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Physical Principles and Extant Biology Reveal Roles for RNA-Containing Membraneless Compartments in Origins of Life Chemistry

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Abstract

This Perspective focuses on RNA in biological and non-biological compartments resulting from liquid-liquid phase-separation (LLPS), with an emphasis on origins of life. In extant cells, intracellular liquid condensates, many of which are rich in RNAs and intrinsically disordered proteins, provide spatial regulation of biomolecular interactions that can result in altered gene expression. Given the diversity of biogenic and abiogenic molecules that undergo LLPS, such membrane-less compartments may have also played key roles in prebiotic chemistries relevant to the origins of life. The RNA World hypothesis posits that RNA may have served as both genetic information carrier and catalyst during the origin of life. Due to its polyanionic backbone, RNA can undergo LLPS by complex coacervation in the presence of polycations. Phase separation could provide a mechanism to concentrate monomers for RNA synthesis and selectively partition longer RNAs with enzymatic functions, thus driving prebiotic evolution. We introduce several types of LLPS that could lead to compartmentalization and discuss potential roles in template-mediated non-enzymatic polymerization of RNA and other related biomolecules, functions of ribozymes and aptamers, and benefits or penalties imparted by liquid-demixing. We conclude that tiny liquid droplets may have concentrated precious biomolecules and acted as bioreactors in the RNA World.

Graphical Abstract

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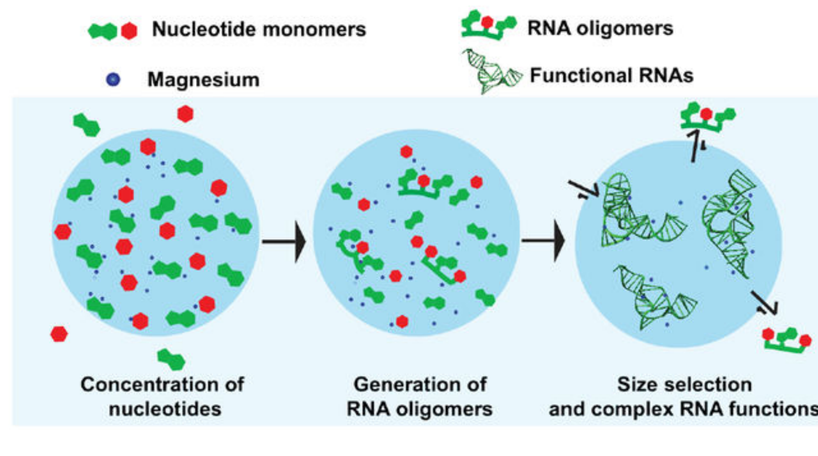

Introduction

Liquid-liquid phase coexistence in aqueous macromolecule solutions has been known for many years and its possible involvement in both living cells and prebiotic chemistry was considered early on. In 1929, Bungenberg de Jong and Kruyt coined the term "coacervate" to describe macromolecule-rich droplets that formed by associative phase separation. Commenting on the potential biological significance of these structures, they wrote, "*On closer inspection of the ground mass of the protoplasm, it strikes one that this has some properties in common with the coacervates, so that there is a possibility that this ground mass may be considered as a coacervate or as a system of coacervates.*"[1] Oparin postulated that coacervates served an important role in the origin of life, ultimately evolving to form heterotropic microbes.[2] As science progressed and roles for DNA as an informational molecule and lipid self-assembly as a means of providing compartmentalization became apparent, attention shifted away from coacervation.[3] With the recent discovery that membraneless organelles including P granules and the nucleolus are in fact liquid-like phases,[4–6] we now know that liquid-liquid phase separation (LLPS) is important in extant biology as a previously unappreciated strategy for subcellular compartmentalization. The prevalence and simplicity of phase separation in aqueous solutions of biogenic and abiogenic polymers,[7] particularly as oligomer length, complexity, and concentration increase,[8] suggests that these structures could have occurred early in prebiotic systems and cellular evolution.[9] Indeed, intrinsically disordered proteins (IDPs), which are major components of extant membraneless organelles, are present in primitive organisms suggesting an early origin.[10] Furthermore, disordered and conformationally dynamic regions of proteins have been hypothesized to have played key roles in evolution of functional proteins.[11–12]

In this Perspective, we consider the physical chemistry of liquid condensate formation and the roles of such membraneless compartments in extant biology and prebiotic chemistry as protocells (Figure 1, left). We examine formation of condensates comprised of IDPs and the biomolecules that partition into them. In extant biology, condensates can form in cytoplasm and the nucleolus where they partition mRNAs,[13] lncRNAs[14] and ribosomal RNAs[15] among others, while in protocells, such membraneless compartments can form under diverse

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conditions and partition RNA oligonucleotides and functional RNAs. We then discuss possible roles of liquid condensates in chemistries that have been implicated in origin of life (Figure 1, right). Specifically, we explore advantages and limitations that are the result of LLPS on relevant prebiotic chemistries such as non-enzymatic polymerization of RNA and activities of RNA enzymes (ribozymes) and other functional RNAs. Overall, LLPS leads to the formation of membraneless organelles in extant cells and we hypothesize that it could have also provided compartmentalization on primordial Earth.

PHYSICAL CHEMISTRY OF LIQUID-LIQUID PHASE SEPARATION

Classes of LLPS.—Many types of phase separation occur in macromolecule-containing aqueous systems. In considering the consequences of LLPS for extant biology and the RNA World, it is useful to distinguish between non-associative and associative phase separation, and to first consider simple, limiting cases (Figure 2). In non-associative, or segregative, phase separation, each phase is enriched in a different component.[1,7] This is common in solutions containing two or more nonionic polymers such as polyethylene glycols (PEGs) mixed with polysaccharides such as dextran, and in solutions with one or more nonionic polymers and a high concentration of salt (e.g., ~0.5 M potassium phosphate).[16] Various PEG/dextran and PEG/salt aqueous two-phase system have been used extensively for bioseparations.[7,17–18] Due to their ability to generate coexisting phase compartments that provide macromolecular crowding and to localize biomolecules on the basis of their size and chemical structure, PEG/dextran aqueous two-phase systems (ATPS) with 5–10 weight percent of both PEG 8 kDa and dextran 10 or 500 kDa have also been used to mimic compartmentalization in extant biology.[19,20] A possible limitation of non-associative phase separation as a model for prebiotic compartmentalization is that it can require relatively high total polymer concentrations.[7] Therefore, it may best be considered for scenarios where organic concentrations are locally higher, for example due to wet/dry cycles.

