Chemical Evolution

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RNA–DNA Chimeras in the Context of an RNA World Transition to an RNA/DNA World

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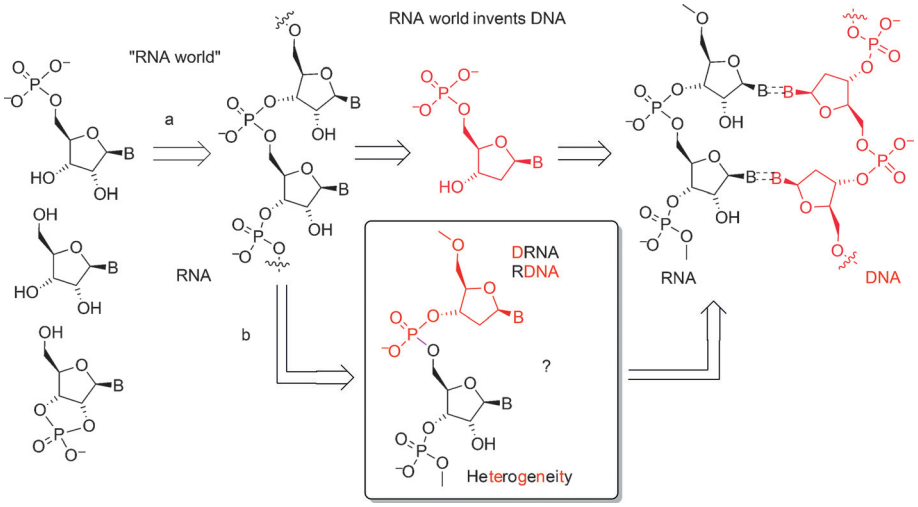
In memory of James P. Ferris

Abstract: *The RNA world hypothesis posits that DNA and proteins were later inventions of early life, or the chemistry that gave rise to life. Most scenarios put forth for the emergence of DNA assume a clean separation of RNA and DNA polymer, and a smooth transition between RNA and DNA. However, based on the reality of "clutter" and lack of sophisticated separation/discrimination mechanisms in a protobiological (and/or prebiological) world, heterogeneous RNA–DNA backbone containing chimeric sequences could have been common—and have not been fully considered in models transitioning from an RNA world to an RNA–DNA world. Herein we show that there is a significant decrease in Watson–Crick duplex stability of the heterogeneous backbone chimeric duplexes that would impede base-pair mediated interactions (and functions). These results point to the difficulties for the transition from one homogeneous system (RNA) to another (RNA/DNA) in an RNA world with a heterogeneous mixture of ribo- and deoxyribonucleotides and sequences, while suggesting an alternative scenario of prebiological accumulation and co-evolution of homogeneous systems (RNA and DNA).*



Scheme 1. The RNA world concept. a) Transition from a homogeneous RNA backbone to a homogeneous DNA backbone is assumed during the invention of DNA by RNA, b) without consideration of the role of heterogeneous backbone chimeric sequences that would be formed.

The RNA world hypothesis[1–3] postulates that RNA played the role of DNA (genotype) and proteins (phenotype) and gave rise later to an RNA/DNA/Protein world, implying that proteins and DNA were the inventions of an RNA world (Scheme 1 a).[4–7] This hypothesis is largely based on the analysis of extant biochemical machinery and that deoxyribonucleotides are formed by the enzyme-catalyzed reduction of ribonucleotides.[8–10] However, this is countered by the arguments that DNA was earlier, or arose concurrent with RNA, supported by plausible prebiotic routes to deoxyribonucleosides or its precursors.[11–17] That RNA led to DNA, or vice versa, or could have coexisted before the sophisticated biochemical machinery came into existence, raises the question of how one homogeneous backbone system (RNA) gave rise to another homogeneous backbone (DNA) in a protobiological (and/or prebiological) world[17–19] without encountering heterogeneous backbones (Scheme 1 b). In extant biology, there are instances of RNA–DNA heterogeneity in nuclear DNA due to the misincorporation of rNMP by DNA polymerase.[20–22] Moreover, some RNA polymerases and their mutants are shown to accept dNMPs and tolerate sugar heterogeneity sites in a DNA- or RNA- or hybrid template-mediated replication.[23–26] It has been suggested these observations imply that backbone heterogeneity was part of the evolutionary process, still manifested in extant biology.[23] In current biology, misincorporation of rNMP in DNA and dNMP in RNA are carefully guarded against by utilizing evolved repair, editing and proof-reading enzymes.[27,28] Absent alternative mechanisms (primitive catalysts or compartments) that were able to distinguish between the nucle-

