silicateins, which have a positive charge at neutral pH (Shimizu et al., 1998). Negatively charged silicates in vitro also form composites with positively charged proteins (Bassindale et al., 2009).

In contrast, in the mica hypothesis, mobile inorganic cations bridge the anionic prebiotic polymers to the life-supporting anionic mineral surface. Instead of a stable electrostatic attraction, mica provides transient interactions with anionic polymers via a variety of mobile inorganic cations and a gentle entrapment of the anionic polymers within the physical space between the mica sheets. Transient interactions can have sensitive responses to the environment and are seen today in the many transient interactions that regulate life, such as the DNA–ligand interactions that regulate gene expression. Therefore, according to the mica hypothesis, the anionic polymers of life originated on an anionic mineral surface.

4. Mica world

The following sections describe the hypothetical Mica World and the relationship of prior research to the origin of life in a mica world. In the mica world, life evolved in large part from the organic molecules that were synthesized in 'molecule mashers.' These molecule mashers are pairs of mica sheets that move up and down, towards each other and away from each other, mashing together and pulling apart whatever molecules are in the way. The energy input to the molecules is mechanical, in the form of work.

4.1. Energy, work, and mechanochemistry

Energy input is usually necessary for chemical reactions to occur and for physical processes to occur that increase the order of a system. In the mica world, a major energy input is mechanical energy from moving mica sheets. In thermodynamics, Free energy (ΔG) is a measure of the ease with which a chemical reaction will occur. Thermodynamic free energy is the sum of enthalpy and entropy terms ($\Delta G = \Delta H - T\Delta S$, where ΔH is the enthalpy change and $T\Delta S$ is the product of entropy change and temperature). Energy is used, in this paper, to refer to the free energy of a reaction or process, as in the schematic free energy diagram of (Schnitzer et al., 2000) and in Fig. 4A. In at least one system (Best and Clarke, 2002), free energy includes solvation, electrostatic

effects, disperson forces, and entropy of the polymer chain. The relationship between free energy and mechanochemistry is analyzed in (Keller and Bustamante, 2000), which points out that mechanochemistry is involved in any enzymatic reaction that involves a conformation change of the enzyme.

The following discussion of work from mica sheets is quite lacking in quantitative analysis, partly due to the properties of mica. Work is the product of a force and a distance. The force, F, exerted by the mica depends on the spring constant, k, of the mica: $F = kx$, where x is the distance of the displacement of the mica. The spring constant depends on the thickness and length of the mica lever. Thickness will vary over a wide range, depending on the number of nm-thick mica sheets in each mica lever, and length will vary among mica levers and even within a single mica lever, depending on the energy of the water or heat that pushes the mica sheets apart and the strength of adhesion between the mica sheets that are being pushed apart. These sheets, in Fig. 3A, are labeled 'y' and 'z' in the regions of the sheets that affect the length of the mica lever. The mica sheets at y and z will interact more or less strongly as K$^+$ exchanges with other ions and as organic and inorganic matter adsorb to or desorb from the mica sheets. The length of the mica lever will be shorter if the mica sheets interact strongly at y and z (i.e., are 'stuck' together), and the length of lever will be longer if the mica sheets can separate at positions y and z and at positions on the sheets that are farther into the mica 'book.'

Simple up-and-down movements of mica sheets may have been a major form of energy for the origins of life (Fig. 3). This energy is endlessly renewable and potentially capable of carrying out many types of chemistry. Work from moving mica sheets might have formed many of the prebiotic covalent bonds. These covalent bonds would have formed at stages in the synthesis of monomers and/or in the joining of monomers to form oligomers and larger polymers. Any small or large molecules with sufficient affinity to mica should be able to form covalent bonds with each other through mica's movements if sufficient energy from mica's movements pushed the molecules into each other as opposed to dislodging the molecules from the mica sheets.