Associative LLPS, or coacervation, results in a macromolecule-dense phase termed the coacervate phase (Figure 2 right) along with a dilute supernatant phase.[1] As an example, the IDP LAF-1 phase separates at concentrations above ~0.8 μM LAF-1 *in vitro*,[21] generating dense protein-rich droplets related to intracellular membraneless organelles. Water is considered a poor solvent for many polypeptides, including IDPs with sequences rich in Gly and the polar uncharged residues Gln, Asn, and Ser.[22–23] Such IDPs often exhibit concentration- and temperature-dependent phase transitions that can include coacervation as well as gelation or fibrillation.[6] Associative LLPS for which ion pairing ("*complexation*") is important is termed complex coacervation.[1] With respect to prebiotic compartmentalization, this type of LLPS is of particular interest for concentrating nucleic acids due to their negatively-charged phosphodiester backbone. Several synthetic and biological polyelectrolytes that have been used to form complex coacervates are illustrated in Figure 3. Features that affect complex coacervation include the number, distribution, and strength of the ion pairing interactions, which can depend not only on functional group identity but also on polymer chain conformation and flexibility.[24–25,26] Solution conditions can play a role as well, with pH determining the charge state of weak acid/base groups and ionic strength tuning the extent of polyelectrolyte/polyelectrolyte ion pairing for a given pH.[27] These parameters also control coacervate water content and viscosity.[28] Although multivalency

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drives polyelectrolyte association and is important for phase separation,[8] complex coacervation can be achieved even for low molecular weight polyions, which is important in a prebiotic milieu where high molecular weight organic polymers may have been scarce. For example, Mann and coworkers demonstrated droplet formation in solutions of mononucleotides and oligolysine peptides.[29]

Due to the chemical complexity of biopolymers, multiple types of interaction can contribute to their phase behavior. In addition to simple charge-charge contacts, other polymer-polymer and polymer-solvent chemical interactions can be important for LLPS, including cation-p, dipole-dipole, and p - p stacking.[23] *In vitro* LLPS of certain IDPs associated with membraneless organelles has shown sensitivity to increasing solution ionic strength. This observation, particularly coupled with the presence of structural domains rich in charged amino acid residues, suggests a complex coacervation mechanism.[21,30–31] These systems also commonly exhibit temperature sensitivity; for example, formation of stress granules is favored at elevated temperature,[32–33] while coacervate droplets rich in the disordered N-terminus of the protein Ddx4 are destabilized by both increased ionic strength and temperature.[30] Specific biorecognition, for instance between multivalent protein-RNA or protein-protein binding partners, can also be important in phase separation and phase occupancy.[34]

The presence of additional molecules increases the complexity of biomolecular interactions and impacts phase behavior as well. RNA in particular can substantially impact LLPS of IDPs *in vitro* and is thought to be important *in vivo* due to the prevalence of RNAs in many membraneless organelles.[35] Binding of RNA to RNA recognition motifs (RRMs) can drive phase separation of a number of proteins including hnRNPA1 and polypyrimidine tract-binding protein.[36] Polymeric crowding agents such as PEG and ficoll have been shown to favor coacervation of some proteins, including IDPs[37] and RNA/spermine systems.[38] This is potentially relevant both for LLPS in the crowded interior of living cells, and for prebiotic coacervation under drying conditions where total polymer concentrations would be increased.[39–40]

Associative phase separation provides a potential mechanism for concentrating rare organic polymers into small-volume compartments in a prebiotic context. Although most studies have been conducted with relatively pure synthetic or biological polymers, the generality of the LLPS mechanisms indicates that polymer mixtures heterogeneous in length or composition can also be expected to undergo coacervation with different properties based on their compositions. Phase transitions in response to changes in temperature, pH or dilution/concentration could have provided means of cycling between compartmentalized and non-compartmentalized solutions. This would have not only allowed compartment-specific reactions and interactions to take place, but also diffusion of informational and small molecules during the non-compartmentalized state.

Physical chemistry of compartments formed by LLPS.—Characteristics of intracellular liquid organelles include surface tension-minimizing spherical shapes, coalescence upon contact, and often rapid exchange of biomolecules with the surrounding cytoplasm or nucleoplasm.[13] These properties are consistent with formation by LLPS and in

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sharp contrast to membrane-bounded organelles that require specialized transport mechanisms to allow entry and egress of biomolecular solutes. Structures of liquid organelles provide a different type of intracellular compartment that can be more readily accessed and retains its major and minor components by equilibrium partitioning rather than a physical barrier such as a lipid bilayer membrane. In thinking about protocells, such an approach does not require energy input nor specialized transport mechanisms to collect and maintain locally higher concentrations of organic oligomers; hence its simplicity could offer advantages compared to lipid vesicles, particularly at the earliest stages of prebiotic evolution.

Coexisting aqueous phases provide distinct physicochemical environments that depend on phase composition. In addition to the obvious differences based on the distribution of phase-forming molecules (e.g., the dextran-rich phase of a PEG/dextran ATPS is enriched in dextran, or a complex coacervate is enriched in oppositely-charged polyelectrolytes), different phases can have a unique dielectric constant, viscosity, ionic strength, and water activity. This in turn affects the distribution of other solutes in the phase-separated medium and can alter reaction rates and equilibria.[41–42] For example, in a 10 wt% PEG 8kDa / 10 wt % Dextran 10 kDa system, the dextran-rich phase has ~7x higher dextran concentration, nearly 5x lower PEG concentration, and 2-fold greater viscosity than the PEG-rich phase.[43–44] Local concentrations of charged groups within complex coacervates can be in the molar range, and water content as low as ~40 wt %.[8,24,45] Mann and coworkers have reported an apparent dielectric constant (ε) of ~60 inside peptide/nucleotide coacervate droplets (as compared with $\varepsilon = 80$ for water at 20 °C).[29]

Many membraneless organelles in extant Biology contain both RNA and proteins (ribonucleoprotein (RNP) bodies). A growing body of literature shows that the presence of RNA in IDP-based coacervates can alter the physical properties of these droplets; for example, resulting droplets have reduced local protein concentration and decreased viscosity.[46] The physical properties of these compartments can also have important consequences for RNA structure, as will be discussed below.[42]

COMPARTMENTALIZATION BY LLPS

Increased local concentration of different biomolecules assists in association between them, ultimately allowing downstream reactions. For example, related genes of metabolic pathways in bacteria are often polycistronic wherein they are translated from the same mRNA.[47] This strategy helps co-localize enzymes for downstream reactions and substrates are channeled efficiently. For instance, polyketide synthases consist of several distinct catalytic domains that are spatially organized through specific protein-protein interactions.[48] Similar distinct interactions between “client” and “scaffold” proteins have been shown to effectively recruit proteins inside compartments formed by LLPS.[34] Localization of biomolecules in ATPS, membraneless organelles, and complex coacervates can potentially assist in chemical reactions, as they can concentrate ions, small molecules, and large polymers.

