Origins of Life and Evolution of Biospheres (2019) 49:105–109
https://doi.org/10.1007/s11084-019-09576-7

THEORETICAL PAPER

The RNA i-Motif in the Primordial RNA World

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Received: 18 February 2019 / Accepted: 26 April 2019 /
Published online: 25 May 2019

Abstract

The primordial RNA world is a hypothetical era prior to the appearance of protein and DNA, when RNA molecules were the sole building blocks for early forms of life on Earth. A critical concern with the RNA-world hypothesis is the instability of the cytosine nucleobase compared to the other three bases (adenine, guanine, and uracil). The author proposes that cytosine residues could have stably existed in the primordial world in the RNA i-motif, a four-stranded quadruplex structure formed by base-pairing of protonated and unprotonated cytosine residues under acidic conditions. The i-motif structure not only increases the lifetime of cytosine residues by slowing their deamination rate, but could also allow RNA polymers to bind to certain ligands (e.g., anions) to perform critical functions. Future studies focused on determining the rate of cytosine deamination in RNA i-motifs over a range of pH, temperature, and pressure conditions, and on interrogating the interactions between ligands and RNA i-motifs, could uncover new evidence of the origin of life on Earth.

Keywords Primordial RNA world · i-motif · Cytosine deamination · Protonated cytosine · Origin of life

The "intercalated motif (i-motif)" is a four-stranded quadruplex structure formed by cytosine-rich DNA or RNA sequences (Abou Assi et al. 2018; Zeraati et al. 2018). A cytosine nucleobase may protonate at its N3 position under acidic conditions, thus allowing the formation of a C^+:C base pair between one protonated and one unprotonated cytosine via three hydrogen bonds (Fig. 1a). The C^+:C base pairs hold two cytosine tracts together, which intercalate in an antiparallel-oriented C^+:C duplex to form a four-stranded i-motif (Fig. 1b). An intramolecular i-motif is formed through the spatial arrangement of four cytosine tracts within a single DNA or RNA strand, whereas an intermolecular i-motif is formed through the association of two or four separate cytosine-containing DNA or RNA strands. The stability of an i-motif depends on factors such as the pH of the solution, the DNA or RNA sequence, the length of the cytosine tract, and temperature.

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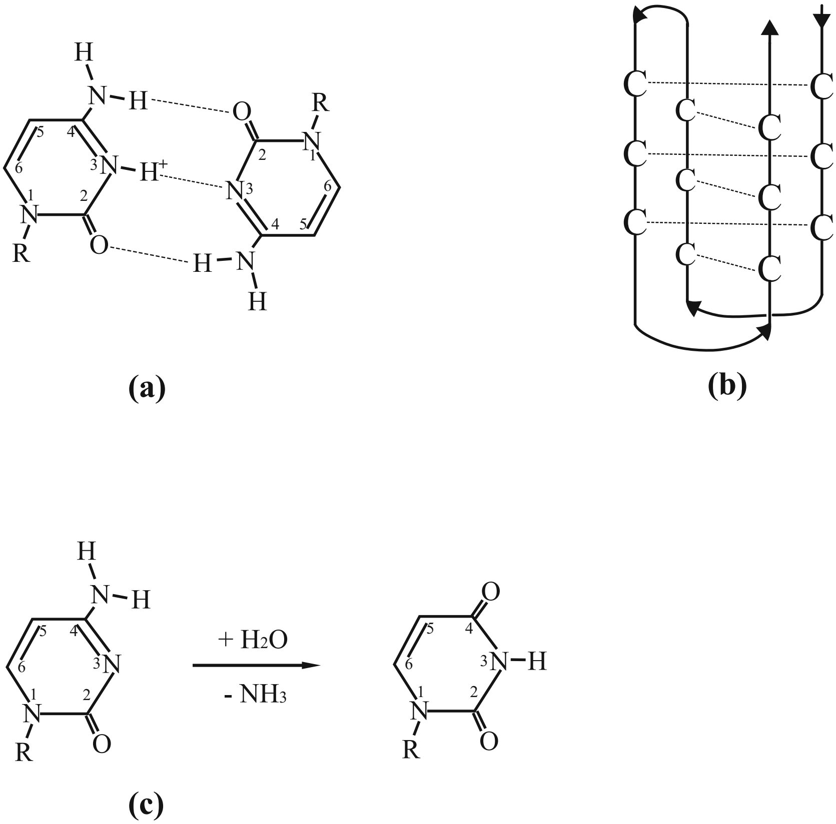

Fig. 1 **a** An illustration of a C⁺:C base pair formed via three hydrogen bonds; **b** an illustration of an intramolecular i-motif; and **c** the deamination of cytosine to uracil

Fifty years ago, it was hypothesized that the evolutionary history of life on Earth included a primordial RNA world where RNA molecules served both to store genetic information and to catalyze chemical reactions (Crick 1968; Gilbert 1986; Orgel 1968). The discovery of ribozymes strongly supported this hypothesis (Guerrier-Takada et al. 1983; Kruger et al. 1982). Nonetheless, other studies took issue with the hypothesis, including critical concerns regarding the origin and stability of cytosine nucleobases (Shapiro 1999).