The up-and-down movements of mica sheets would have been powered by water motion and cyclic solar energy (Hansma, 2009). As described above, some of the resulting mechanical energy might be used to form bonds between molecules. This is mechanochemistry (Figs. 3 and 4). Mechanochemistry is a growing field, in which mechanical energy is being used to make or break bonds (Hickenboth et al., 2007; Rosen and Percec, 2007), sometimes using single-molecule techniques (Grandbois et al., 1999; Lenhardt and Craig, 2009; Yang et al., 2009). Angstrom-sized motions can form covalent bonds, as envisioned in Fig. 4. Nanometer-sized motions alter the conformations of polymers (Fig. 3). Micron-sized motions can bleb off vesicles (Fig. 2A) or protocells (Fig. 2B) between mica sheets and would also manipulate the macromolecular aggregates or 'blobs of goo' that often result from prebiotic chemistry experiments in the lab (Hazen, 2005). Mechanical manipulation might rearrange the goo into primitive structures.

Two sources of energy for mechanochemistry are shown in Fig. 3. Water movements in and out at the edges of mica sheets push the mica sheets apart and pull them together (Fig. 3A). Bubbles in mica sheets expand and contract in response to the heating and cooling of day and night (Fig. 3B). These cycling bubbles are heat pumps, which have been proposed as an energy source for life's origins (Schulze-Makuch and Irwin, 2006). Another source of energy between mica sheets comes from meniscus forces, caused by surface tension at air-water interfaces, such as those indicated by the arrows in Fig. 6C. Air–water interfaces appear when water recedes or advances at the edges or in the interstitial spaces of mica sheets. Meniscus forces were attractive

forces of 10–100 nN when measured between mica and the probe tip of an atomic force microscope (Weisenhorn et al., 1989). Therefore meniscus forces are strong enough to make and break covalent bonds, which rupture at ~4 nN, according to theoretical modeling for C–C and C–N bonds (Grandbois et al., 1999). Meniscus forces or convection forces in an evaporating droplet have been used to stretch DNA molecules (Wang et al., 1998).

4.1.1. Mechanochemistry research

In the lab, mechanochemical research usually results in bonds being broken, due to technical limitations; but bond formation is also possible (Beyer and Clausen-Schaumann, 2005). When the energy source is mechanical as opposed to thermal, the relative bond strengths are sometimes significantly different. This is demonstrated by Molecular Dynamics simulations of thiols on copper, in which carbon–sulfur bonds cleave when heat is the energy source, while copper–copper bonds cleave when the energy source is an upward-moving mechanical force (Konopka et al., 2008). In another example of mechanochemistry, ultrasonic forces are better than heat at activating a catalyst by dissociating its ligand (Piermattei et al., 2009). Disadvantages of ultrasonic mechanochemistry are its irreversibility and lack of control over the mechanochemical forces. Simple up-and-down movements of mica sheets would provide more reproducible mechanical forces, distances, and directions, as compared with ultrasonic mechanochemistry.

4.1.2. Entropy

Entropy is discussed separately from energy, even though entropy is a term in the thermodynamic equation for free energy. Thermodynamics describes systems at equilibrium, and life is not at equilibrium. One example where entropy is not described well by the equation for free energy ($\Delta G = \Delta H - T\Delta S$) is in single-molecule pulling experiments: The energy to stretch a polymer is typically an order of magnitude larger than the typical free energy of unfolding, as measured from the area under the force-vs.-distance pulling curve (Thompson et al., 2002). The experiment was done by pulling a protein molecule with the probe tip of an AFM, stretching the molecule between the surface of the substrate and the tip of the AFM probe. The best explanation for the peculiarly high energy is that there is a huge reduction in entropy as the protein molecule is extended nearly completely. The protein molecule experiences changes in entropy rather like the hypothetical polymer molecule in Fig. 5, as it goes from being free in solution to extended between mica sheets.

One challenge with origins-of-life hypotheses is that entropy is low in living systems (Arrhenius et al., 1997; Root-Bernstein and Dillon, 1997). As the diagram in Fig. 5 shows, the entropy of polymers decreases progressively as they move from being free in solution, to tethered on a surface, to confined between sheets, such as mica. Entropy measures molecules' freedom of motion, both internal and translational, and this freedom of motion is much lower in the crowded intracellular environment of a unicellular organism than in its aqueous extracellular environment. Protein molecules in a cell are typically so close that there is only a space equal to the size of one protein molecule between them (Phillips et al., 2008). Entropy reduction in today's living cells requires enzymatic systems with complex coupling to chemical energy. Confinement between mica sheets may have been a mechanism for reducing entropy before complex enzymatic systems existed.