In addition to LLPS, mineral surfaces,[49] aerosols,[50] and fatty-acid vesicles[51] have been proposed as prebiotic compartmentalization strategies, and indeed multiple

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compartmentalization mechanisms likely coexisted, which could have facilitated prebiotic chemistry and chemical evolution. Compartmentalization through LLPS has several unique properties that would have been highly desirable in a prebiotic protocell. Specifically, LLPS allows uptake of freely diffusing solutes such as Mg^{2+} from the other phase since both phases coexist as liquid without the need of dedicated transporters that are common in modern biology. High concentrations of polymers and ions in LLPS also mimics biological intracellular environment which tends to be crowded.[52] Furthermore, accumulation and concentration of larger molecules from a dilute external phase tends to be difficult in some fatty acid and lipid vesicles. While the absence of a physical barrier to solute entry/egress can be an advantage for compartmentalization, genetically distinct protocells require that informational molecules are not rapidly exchanged. Exchange rates for molecules encapsulated by LLPS can vary extensively with composition. For example, in ATP/oligolysine coacervates, an RNA oligomer was shown to exchange rapidly,[53] while for PDDA/ATP coacervates generated microfluidically, a single-stranded DNA oligomer of similar length was shown not to exchange over 48 hr.[54] In general, rates of RNA exchange between droplets will depend on coacervate composition, solution conditions (e.g., temperature, ionic strength), and RNA sequence and length, where longer RNAs are slower to exchange.[53,55] Thus membraneless compartments can potentially serve as protocells, which have unique genetic identities depending on solution conditions. While a variety of molecules and ions can be compartmentalized by LLPS,[9,18,29] in this Perspective we focus on partitioning of RNA and Mg^{2+} ions because of their potential roles in the origin of life on Earth in the context of RNA World hypothesis.

ROLES FOR MEMBRANELESS COMPARTMENTS IN ORIGINS OF LIFE CHEMISTRY

Protocells: Oligomerization of Monomers to Functional RNA Polymers.—The RNA World hypothesis has been investigated as a model for origin of functional biopolymers, prebiotic evolution, and subsequent origin of life on Earth (reviewed extensively).[56–57] The origin of RNA polymers from non-enzymatic polymerization of monomers is still an active area of origins of life research. The anionic phosphoric acid moieties in both monomers and oligonucleotides of RNA and other alternative nucleic-acid molecules[58] are ideal for complex coacervation driven by ion pairing interactions. Indeed, studies on ATP/polylysine coacervates[53] revealed high partitioning of RNA oligonucleotides into the condensed phase. As shown in Figure 4, solutions of coacervates can be centrifuged to separate the condensed coacervate phase from the bulk solution. The concentration of analytes in the coacervate phase can be then determined by subtracting the amount of analyte in the bulk phase, which can be readily measured, from the known total amount of analyte. We used this method to study ATP- poly(allyl)amine (PAH) coacervates,[45] where ATP and ADP concentrations within the condensed phase were found to be on the order of molar (Figure 4). Similar reports of enriched nucleotide concentrations have also been reported for coacervates comprised of ATP and polylysine.[29]

Increasing the concentration of reactants is one of the major ways that modern enzymes operate, through the so-called principle of approximation,[59] and it is likely that coacervates can capture some of these same principles. For instance, non-enzymatic polymerization of RNA reactions are sensitive to concentrations of monomers, and experiments to synthesize

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functional RNA molecules via template-mediated non-enzymatic polymerization typically use very high concentrations (~50 mM) of activated nucleotide monomers.[60–61] Mechanisms for attaining such high concentrations of monomers, which were presumably rare, outside of the laboratory setting have not been well explored. Furthermore, both experimental[62–63] and recent *in silico*[64] studies have shown that concentration and organization of amino acids along mineral surfaces can enhance polymerization under prebiotic conditions. Complex coacervates may have performed similar roles perhaps concentrating amino acids to provide early peptides to provide an exit from the RNA world.

Concentrating RNA by partitioning.—The first RNA polymers were likely generated by non-enzymatic polymerization of nucleotides,[65] these reactions tend to proceed at timescales where degradation of the newly synthesized RNA and activated monomers becomes a major issue.[65] Thus, compartmentalization of scarce and dilute RNA polymers would have been extremely beneficial to increase the local concentration of functional RNA molecules and protect them from destructive chemicals and radiation. Partitioning of solutes and polymers in ATPS is largely dependent on their respective chemical and physical properties. For example, in PEGDextran ATPS, hydrophobic peptides/denatured proteins tend to partition in the more hydrophobic PEG phase,[9] whereas the anionic backbone of RNA makes it more favorable to partition in the dextran phase. For larger RNAs, the contact area with the dextran increases, thus they tend to show increased partitioning in dextran phase. Indeed, in PEG/dextran ATPS, increased-partitioning of RNA in the dextran phase strongly correlates with the size of the RNA.[41]

Although partitioning of RNAs inside complex coacervates is driven by ion pairing interactions, additional interactions can occur between the RNA and other molecules within the condensed phase. For example, in case of polyU RNA/spermine coacervates, A15 RNA partitions much strongly in the condensed phase compared to U15 or N15 RNAs.[55] This is likely due to sequence-specific interactions between polyU RNA/spermine coacervates and A15 RNA.

It is helpful to consider how RNA partitions inside biological intracellular condensates and abiological complex coacervate. As mentioned earlier, intracellular condensates comprised of proteins have been found to contain RNA. While it is known that RNA functions are closely tied with their structures, detailed studies of RNA functions and structures inside condensed phases of coacervates are sparse. For example, in Ddx4-containing organelles, it was found that single-stranded RNA and DNA were partitioned in the interior of condensate irrespective of length.[42] However, long double-stranded DNA and RNA were excluded, while short ones were only moderately absorbed (Figure 5 A). This partitioning behavior was rationalized in terms of the distortion produced by the nucleic acids on the underlying structure of the organelle interior (Figure 5A right). The authors posit that single-stranded nucleic acids, short double-stranded oligonucleotides, and compact RNA folds exert only minimum distortion on the "mesh-like weave" of the membraneless organelle, whereas long double-stranded nucleic acids distort the interior structure of the organelle and are thus excluded.[42] Mechanisms for absorption nucleic acids are highly dependent on the constituents that make up the coacervate; therefore, this distortion of interior structures may not apply to other phase separated systems.

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In case of the poly(allyl)amine hydrochloride (PAH)-ATP coacervate system,[45] we found that different lengths of polyA RNA partition similarly (Figure 5B). Furthermore, RNAs containing 54 random nucleotides and the structured HDV ribozyme were absorbed in the condensed phase to similar extent as polyA RNA. Since the charge-charge interaction is the main driver of the PAH-ATP coacervate system, partitioning of RNAs is likely from the displacement of ATP (Figure 5B right), which has smaller charge valency. This mechanism also appears to serve as the basis for selection of longer RNA polymers in the coacervates.

There are large differences in partitioning coefficients for RNA in Ddx4 organelles and in PAH-ATP coacervates. For example, there is ~3000-fold smaller K_{eq} values for RNAs of similar lengths in Ddx organelles. Since Ddx organelles are biological, there are likely other constraints such as structure and sequence that not only dictate partitioning, but also have biological functions. The PAH-ATP coacervates, on the other hand, are abiological, and the partitioning of RNAs are strictly based on the chemical interactions without any evolutionary history.