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otides of DNA and RNA and/or keep them spatially separated, oligomeric sequences containing random mixtures of RNA and DNA residues would have been inevitable.[14–19]

From previous studies of homogeneous backbone sequences, the thermal stability of hybrid duplexes follows the general trend RNA–RNA > RNA–DNA > DNA–DNA (or DNA–DNA > DNA–RNA) depending on the sequence context.[29–33] Based on such studies and others involving homogeneous backbones of XNAs, there is an implicit assumption in the RNA world approach that mixed RNA–DNA polymers containing monomers of each type could still engage in base-pair mediated replication and tolerate chemical heterogeneity.[18] Whether this enables a smooth transfer of information going from a homogeneous RNA backbone to homogenous DNA backbone, via "heterogeneous backbone" systems, was the motivation for this study (Scheme 1 b). Since Watson–Crick base-pair mediated interactions are at the heart of RNA-to-DNA information transfer and fidelity (via template-mediated replication), the base-pairing behavior of heterogeneous backbone chimeric sequences of RNA and DNA was investigated and compared to parent RNA and DNA sequences.

Four sets of sequences, self-complementary and non-self-complementary A–T/u 16-mer, non-self-complementary A–T/u–G–C 10-mer, and self-complementary C–G 6-mer were chosen as representative systems (lower case indicates ribonucleotide) (Figure 1). In order to constrain the space of possible combinations, a set of heterogeneous backbone chimeric sequences representative of transitioning from RNA to DNA was generated by systematically changing a) RNA-pyrimidine(r-Py) to DNA-pyrimidine(d-Py) and b) RNA-purine(r-Pu) to DNA-purine(d-Pu). This led to 8, 32 and 16 (r/d)Pu–(r/d)Py duplex combinations for the first 3 sets of sequences. The smaller number of nucleotides in the C–G 6-mer sequences allowed investigation of more diverse backbone patterns. We measured the base-pairing propensity of all sequences via UV-T_m thermal melts and, for selected sequences, the temperature-dependent CD spectra to gain insight into how some of these chimeric duplexes compare with their unmodified parent duplexes in overall helical structure.

The thermal melt studies of the self-complementary A–T/u system, 5′-$(au)_8$-3′ to 5′-$(AT)_8$-3′ indicated that the homogeneous backbone combinations were the most stable duplexes (Figure 1 a). The heterogeneous backbone chimeric duplexes had significantly reduced thermal stabilities compared to the homogeneous parent DNA or RNA duplexes (Figure S1 and Table S1 in the Supporting Information).

There was little variance due to the directionality of either nucleobase or backbone sequences (e.g. 5′-$(Pu\text{-}Py)_n$-3′ vs. 5′-$(Py\text{-}Pu)_n$-3′ and 5′-$(ribose\text{-}deoxyribose)_n$-3′ vs. 5′-$(deoxyribose\text{-}ribose)_n$-3′) in these destabilized duplexes (Figure 1 a). The detrimental impact of backbone heterogeneity on base-pairing stability in this simple duplex system was significant, much more than what was expected from previous studies.[29–31]

The non-self-complementary system 5′-$a_4u_3auau_2au_2a$-3′ + 3′-$u_4a_3uaua_2ua_2u$-5′ provided the possibility for varying the