Entropy also may have driven some molecular ordering during prebiotic molecular evolution between mica sheets. Overcrowding can produce entropy-driven molecular order, as in the nematic-to-smectic phase transitions of liquid crystals and the alignment of elongated molecules on a membrane (Almeida and Wiegel, 2006).

4.1.3. Confinement effects

Entropy reduction between mica sheets also produces chemical confinement effects. Chemistry in confined spaces is more selective than chemistry in solution. Zeolites, for example, are porous crystalline alumino-silicates that produce fewer products from reactants, as compared with the reaction in solution (Turro, 2000). Proteins in confined spaces are stabilized against denaturation (Thirumalai et al., 2003; Zhou and Dill, 2001). Pressure is a form of confinement that induces actin polymerization and glycine polymerization (Cipolla et al., 2002; Ohara et al., 2007).

Confinement between mineral sheets may also favor some chemical reactions over others, thus reducing the number of reaction products, as compared with the same reaction in a fluid or on a surface. One problem with the origins of life is the large number of possible organic molecules as opposed to the smaller number of molecules actually found in living systems. Amino acids produced in the Urey–Miller electric discharge experiments, for example, are primarily amino acids not found in proteins, such as β-alanine and norvaline (Johnson et al., 2008). Somewhere in the origins of life, the possible diversity of amino acids in proteins was reduced to a subset of ~20 different L-α-amino acids.

4.1.4. Chirality

Confinement between mica sheets might be the way in which life evolved with chiral polymers. Adjacent mica sheets impose steric and chemical constraints on the molecules between them. These constraints might bias monomer packing such that monomers of the same handedness would pack closer on mica sheets than their mirror–image enantiomers, thus facilitating bond formation between same-handed monomers. This argument, that chirality can come from confinement, is an alternative to other work on the origins of chirality. One proposal for the origins of chirality involves chiral mineral surfaces. Chiral surfaces of calcite give a few percent enrichment of the D- or the L-isomer of aspartic acid (Hazen, 2005; Hazen and Sholl, 2003). Another proposal for the origins of chirality involves the differential solubility of chiral and racemic organic crystals (Breslow and Cheng, 2009).

4.1.5. Solid phase synthesis

Mica is a highly structured solid support that provides an amazing environment for solid phase synthesis. Solid phase synthesis is used experimentally and industrially to avoid problems with synthesis in solution (Bergbreiter and Kobayashi, 2009). The mica environment contains a mechanical energy source applied to spaces consisting of parallel structured mineral surfaces. The ceilings and floors of the spaces alternately squeeze and stretch whatever is within the spaces.

The ceilings and floors of the spaces between mica sheets are also anionic grids with periodicities of 0.5 nm, similar to the periodicities of monomers in biopolymers such as nucleic acids and peptides. Therefore the biopolymers being synthesized would be able to interact directly with the mica ceiling and the mica floor of the molecule masher via specific chemical interactions.

4.1.6. Molecular complementarity

Molecular complementarity fits nicely with the mica world. Molecular complementarity is a way of unifying many aspects of life's origins and dealing with their problems by treating molecules not as individuals but as complements to other molecules (Dillon and Root-Bernstein, 1997; Hunding et al., 2006; Root-Bernstein and Dillon, 1997). Molecular complementarity is a ubiquitous phenomenon that stabilizes molecules against degradation, because the molecules are part of larger complexes of molecules. Molecular complementarity results in

Darwinian natural selection in much the same way as the mica world: molecules that associate with other molecules – or with mica sheets – are selected for, and molecules that do not associate with other molecules or with mica sheets are lost. Mica world is an analog system, like living systems, with continuous variations in quantities, rates, lengths and other variables.