Effect of Compartmentalization on Ribozyme Reaction Rates.—Concentrating biomolecules by partitioning can enhance reaction rates. We investigated the effect of compartmentalization on single turnover kinetics for three different hammerhead ribozymes in PEG/dextran systems.[41] Different volume ratios of dextran-rich to PEG-rich phase (V_D:V_P) allowed the effect of compartment size on ribozyme catalysis to be studied without altering the composition of the phases. Apart from the selective RNA partitioning in the dextran phase, we observed the highest reaction rate increase of ~70-fold at a 1:100 (V_D:V_P) volume ratio (Figure 6A), which occurs because substrate and enzyme are concentrated under k_{cat}/K_M conditions. Since ATPS increase polymer concentrations, they can additionally exert the crowding effects to help fold RNAs. Indeed, crowders such as PEG and dextran have been shown to increase cooperative folding in variants of tRNA.[66] The mode for enhanced cooperative folding is RNA and crowder-specific, and can arise due to destabilization of secondary structures or stabilization of tertiary structures.[67] Interestingly, viscous solvents have also been hypothesized to have alleviated strand inhibition during prebiotic RNA replication.[68]

Mg^{2+} Partitioning Inside Coacervates.—It is well established that ribozymes and riboswitches often require Mg^{2+} or other metal ions for catalysis and folding.[69–72] Interactions with components that form ATPS can concentrate metal ions,[73] for example, iminodiacetic acid derivatized PEG has been used in PEG/Dextran ATPS to chelate copper and effectively extract heme proteins.[74] Partitioning of Ca^{2+} ions in coacervates composed of elastin peptides have been known.[75] Recent work has established that the condensed phase of PAH-ATP coacervate phase extensively partition Mg^{2+} ions.[45] We found that Mg^{2+} ions in the condensed phase can reach concentrations in the order of ~1 M (Figure 6B). We reasoned that Mg^{2+} partitioned within the condensed phase while being coordinated by the phosphates of ATP.

Ribozymes and riboswitches often require Mg^{2+} ions for optimal activity.[69–70,76] One of the most striking examples of the interplay between magnesium and ribozyme is the RNase P ribozyme, where both high (~0.2 mM) and low affinity (~3 mM) interactions are involved in

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catalysis.[77] Magnesium ions are believed to stabilize the transition state and neutralize charges to assist in substrate association. While RNase P in bacteria and eukarya alike require associated proteins for function *in vivo,* bacterial RNase P RNA-alone is active at high magnesium concentrations *in vitro*[78] with almost complete cleavage of precursor of bacterial ptRNA^{Gly} in 50 mM Mg^{2+}.[79] Increased Mg^{2+} concentration within the condensed phase of coacervates may have assisted the functional RNAs that would be non-functional in bulk solution with low concentration of required cations. Apart from the RNase P ribozyme, many other natural and artificial ribozymes show magnesium dependent increases in observed rate constants.[80] Notably, high concentration of metals and salts in eutectic ice phase has been shown to assist in ribozyme catalyzed RNA polymerization.[81]

Apart from enhancement in ribozyme catalysis, hydrolysis of activated nucleotides used for non-enzymatic polymerization of RNA is also highly dependent on Mg^{2+} [82] and would disfavor function. It remains to be known how condensed phases rich in monomers, RNA primers and template, and Mg^{2+} conspire to affect the many rates and fidelity of non-enzymatic RNA polymerization.

Covalent and Non-covalent Control of Coacervation.—A key component of all of biology is regulation of function, turning on genes only when needed. The constituents of coacervates themselves can provide some level of control on formation and dissolution of these compartments. One of the most fundamental reactions in all domains of life is phosphoryl transfer, which is a common post-translational modification (PTM). Ribozymes with polynucleotide kinase activity have been discovered from multiple *in vitro* evolution experiments and they have been found to be dependent upon diverse metal ions.[80,83–84] Furthermore, ribozyme-catalyzed covalent modification can selectively inactivate functional RNAs,[85] analogous to differential protein regulation by PTMs. Notably, deoxyribozymes with kinase and phosphatase activities have also been isolated.[86–87] Each phosphate group added to a substrate's sidechains changes the charge density, thus affecting the propensity of phase separation.

While phase-separation through ribozyme/deoxyribozyme-mediated covalent modifications has not been explored yet, protein enzymes have been shown to actively induce or prevent phase separation through phosphoryl transfer. This has been demonstrated in coacervate systems made with polyU RNA and the cationic peptide RRASLRRASL.[88] The phosphorylated form of this peptide, RRApSLRRApSL, cannot undergo coacervation with polyU RNA; coacervates form only after a phosphatase enzyme removes the two phosphates (Figure 7 A and B). This is because as the phosphate groups are lost from the peptide, there is a net increase in positive charge, which consequently increases interaction with the polyU RNA. Conversely, protein kinase A is able to transfer phosphoryl groups to unphosphorylated peptide RRASLRRASL causing a net reduction in positive charge, which weakens the polyelectrolyte interactions and results in dissolution of coacervates (Figure 7 C and D). Reversible coacervate systems of polylysine and ATP have also very recently been programmed by enzymatically converting between ATP, which could form coacervates with polylysine, and ADP, which could not.[25] This system is notable in that both enzymes can remain active in the reaction mixture simultaneously, facilitating control over coacervate formation and dissolution.[25]

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An important issue in protocells is protection of the biomolecules from degradation. Montmorillonite clay has been found to protect ribozymes from UV-irradiations.[49] Interior of coacervates may also physically protect RNAs from factors that could destroy its integrity. This has been demonstrated for glycine and diglycine in coacervates made with partially sulfated and aminoacetylated polyvinyl alcohols.[89] RNA molecules may be similarly protected from the insults in the bulk solution that would otherwise degrade or modify RNAs when inside coacervates.

While partitioning of RNA inside the condensed phase would have been advantageous for the RNA World, recycling and reshuffling of functional and non-functional RNA molecules is important too. Exchange of RNA[53] and DNA[90] molecules between coacervate droplets and dilute solutions have been shown. Dissolution of coacervates can readily release biomolecules into the bulk solution. This would allow specific functional RNAs to be taken up by other coacervates, where they can impart functions. Simple environmental changes such as temperature can significantly impact formation of coacervates. Recent example of this was demonstrated in the polyuridylic acid-spermine/spermidine coacervates,[55] which showed a characteristic lower critical solution temperature (LCST) of ~20°C. Formation of coacervates were only observed at temperature greater than the LCST (Figure 7 E and F). Depending on the composition, coacervates can also have an upper critical solution temperature (UCST),[91] above which they cannot phase separate. Dynamic coacervate systems[92] that form or dissolve based on surrounding temperature could have been prevalent in early earth where temperature changes in diurnal cycle would have driven transient coacervation allowing coacervate-dependent functions of RNA such as ribozyme or aptamer functions, and then dissolve to allow these molecules to diffuse in the solution.