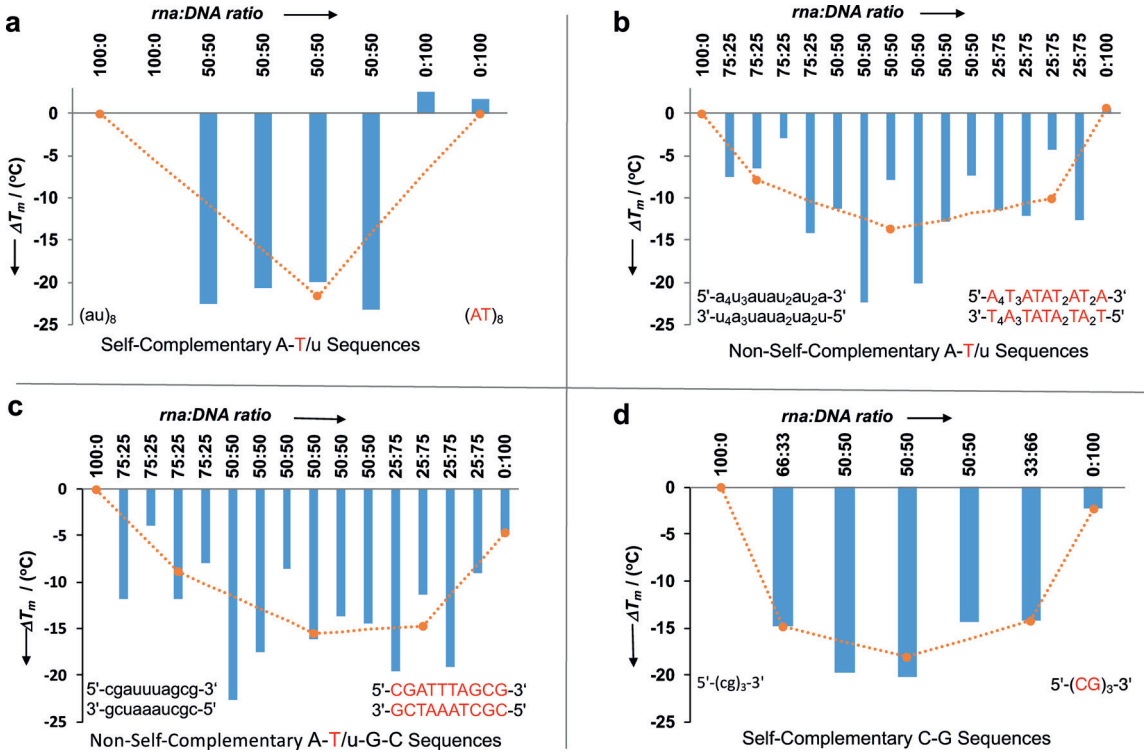


Figure 1. Charts displaying base-pairing stability relative to RNA (ΔT_m (°C)) for each duplex (blue bars) and for the average of a given RNA:DNA ratio (orange line and dots). a) self-complementary A–T/u, b) non-self-complementary A–T/u, c) non-self-complementary A–T/u–G–C and d) self-complementary C–G sequences. a, u, c, g represent RNA and A, T, C, G represent DNA. For conditions of measurements and T_m values see the Supporting Information (Tables S1–S5).

ratios of RNA:DNA nucleotides within a duplex and the opportunity to study non-symmetric chimeric backbone sequences (Figure 1b and Figures S2, S3). Similar to the self-complementary system, duplexes with homogeneous ribose and deoxyribose backbones had the highest thermal stabilities (Figure 1b, Table S2). The heterogeneous backbone chimeric systems formed a pool of relatively destabilized duplexes. Simulating the transition from the full RNA duplex to the fully mixed duplexes via "modification" of ribose-to-deoxyribose consistently led to decreases in duplex stability. The first round of modification, to either of the two strands leading to the 75%/25% RNA/DNA duplexes, were generally all disruptive giving T_m values 3–14 °C lower than the 100% RNA duplex. Further modification to duplexes where both strands were heterogeneous caused further reduction in duplex thermal stability (Figures S2, S3, Table S2). Similarly, continued infiltration of rNMP into DNA to form the 25%/75% RNA/DNA duplexes, irrespective of whether it is pyrimidine or purine residues decreased duplex stability. Further incorporation of rNMP into DNA led to varying decreases in duplex stability depending on whether it was a purine or a pyrimidine residue. In general terms, the thermal stability lost from the transition from RNA (or DNA) to the 50%/50% RNA/DNA chimeras is about −1 °C per modification. The same was true for the reverse combination 5′-$u_4a_3uaua_2ua_2u$-3′ + 3′-$a_4u_3auau_2au_2a$-5′ (Figures S4, S5, Table S3). There did not appear to be any discernable thermal stability trend due to inter-strand nucleotide backbone pairing patterns (e.g. d- with d- and r- with r- vs. d- with r- and r- with d-) nor for regular vs. irregular insertions within the chimeric duplexes (Tables S2, S3). In nearly all cases, the modification of ribopurines to deoxyribopurines was more disruptive to duplex thermal stability than similar modification of the pyrimidines. The weakest base-pairing for the fully chimeric duplex was when all of the ribopurines were modified to deoxyribopurines, while the strongest base-pairing for the chimeric duplex was when all of the ribopurines were retained (Figures S7, S8), consistent with previous observations for the homogeneous backbone chimeric duplexes.[32,33]