4.2. Wet–dry cycles in the origin of life

Cycles of wetting and drying are ubiquitous on earth today and were likely to be present on the primordial earth as well. The absence of water favors polymerization, and the presence of water favors hydrolysis of polymers. For example, drying of the amino acid alanine produces alanine di-peptides (Napier and Yin, 2006). Origins research has used wet–dry cycles to encapsulate an RNA polymerase in lipid vesicles and to synthesize an RNA-like polymer non-enzymatically from a nucleotide monophosphate, when stabilized in layers with lipids (Chakrabarti et al., 1994; Rajamani et al., 2008).

Although water is necessary to life, cells regulate their water content to prevent the accumulation of water to toxic levels. High concentrations of solute and careful regulation of water content in the cytoplasms of living cells are consistent with life's origins in limited water, not exposed to prolonged wet and dry periods.

4.2.1. Fluid flow
Besides applying mechanical energy to the material between the mica sheets, the squeezing and stretching of material between mica sheets involves fluid flow between the sheets (Fig. 3). Fluid flow would exchange unbound or soluble material between the sheets with material in the fluid outside the mica. Such fluid flow would remove molecules not bound to mica and might bring in new monomers or other molecules for reaction. Such 'feeding' and washing has been used to synthesize biopolymers on clays to lengths greater than the lengths synthesized in solution (Ferris et al., 1996).

Water does not enter the spaces between mica sheets at a visible level even after long incubation times (Gaines, 1957). There are exceptions, however. Water rapidly entered between the sheets of the low-grade mica in Fig. 1D, which was collected from an abandoned mica mine. Water slowly entered between the sheets of high-grade mica when the mica was submerged in water or aqueous salt solutions and subjected to daily temperature changes (data not shown). After two weeks of daily cycling between room temperature and ~4 °C, water or salt solution entered a few millimeters into the spaces between the sheets. Water entered farther into the mica sheets when the submerged sheets were cycled daily between freezing and boiling water temperatures. With these temperature extremes, water entered a centimeter or more into the spaces between the sheets. The mica used in these experiments was like the mica pieces in Fig. 6.

The mica pieces in Fig. 6 were treated in the opposite way: drying was measured instead of wetting. The mica pieces were sandwiched together with water and allowed to dry in air. Water evaporated slowly from the spaces between mica sheets, often forming intricate patterns of wet and dry areas in the process (Fig. 6). The water evaporated completely within $12-24$ h. These results show how mica provides both wet environments and dry environments simultaneously in close proximity.

4.3. Internal potassium ions

Living cells have high internal K^+ concentrations. Where did this come from? Blood and extracellular fluids are high in Na^+, supposedly because life originated in the Na^+-rich ocean, but there is no corresponding theory for why the intracellular fluid is high in K^+.

Mica sheets are held together by K^+, which provides a good environment for molecules destined to exist in a K^+-rich cytoplasm. Muscovite mica has a K^+ concentration of ~100 mM between adjacent sheets that are separated to a distance of ~0.7 nm. This is calculated from the 0.5-nm hexagonal spacing of K^+ between mica sheets. Intracellular K^+ concentrations in living cells are ~100 mM. Although some clay minerals are also rich in K^+, these clays only have small micron-sized sheets.

4.4. Possible synthesis of biomolecules between mica sheets

The spaces between mica sheets, their 0.5-nm anionic grid, and their chemical composition may have supported the synthesis of the earliest biomolecules and biopolymers, with energy supplied by mechanochemistry in the mica molecule mashers. In this respect, the mica hypothesis resembles the metabolic origins proposed by others, in which a useful subset of small biomolecules were synthesized from smaller organic and inorganic molecules (Smith et al., 2009; Wachtershauser, 2007). Transition metal catalysis is key to these approaches, and life is viewed as a natural outcome of the chemical and energetic processes on the early earth.

For example, Lewis acid sites provided by a divalent metal cation on a surface can be a component for non-enzymatic catalysis of thiol-mediated reduction reactions in the reductive tri-carboxylic acid (rTCA) cycle proposed for life's origins (Smith et al., 2009). Divalent metal cations stabilize negative charges, thus facilitating some substitution and elimination reactions of organic molecules.

The following sections discuss the possible synthesis of peptides, nucleotides, oligonucleotides, and ribozymes between mica sheets.