Coacervates and Catalytic Strategies.—All of the coacervate-forming molecules provided in Figure 3 have chemical moieties that can participate in one or several of the catalytic strategies described for naturally occurring self-cleaving ribozymes (Figure 8A)[93]. These catalytic strategies involve deprotonation of the O2′ nucleophile, ‘γ’; neutralization of the non-bridging oxygen (NBO) atoms of the scissile phosphate, ‘β’; protonation of the incipient oxyanion on the O5′, ‘δ’; and orientation of the in-line nucleophilic attack, ‘α’. Recently, we described two other catalytic strategies, which focus on activation of the O2′ nucleophile:[94] a ‘γ'’ strategy that involves direct acidification of the O2′ by hydrogen bond donation, and a ‘γ''’ strategy that releases the O2′ nucleophile from inhibitory interactions (Figure 8A). These catalytic strategies can be facilitated by the components of the coacervates themselves. For example, arginine has the guanidinium group that resembles the N1-C2-N3 part of guanosine molecule that is involved in γ, γ', and the γ'' catalytic strategies. On prebiotic earth, the nonenzymatic polymerization of RNA likely yielded some level of backbone heterogeneity, where some of the critical functional groups utilized for catalysis could have been mis-oriented; in such cases, components of the coacervates may have assisted in catalysis. These issues await further experimentation.

Apart from coacervates enhancing catalytic potential of ribozymes, they could have also served as a basis for origin and selection of functional RNAs by taking advantage of the partitioning principles described above. A scheme for complex coacervate-based selection of longer and functional RNAs is shown in Figure 8B. As nucleotides concentrated within the

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coacervates, non-enzymatic polymerization of RNA would yield RNA oligonucleotides. Since the longer RNA molecules have more net charge, they would outcompete smaller oligonucleotides to remain inside complex coacervates. Because longer RNA molecules are more likely to fold into complex structures, functional RNA molecules such as aptamers and ribozymes would be more likely to originate within the condensed phase.

CONCLUSIONS

Phase-separated condensates are common in modern biology. While LLPS was implicated in origins of life on Earth by Oparin before the discovery of the genetic code and the RNA World hypothesis, relevance of such systems in prebiotic chemistry remains largely unexplored. Unique microenvironments that concentrate specific biomolecules are provided by LLPS. Both of these characteristics would have been beneficial during the origins of life on Earth where molecules would have been otherwise dilute and unfolded. As a later step to modern cells, which contain semi-permeable membranes, complex coacervates could have interacted with other prebiotic compartments, ultimately forming outer coatings at the liquid-liquid interface. For example, Mann and coworkers reported assembly of multilamellar fatty acid membranes around PDADMAC/ATP and oligolysine/RNA coacervates,[95] and we have observed self-assembly of pre-formed liposomes at the surface of spermine/polyU RNA coacervate droplets.[55]

Since RNA molecules are highly polyanionic, they make excellent counterions for cationic polymers that are involved in coacervation. Strikingly, prebiotic syntheses of some RNA monomers has already been demonstrated;[96–97] furthermore, oligonucleotides of RNA have been generated from mononucleotides in wet and dry cycles.[98] Therefore, LLPS involving RNA molecules could have been highly relevant during the RNA World. Phase-separation-assisted synthesis of RNA oligonucleotides, selection of longer RNA molecules by partitioning, enhancement of ribozyme catalysis, and protection from a harsh and unfavorable external environment are all plausible roles for LLPS in the primordial Earth. Further investigations of RNA-containing LLPS systems in extant biology and simulated early Earth conditions promise to unravel new insights into the interplay between LLPS and RNA structure-functions.

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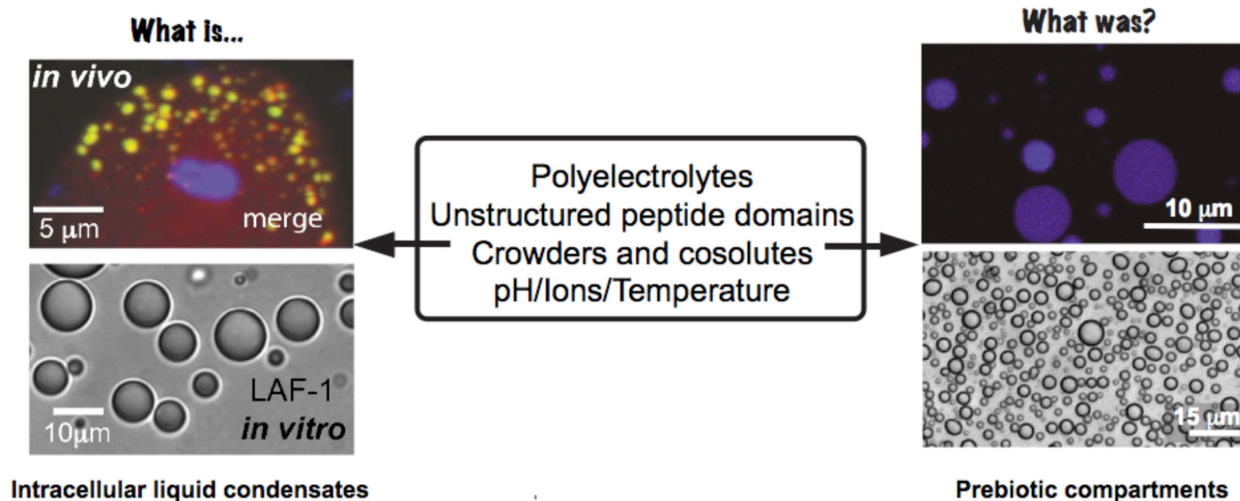

Figure 1:

Phase separation in extant biology (left) and prebiotic chemistry (right). Low complexity regions of the LAF-1 helicase protein, charged peptides and RNA are involved in liquid-liquid phase separation inside cells (left)[21]. Similar interactions could have produced non-membranous compartments in the primordial earth that partition molecules (right). Spermine/polyU RNA coacervates (top)[55] and PAH-ADP coacervates (bottom).[45]

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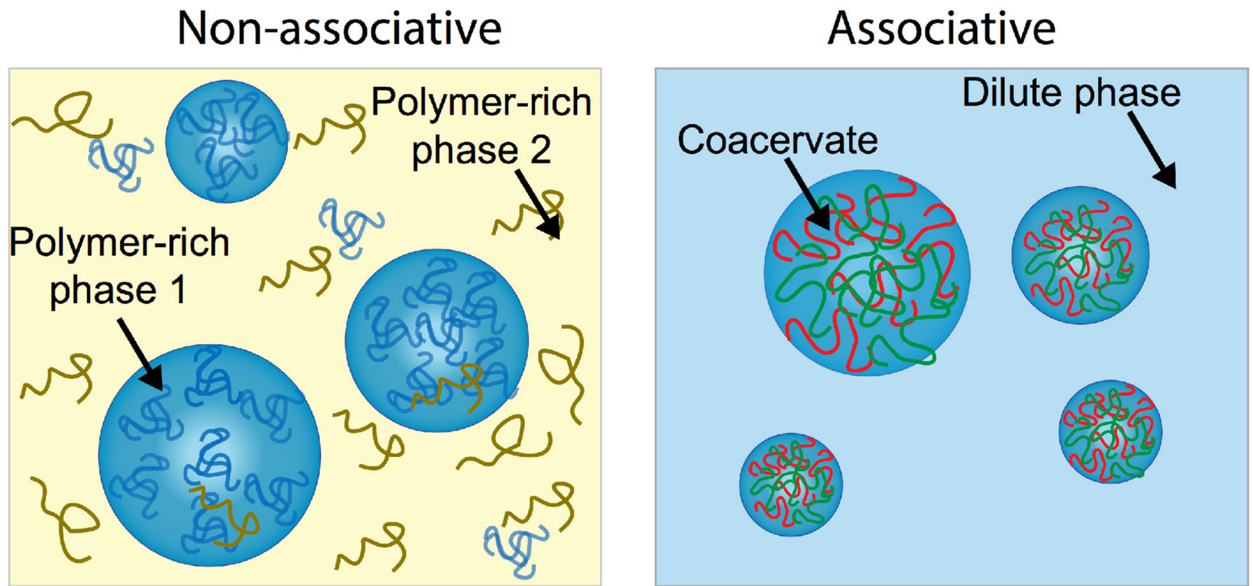