With the expectation that a stronger C–G base-pair might mitigate the drastic weakening in thermal stability due to RNA–DNA backbone heterogeneity, we studied a 10-mer duplex 5′-cga$u_3$agcg-3′ + 3′-gcu$a_3$ucgc-5′ containing all four canonical nucleobases (Figure 1c, Figures S9, S10). The duplexes were designed to have an equal number of C–G and A–T/u base pairs, and an equal number of pyrimidines and purines on each strand. This provided a series of intermediate chimeric duplexes with varying percentages of ribose or deoxyribose to span the landscape between the extremes of homogeneous RNA and DNA. In spite of the expanded nucleobase heterogeneity (A, T/u, G, C), the trends in thermal instability of chimeric duplexes paralleled the observations for the two-base A–T/u chimeric sequences. Doping dNMP (or rNMP) into one strand reduced the duplex thermal stability (4–12 °C lower) relative to the homogeneous RNA (or DNA) duplex, with the magnitude depending on whether modifications occurred at the pyrimidine or purine sites (Table S4, Figure S11). Introducing C–G pairs into the chimeric duplexes appears to enhance the destabilizing effects of backbone heterogeneity on base-pairing. The ΔT_m/modification for RNA and DNA going to 50%/50% chimera were, on average −1.6 °C and −1.2 °C, respectively, indicating that modification of RNA causes more significant destabilization than what is gained back in duplex stabilization in modification from chimera to DNA in the A–T/u–G–C pairing system. For the CG-only containing self-complementary sequences 5′-$(cg)_3$-3′, the same trends were observed (Figure 1d, Figure S12); the homogeneous backbone RNA and DNA sequences had the highest stabilities while the mixed backbone sequences experienced a deep drop (ca. −20 °C, Table S5).

The CD spectra for all heterogeneous backbone A–T/u–G–C chimeric duplexes display features that fall in between those for the parent RNA and DNA spectra (Figure S13). The general line shapes and peak shifts of the theoretical and experimental spectra correlate well, further supporting the intermediate helical nature of the chimeras similar to previous RNA–DNA chimeric systems.[34,35]

A van't Hoff analysis was undertaken to assess the influence of backbone heterogeneity on the A–T/u–G–C base-pairing system in terms of thermodynamic stability.[36] The thermodynamic contributions of each duplex as well as the change in free energy ($\Delta\Delta G_{298}$) relative to homogeneous RNA were determined (Table S4, Figures S14–S16). These values have similar trends as those observed for the T_m values: as the backbones tend towards heterogeneity (50% RNA/50% DNA) there is a steady increase in the thermodynamic instability, pointing to the existence of a free-energy barrier in the energy landscape of RNA-to-DNA transition (Figure 2). Within each duplex class (differing ratios of ribose to deoxyribose) the most favorable free energies (lowest ΔG_{298}) correspond to the duplexes with greatest proportion of ribopurines. Additionally, the duplexes with the most favorable enthalpic component have higher amounts of ribopurine, whereas the most entropically favored duplexes tend to be richer in deoxyribopurines.

The thermal stability decreases consistently with accruing numbers of chimeric RNA–DNA junctions; however, for the same number of chimeric junctions, it also depends very much on which backbone unit carries the pyrimidine or purine nucleobase (Figures S7, S8 and S11). As the systems tend towards homogeneity in the backbone, both of these effects vanish and the thermal stabilities of the duplexes increase.[29] The significant and surprising destabilization of the highly heterogeneous chimeric duplexes, while requiring an in-depth structural analysis,[37] seems to stem from the conflicting conformational tendencies of ribose ("structural conservatism of RNA" favoring the A-form, restricted to a 3′-*endo* sugar pucker) versus deoxyribose ("polymorphism of DNA" favoring the B-form, preferring a 2′-*endo* sugar pucker).[38] This conflicting sugar heterogeneity within the same backbone sequence gives rise to divergent inter-phosphate distances (5.9 Å in A-type versus 7 Å in B-type duplex), differing dislocation of helix axis (4.4 to 4.9 Å versus −0.2 to −1.8 Å), opposing base-pair tilt (+10° to +20.2° versus −5.9° to −16.4°) and inconsistent rotation per residue (30° to 32.7° versus 36° to 45°).[38] Such local effects when coupled with