4.4.1. Peptides
Peptide synthesis, when confined between mica sheets, may favor the polymerization of only α-amino acids. This hypothesis is diagrammed in Fig. 4 for alanine and polyalanine but is applicable to other amino acids, too. If moving mica sheets can force molecules close enough to reach the attractive regime of the potential energy well, then covalent bonds may form. In Fig. 4, a covalent peptide bond forms by mechanochemistry. Whether or not this is possible, moving mica sheets would move molecules on adjacent sheets toward and away from each other.

The free energy of hydrolysis of the peptide amide bond (ΔG_m) actually favors the *synthesis* of amide bonds: $\Delta G_m = 5$–6 kcal/mol at 25 °C or 37 °C (Martin, 1998). The driving energy for hydrolysis is the ionization of the carboxyl and amino groups produced by hydrolysis of the amide bond, for which $\Delta G_i \sim -8$ kcal/mol. These results are for several hydrolysis reactions of glycine (G) dimers and glycine oligomers, such as

$$GG \rightarrow G+G$$

$$GGGG \rightarrow GG+GG$$

$$GGGG \rightarrow GGG+G$$
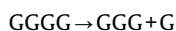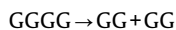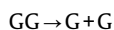
Considering the reverse reactions, as in prebiotic syntheses, the most energetically favorable reaction is the joining of 2 peptides, each of which is a dimer or larger. Forming the dimers is the most energetically difficult. Although the calculations are for glycine and its oligomers, other amino acids should behave similarly (Martin, 1998).

Good sites for amino acid polymerization on alumino-silicates consist of adjacent Lewis acid and Bronsted acid sites (Rimola et al., 2007). This was modeled *ab initio* for the formation of a simplified analog of a glycine–glycine dipeptide. The authors propose that the dipeptide chain would proceed to elongate on the silicate surface due to the dipeptide's affinity for the silicate surface.

4.4.2. Nucleotides

Nucleotides contain a purine or pyrimidine base and a phosphate attached to a sugar, which is ribose in RNA and deoxyribose in DNA. Prebiotic synthesis of these complex molecules is an unanswered question in the origins of life. Even the prebiotic syntheses of the bases and ribose are unanswered questions.

Ribose and other sugars could have been synthesized prebiotically via the formose reaction. The formose reaction is an autocatalytic reaction that produces, in solution, a large and unstable diversity of racemic branched and unbranched sugars (Joyce, 2002b; Ricardo et al., 2004). Aqueous silicate solutions have recently been shown to benefit the formose reaction in various ways related to the origins of life (Lambert et al., 2010). Silicate stabilizes some products of the formose reaction; silicate reduces the complexity of the reaction products, and silicate even selects for some stereoisomers. Five-carbon (5-C) sugars including ribose are a large part of the product when the reaction mixture starts with equimolar concentrations of 2-C and 3-C sugars.

Mica, being a highly structured aluminosilicate mineral, might confer even greater benefits on the formose reaction, with regard to producing ribose for RNA in life's origins. In another recent solution to the ribose problem, direct ribose synthesis is eliminated altogether in a reaction pathway for synthesizing entire nucleotides (Powner et al., 2009).

4.4.3. Oligonucleotides

Nucleotide polymerization produces oligonucleotides and larger molecules of RNA and DNA. Nucleotide polymerization may also benefit from the confines of mica sheets and their clay-mineral lattice structures (Fig. 7). Nucleotide polymerization in solution often produces bent oligonucleotides with unnatural linkages in addition to the 3′-5′ linkage found in nucleic acids (Joyce and Orgel, 2006). These unnatural linkages include 5′, 5′ pyrophosphate linkages and 2′-5′ linkages.

The periodicity of phosphate groups in extended oligonucleotides and single-stranded nucleic acids (0.6 nm) is similar to the 0.5-nm periodicity of anionic sites on mica sheets (Calladine and Drew, 1997). Therefore one might expect better binding to mica for linear oligonucleotides having only a single phosphate between each pair of nucleotides. Polymerization of nucleotides arrayed on the 0.5-nm mica lattices and confined between mica sheets might produce mostly linear unbent oligonucleotides. Thus, the confines of mica sheets and their clay-mineral lattice chemistry may function to reduce the number of different oligonucleotide linkages that form during nucleotide polymerization.