Figure 2:
Non-associative and associative phase separation. In non-associative phase separation, solutions rich in two “incompatible” aqueous polymers form two distinct crowded phases (left). In associative phase separation, polymers interact and associate to form a very crowded polymer-rich phase and separate from the dilute bulk solution (right).

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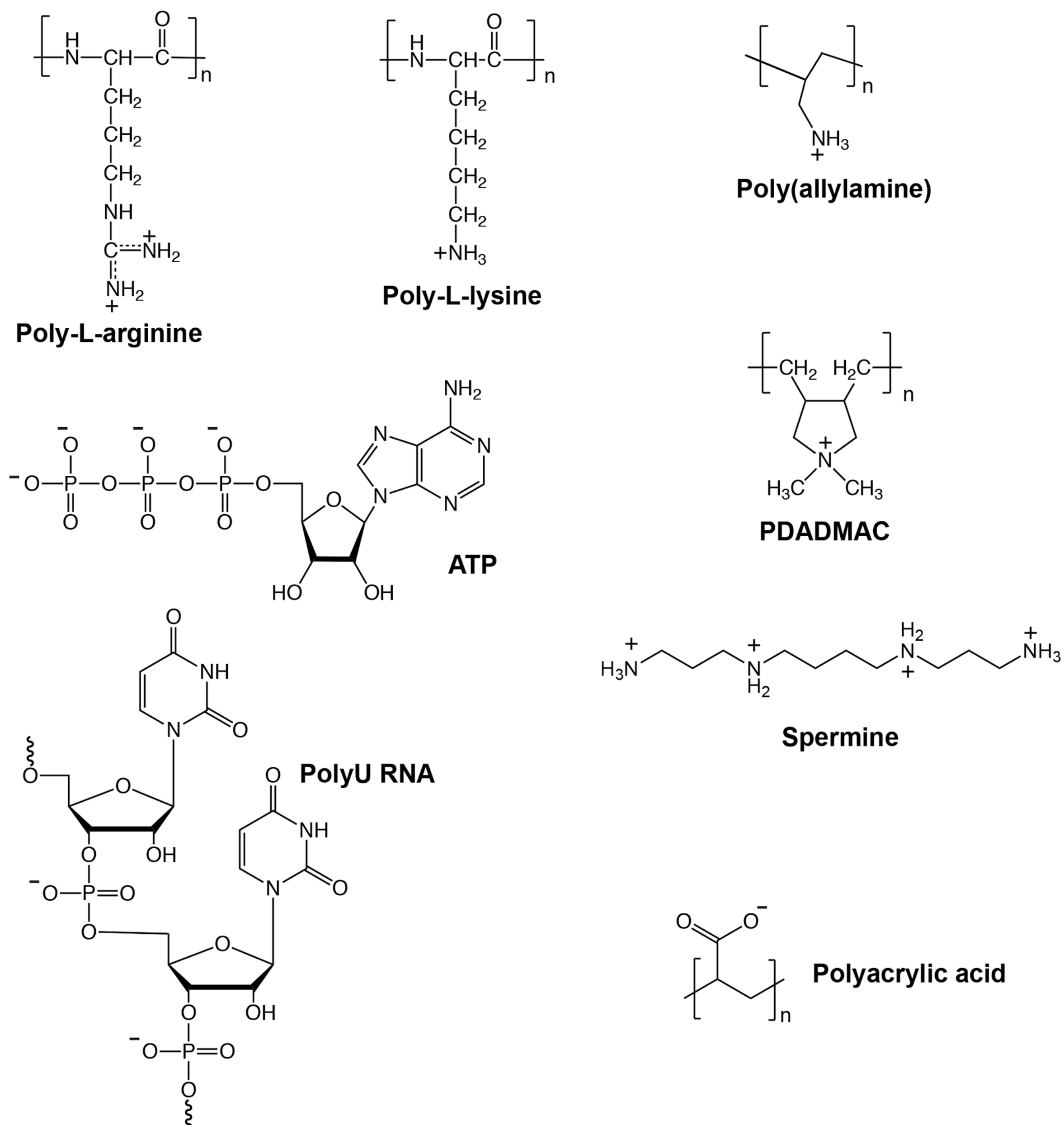

Figure 3:
Structures of molecules discussed in this perspective that are involved in associative phase separation.

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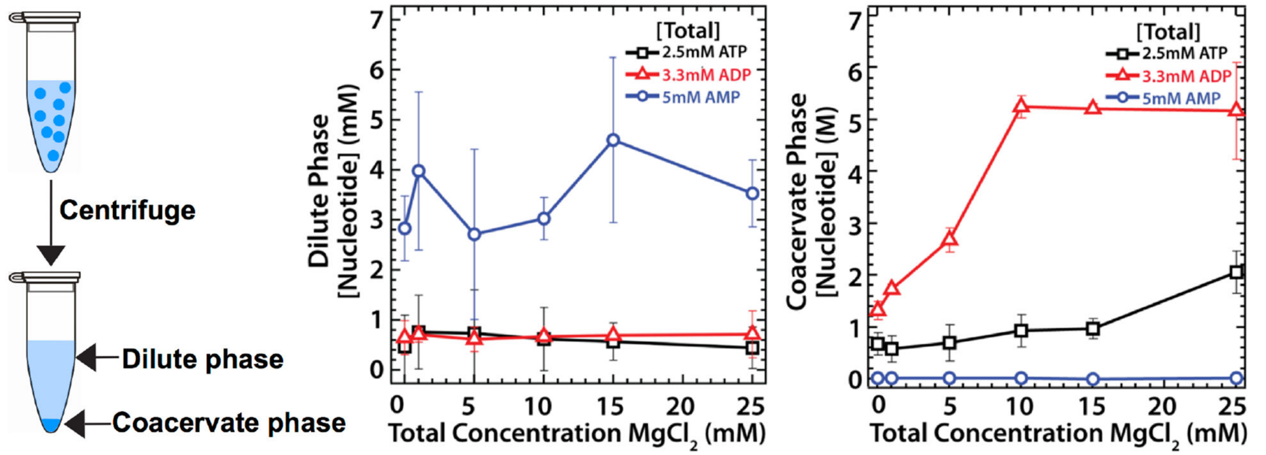