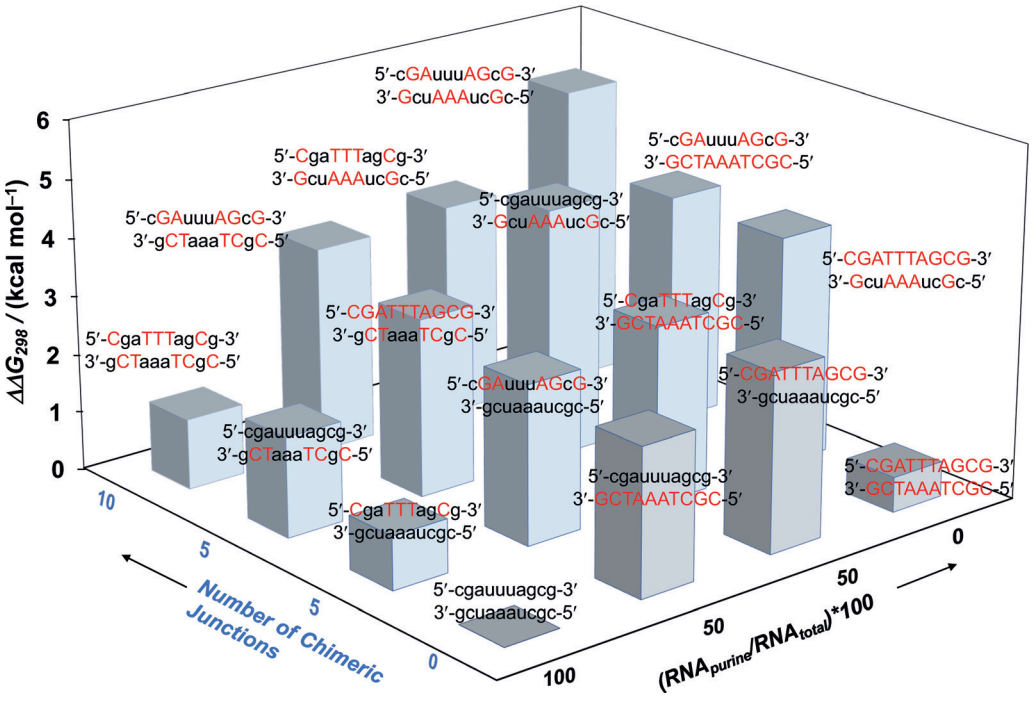


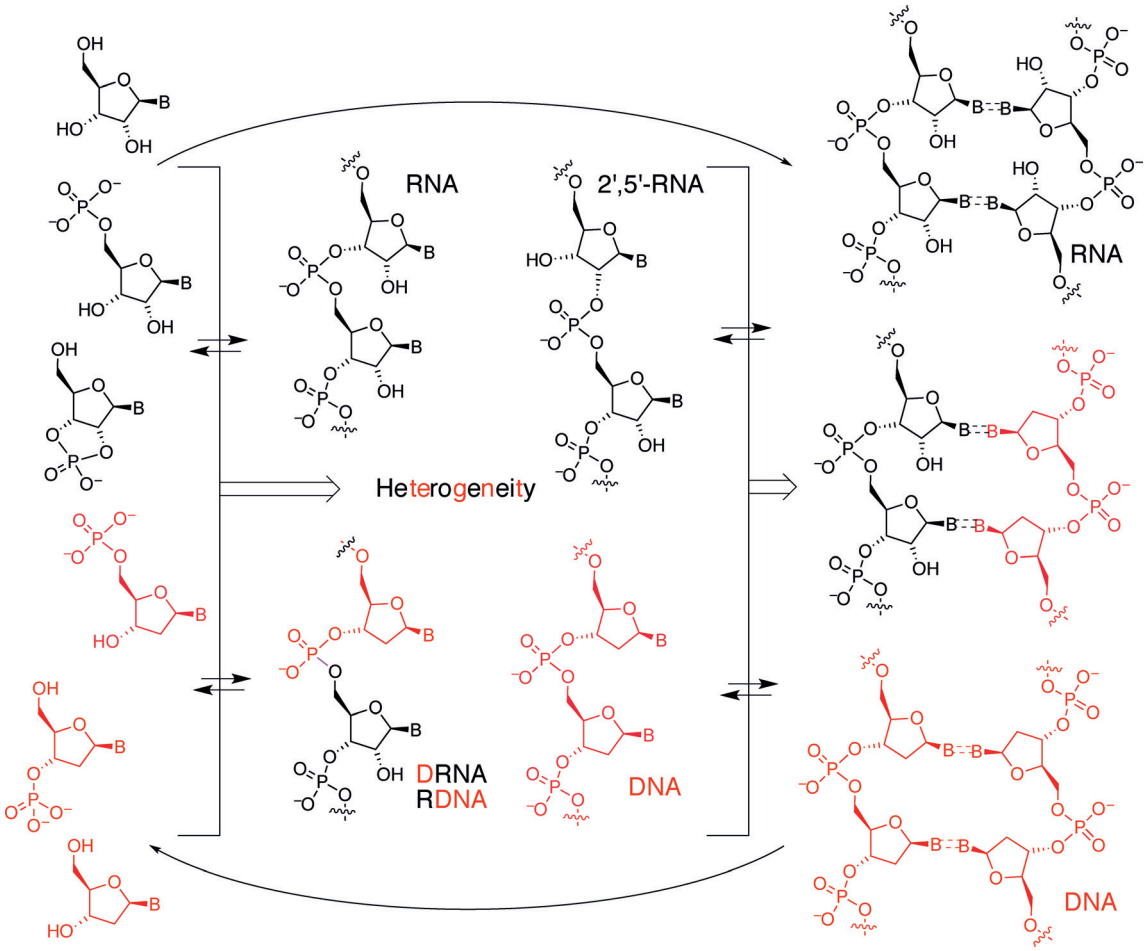
Figure 2. Bar graph of relative free energies ($\Delta\Delta G$) of chimeric A–T/u–G–C duplexes. The transition of RNA to DNA encounters thermodynamically unstable intermediate heterogeneous chimeric duplexes which vary in their relative stability depending on the number of chimeric junctions and content of purine-RNA (r-Pu). For conditions of measurements see the Supporting Information.