Since the Watson-Crick pairing of adenine (A) with uracil (U) and of guanine (G) with cytosine (C) is the basis of genetic template recognition, it was originally presumed that the nucleobases A, U, G, and C were readily available in the primordial RNA world. However, later studies indicate that the proposed prebiotic syntheses of cytosine are implausible (Ferris et al. 1968; Shapiro 1999). In addition, although pyrimidine, the central building block of cytosine, has been found in meteorites, cytosine itself has not been.

In 2014, NASA scientists demonstrated that a pyrimidine-containing ice sample (mostly water, containing methanol and ammonia) produced cytosine when exposed to high energy ultraviolet radiation under space-like conditions (15–20 K in a vacuum chamber) (Nuevo et al. 2014). Although cytosine has not been found in meteorites due to its rapid decay in Earth's environment, the NASA experiment strongly suggests that cytosine was created in space and would have been present from the beginning of

Earth's history. Since adenine, guanine, and uracil have long been reported from meteorites (Hayatsu et al. 1975; Stoks and Schwartz 1979; Stoks and Schwartz 1981), NASA's 2014 discovery gave support to the RNA world hypothesis.

Cytosine monomers are unstable, with a half-life much shorter than those of the other three bases, thus seeming to exclude the possibility of GC base pairing in the primordial world (Levy and Miller 1998; Shapiro 1999). Some scientists proposed that a two-letter code (A and U) or an alternative base pair may have existed early in Earth's history (Levy and Miller 1998). The author proposes that cytosine-rich RNA motifs played important roles in the primordial world. This hypothesis is based on the fact that cytosine tracts from a single RNA strand, or from separate RNA strands, can fold into stable i-motif structures under acidic conditions. There is widespread acceptance that acidic oceans existed in the primordial world as a result of the high level of atmospheric CO_2, thus providing an environment suitable for i-motif formation (Bernhardt and Tate 2012; Kua and Bada 2011).

The instability of the cytosine nucleobase is mainly due to its spontaneous deamination by hydrolysis, resulting in the formation of uracil (Fig. 1c). Studies have demonstrated that the hydrolytic deamination of cytosine in single-stranded DNA occurs at a much faster rate at high temperature; in addition, the hydrolysis rate of cytosine monomers increases with increasing pressure (Lepper et al. 2018; Lewis et al. 2016). From these results we could deduce that cytosine in single-stranded RNA would be degraded if early Earth had a high temperature and/or pressure environment. However, the folded conformation of an RNA i-motif could reduce the likelihood of cytosine deamination, allowing an extended lifespan for cytosine-containing RNA molecules.

To the author's knowledge, no research has been conducted to determine the rate of cytosine deamination in RNA i-motifs over a range of pH, temperature, or pressure conditions. Shaw's group investigated cytosine deamination in single-stranded and double-stranded DNA, and found that the deamination rate of cytosine in a double helix was approximately 140-fold slower than in single-stranded DNA (at 37 °C) (Frederico et al. 1990). This difference may be due to the decreased accessibility of the N3 and C4 positions in a cytosine that is paired to guanine via three hydrogen bonds, blocking the attack from water. Similarly, the three hydrogen bonds formed between a protonated and an unprotonated cytosine (Fig. 1a) may significantly reduce the deamination rate of cytosine residues located in an i-motif, thus increasing their lifetimes.

Phan et al. discovered that i-motif and G-quadruplex structures efficiently compete with a C ≡ G double helix in telomeric DNA at lower pH and higher temperature conditions; whereas a C ≡ G duplex structure predominates under near-physiological pH and temperature conditions (Phan and Mergny 2002). This finding hints that RNA i-motifs could have stably existed on early Earth, which is believed to have had a lower pH and higher temperature environment than today. The hydrogen bonds tightly holding the protonated and unprotonated cytosine bases together, combined with the electrostatic attractions between the C^+:C pair and the negatively charged phosphate backbone, stabilize local RNA conformations and guarantee their proper function.

Although RNA polymers are highly negatively charged and prefer cation binding, anions have been observed bound to RNA (Kieft et al. 2010). The author proposes that the C^+:C pair in an i-motif may prefer anion binding. To the author's knowledge, no study has investigated ligand binding to RNA i-motifs, leaving open the promising exploration of the potential critical regulatory roles that i-motif structures may have played in the early world. RNA structure governs its function; without the presence of proteins on early Earth, RNA may have formed assorted motifs to interact with various ligands to perform such functions as control, regulation, and catalysis. Studying the interactions between ligands and RNA i-motifs could uncover new evidence of the origin and early evolution of life on Earth.

In summary, the author proposes that the RNA i-motif was the prevalent conformation cytosine tracts adopted in the primordial RNA world. Such a conformation would not only increase the lifetime of cytosine residues by slowing their deamination rate, but would also allow RNA polymers to bind to certain ligands (e.g., anions) in order to perform the functions necessary to support life on a developing Earth.

Acknowledgements This material is based upon work supported by the National Science Foundation under Award No. OIA-1458952.

Compliance with Ethical Standards

Conflict of Interest The author declares that she has no competing financial interests.

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