Figure 4:

Coacervates concentrate monomers and polymers A) (left) Centrifugation separates condensed phase from the bulk solution. (middle) Concentration of nucleotides in dilute phase (mM). (right) Concentration of nucleotides in condensed phase (M).[45]

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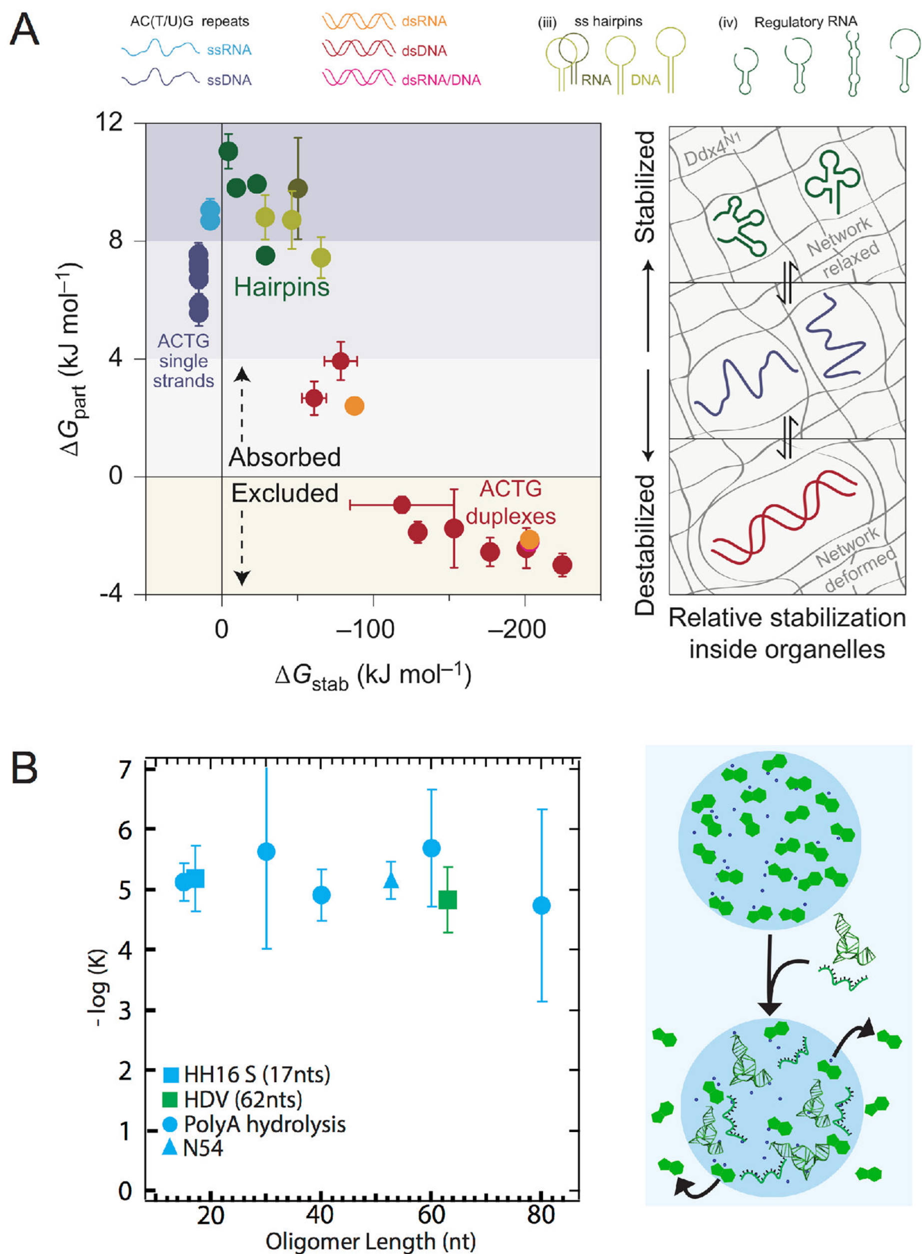

Figure 5:

Multiple mechanisms for RNA partitioning inside membraneless compartments (A) Ddx4 protein condensates differentially exclude long double stranded DNA and RNA while absorbing single stranded nucleic acids and regulatory RNAs (left), G_{part}= -RTln([in]/[out]), where [in] and [out] are concentrations of nucleotides inside and outside of droplets. Constraints in the interior structures of Ddx4 condensates allow a subset of nucleic acid sizes and structures to be absorbed.[42] (B) PAH-ATP coacervates selectively partition RNAs irrespective of the structures and sizes (left)[45]. The mechanism for RNA partitioning is by displacement of ATP.

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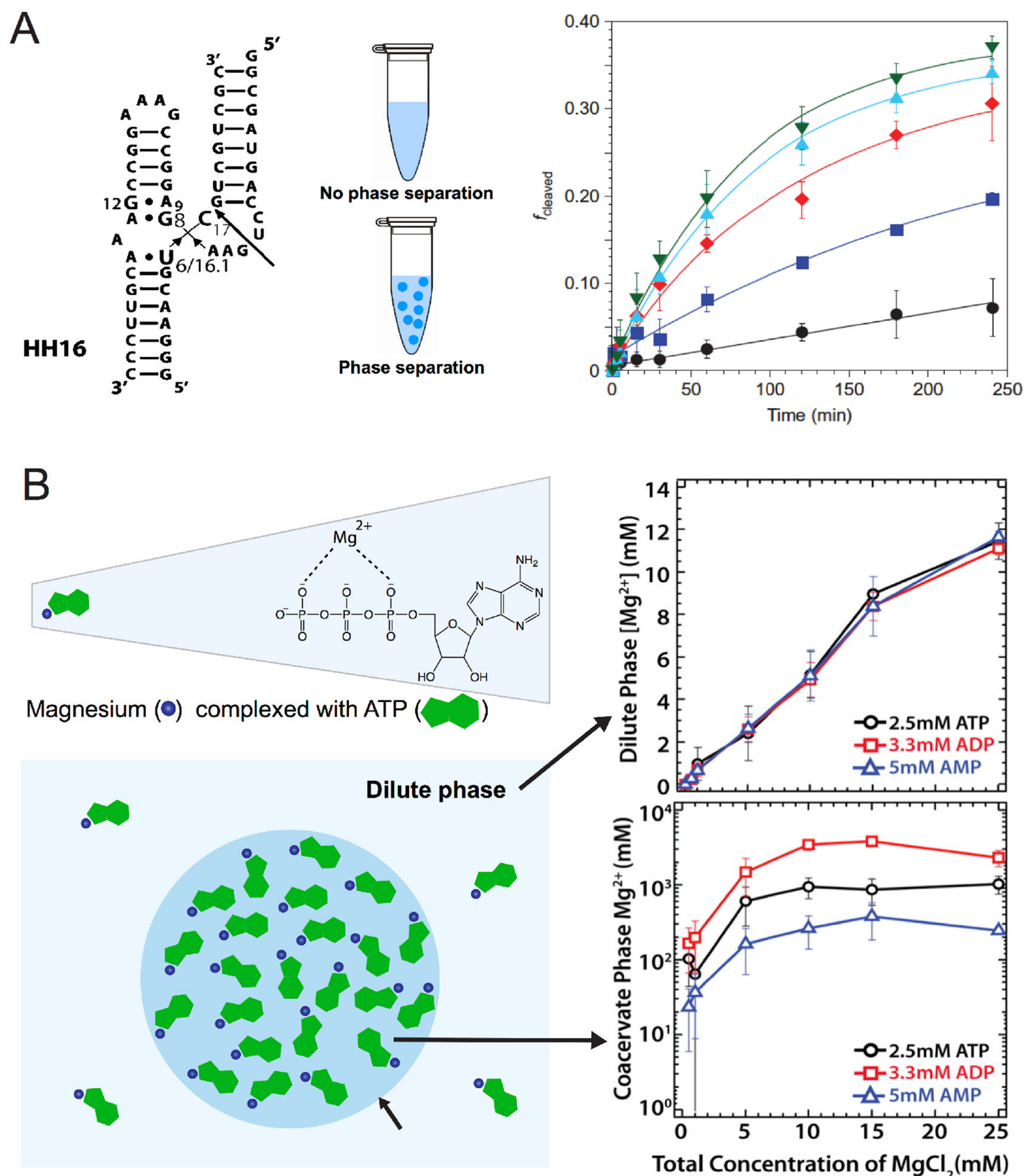