4.4.4. Ribozymes

Ribozymes may have evolved when life originated to do the catalytic work now done by protein enzymes as well as the information storage now done by DNA (Gesteland et al., 2006; Gilbert, 1986). Ribozymes are catalytic RNA molecules that may have catalyzed the synthesis and replication of RNA molecules in an early RNA World. RNA self-replication in solution is hindered by intramolecular base pairing of RNA oligonucleotides (Joyce, 2002b). Confinement between mica sheets could reduce the problem of intramolecular base pairing, as diagrammed in Fig. 7.

Cation-mediated interactions between oligonucleotides and mica might compete successfully with intramolecular oligonucleotide interactions.

4.4.5. RNA world: ribozyme replication and evolution

The many spaces between mica sheets provide niches for the replication and evolution of many different ribozymes at relatively high concentrations and in close proximity (Fig. 8). This might solve a problem with the origin of life: how to generate a large genome without accurate replication machinery, and how to generate accurate replication machinery without having a large genome. Modeling shows that a population of different self-replicating ribozymes in close proximity degenerate, during repeated replication, into the same ribozyme (Joyce, 2002a; Szabo et al., 2002). This happens because a small 'selfish' ribozyme excels at getting itself replicated by all the other ribozymes, which then become extinct. This has now been demonstrated experimentally with pairs of ribozymes that can catalyze each other's replication by ligation (Lincoln and Joyce, 2009). A few of these pairs eventually dominated the ribozyme population. With many adjacent pairs of mica sheets, more pairs of ribozymes would be able to continue replicating, due to their isolation from competing ribozymes. Ribozymes with enough time and space would gradually evolve large genomes with accurate replication machinery. Mica's compartments provide an environment in which multiple ribozymes could self-replicate, migrate, and ligate to evolve large genomes and accurate replication machineries, without facing extinction from competition with other ribozymes (Fig. 8).

Cycles of replication, migration, and ligation could have created great variety and massive redundancy in the ribozyme and RNA populations. Most ligations between RNAs would be expected to produce 'unsuccessful' big RNAs that became extinct, but only an occasional successful ligation would have been enough to generate large enough RNAs for growing a genetic message.

4.5. Prebiotic mica 'cells' and protocells

Before the existence of protocells enclosed by lipid membranes, mica's thin flexible mineral sheets may have provided some of the protection now provided by the lipid membranes. Life is so incredibly cellular today – multi-cellular even – that it makes sense to imagine some form of cellularity in the early stages of life's origins, even before primitive biomolecules were enclosed in lipid bilayers. Forms of multi-cellularity are seen even in bacteria and archaea today. Bacteria form biofilms. Biofilms of *Pseudomonas aeruginosa* resist antibiotic treatment that kills free-living *P. aeruginosa*. An extremophilic archaea, *Pyrodictium abyssi*, produces extracellular cannulae that hold clumps of the cells together in macroscopic cobweb-like flakes in the culture medium (Rieger et al., 1995). In the mica hypothesis, multi-cellularity existed independently of lipids, in the multiple niches between mica sheets, as in the 'RNA world' diagrammed in Fig. 8.

Eventually the hypothetical mica world became populated with lipids. These lipids formed vesicles that encapsulated material between the mica sheets (Fig. 2A). This was at an early stage in the origin of life, and the vesicles were still small. There is nothing resembling cytoplasm in the vesicles; they contain only a collection of molecules and some of the aqueous liquid between the mica sheets. Later in the origins of life, lipids encapsulated larger and more complex biomolecules and biomacromolecules, including biopolymers and complex biomolecular aggregates, a few of which carried out primitive functions. At some point (Fig. 2B), the lipid-encapsulated structures were stable enough

that they began to develop a primitive cytoplasm, quite distinct from the aqueous fluid between the mica sheets. Most of these lipid-encapsulated structures were rather useless, but occasionally some of them contained the necessary ingredients for primitive metabolism and became self-sustaining protocells. We are living proof that these ancient protocells succeeded, in some ancient environment, at replicating and transmitting what we now call genetic information.

Mica also provides a possible solution to the problem of cell division in protocells. Cells of wall-less L-form bacteria divide by blebbing off daughter cells (Leaver et al., 2009). This blebbing process is proposed to be a remnant of the earliest form of cell division; but the question is raised, where did the energy for blebbing off come from? Mica sheets' up-and-down movements could have provided mechanical energy to bleb off daughter cells (Fig. 2B).

Most of the earliest daughter cells may have remained in place, simply pushing the mica sheets farther and farther apart. Daughter cells that drifted away from the 'family' are less likely to have survived, until they had a robust biochemistry capable of self-sustained metabolism and replication, and a robust structure able to resist external environmental forces.

5. Possible mica-origins research

What experimental research is needed to provide direct support for the mica hypothesis? Mechanochemistry is one distinguishing characteristic of the mica hypothesis. Mechanochemistry from moving mica sheets could be investigated in the laboratory with approaches involving the AFM or the SFA. Prebiotic reaction mixtures between mica sheets could be subjected to cycles of pushing the mica surfaces together and pulling them apart. This process could go on for days, which is still a very short period of time, compared with the time scales over which life originated. The SFA's standard mechanism of operation is like the proposed mechanochemistry of moving mica sheets: the SFA pushes mica sheets toward and away from each other, in cycles, to produce data about the interactions of the mica sheets or the materials deposited onto them. The AFM would need a small modification: one could take a cantilever with no tip, glue onto it a piece of mica, and use this as the top sheet of mica, which would press against the mica of the sample surface. This is reminiscent of the early days of AFM, when methods had not yet been developed for integrating tips into the micro-fabricated cantilevers. Fragments of crushed diamond were glued onto the tipless cantilevers, using an eyelash hair glued to a small stick to put the glue on the cantilever (Drake et al., 1989). Nucleotide polymerization is one system worth testing with mechanochemistry and mica sheets, following the observations that nucleotides can polymerize in other confined spaces such as pockets within ice or in combination with lipids (Monnard and Szostak, 2008; Rajamani et al., 2008). The failure rate of mica mechanochemistry experiments will be high, as is typical of research into the origins of life.

Entropy reduction is another characteristic of the mica hypothesis. Entropy reduction by confinement is accompanied by chemical confinement effects, including the prediction that chiral polymer syntheses would occur within the crystalline confines of mica sheets. A prediction of entropy reduction is that the formose reaction for synthesizing sugars would produce fewer reaction products between mica sheets than in solution. The formose reaction is perhaps the easiest place to start mechanochemical research with mica, too, because reaction products are easily obtained with the formose reaction. Are there differences between the products from the formose reaction between moving mica sheets and the products of the formose reaction free in solution?

Other predictions of the mica hypothesis are that mica is the source of high intracellular K^+ levels and that the spaces between mica sheets functioned as primitive cells both before and after lipid membranes. Thus the mica hypothesis opens new research areas for origins of life research, but some of these areas will be very difficult to test.

6. Concluding remarks

Natural complexity often arises from fundamentally simple processes. Capillary action raises water to the tops of tall trees. Stochastic fluctuations in the numbers of molecules turn bacterial genes on and off (Choi et al., 2008; Beaumont et al., 2009). Charge density in the minor groove guides DNA binding proteins to their target site (Rohs et al., 2009; Tullius, 2009). An ion diffuses into an ion channel and stabilizes a conformation of the channel until it diffuses away.

Similarly, in the mica hypothesis, simple processes combine to form progressively more complex bioorganic structures. Mica micro-habitats fostered the evolution of considerable prebiotic diversity in a loose communal system for a long time. The protection afforded by mica sheets evolved into the walls and membranes of cells. This subsequent origin of cells fostered the geographic spread of life and its increasing complexity and diversity.

The mica hypothesis views life's origins as resembling an ecosystem (Hunding et al., 2006; Woese, 2002) that evolved only gradually into the autonomous cells of today's primitive life forms. The mica hypothesis also provides a large error tolerance, through molecular redundancy in the multitudes of spaces between the mica sheets, as in Fig. 8A. Error tolerance is proposed to be the primary requirement for the origins of life, because almost everything will fail (Dyson, 1999).

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