Figure 6:
Ribozyme catalysis in non-associative phase separated system and Mg^{2+} partitioning in associative phase separated system. (A) Structure of the hammerhead ribozyme. Ribozyme catalysis was carried out in different dextran:PEG phase volumes. 1:0 (filled black circles), 1:5 (blue squares), 1:12.5 (red diamonds), 1:50 (blue triangles) and 1:100 (inverted green triangles).[41] (B) Magnesium and other catalytic potentials inside complex coacervates. Magnesium associated with ATP is also partitioned inside the PAH-ATP coacervates.[45]

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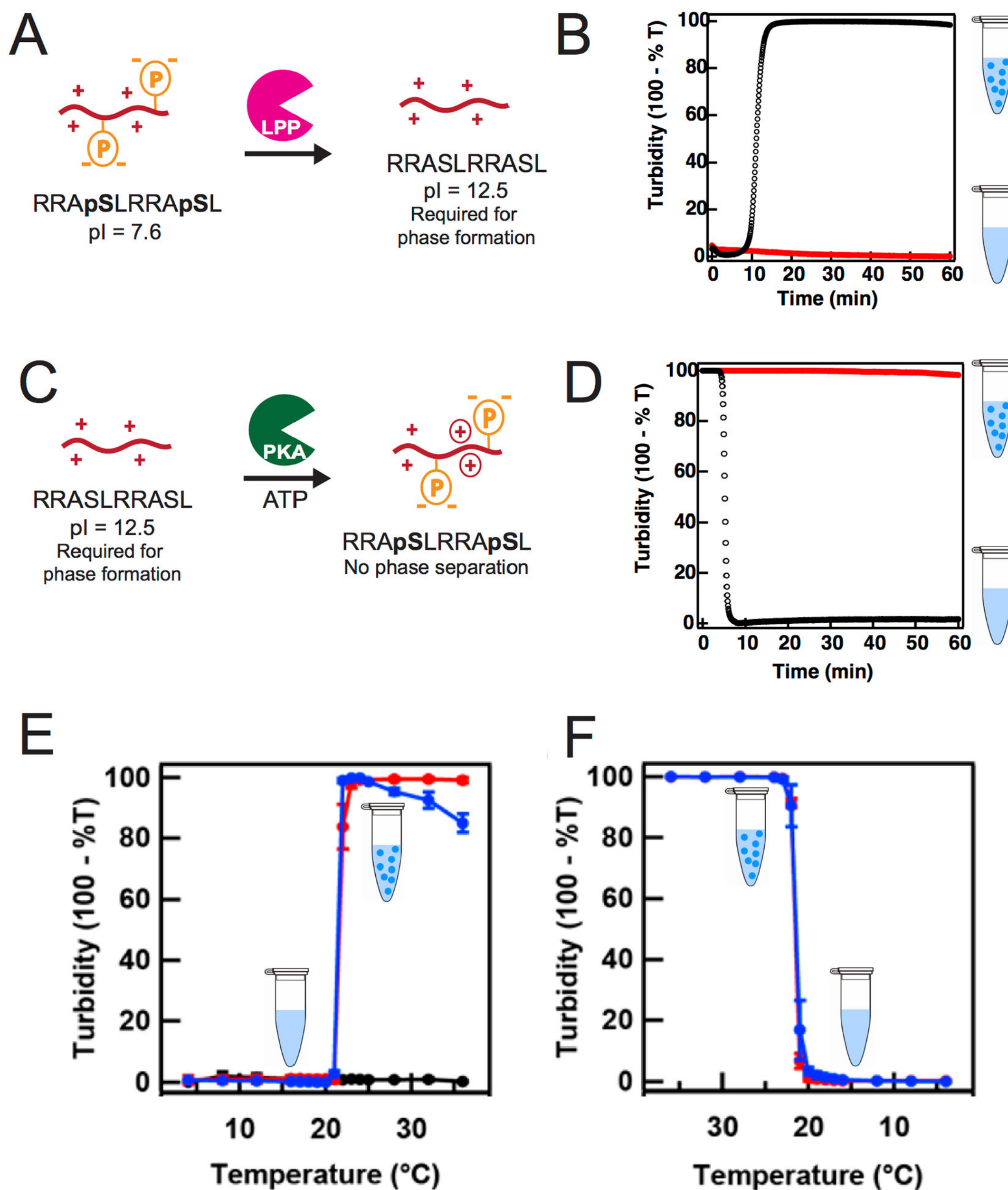

Figure 7:
Molecular and environmental tuning of coacervates (A) and (B) Phosphatase enzyme increase the net positive charge by dephosphorylating RRASpLRRASpL peptide which forms coacervates with polyU RNA. (C) and (D) Kinase enzyme decrease the net positive charge by phosphorylating RRASLRRASL peptide and prevent coacervation with polyU RNA.[88] For (B) and (D) appearance or disappearance of phase-separation is indicated by changes in turbidity measurements. Red trace indicates samples without any enzyme. (E) and (F) Turbidity plots indicating either the appearance or disappearance of PolyU RNA/Spermine coacervates as a function of temperature.[55]

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Figure 8:
Potential contributions of coacervate components to prebiotic catalysis and evolution. (A) Several catalytic strategies used by naturally occurring self-cleaving ribozymes described in ref 94. (B) Scheme for coacervate-assisted RNA oligomerization and partitioning of larger RNAs.