nucleobase heterogeneity alter the overall curvature of the duplex, making it difficult to adopt either of the favored forms, detrimentally impacting the base-pairing symmetry of inter- and intra-strand base-stacking overlaps.[33,38,39]

The current study raises questions regarding the feasibility of a linear transition from a homogeneous RNA world to a RNA/DNA world ("genetic takeover"), given the potential for heterogeneous backbone sequences to be a part of this transition. Some of the heterogeneous sequences themselves could have played a constructive role; it has been proposed that weak base-pairing of polymer strands may have facilitated the strand separation necessary for replication.[18] Depending on the percent incorporation of DNA there could still be an A-form of the heterogeneous sequence that could be conducive to template mediated oligomerization.[40,41] However, the increased destabilization of duplexes above a certain threshold backbone heterogeneity may impede base-pair mediated higher order structure and function leading to "non-inheritable backbones".[18] Chimeric RNA–DNA sequences have been shown to reduce aptamer activity 1000-fold when compared to the parent homogeneous RNA and DNA systems.[18] Similar incorporation of 2′–5′ linkages in RNA has been shown to reduce or even abolish base-pairing[42] and also reduce aptamer activity[43] when compared to homogeneous parent systems. Such heterogeneous backbone induced instability is also true for other systems such as backbones with mixed chirality.[44] All of these results, indicate that a) homogeneous backbones form duplexes that are stable and form catalysts that are functionally superior when compared to the heterogeneous backbone systems, and b) a limited amount of heterogeneous backbones could have played a role in the emergence of homogeneous systems[19,43] by selective pressures that would have been present to move the system from an initially heterogeneous backbone to a homogeneous one.

Apart from base-pairing properties, resistance to hydrolytic degradation is another consideration for oligonucleotides.[45] Base-paired duplexes and tertiary structures are known to be more stable to hydrolytic degradation when compared to the corresponding single-strand oligonucleotides.[46–48] The thermally stable duplexes (composed of homogeneous and limited chimeric sequences) would persist, while the heterogeneous single strands hydrolyze back to their monomeric constituents to be recycled by the non-enzymatic oligomerization process to form oligomers of various compositions. This repetitive process could lead to a gradual stockpiling of the more stable homogeneous backbone (RNA and DNA) duplex systems due to this preferential hydrolysis of the (heterogeneous) single strands. While the rate of deoxyribonucleotide incorporations could be low (lesser nucleophilicity of the 3′-OH) compared to ribonucleotides (higher nucleophilicity of 2′,3′-OH) thus limiting heterogeneity of DNA in RNA, this is countered by the expected accumulation of DNA in a sequence from the higher rates of hydrolysis of RNA over DNA.[11]

The consistent trends observed in the four sets of RNA/DNA duplex systems opens up the possibility that homogeneous backbone systems (RNA and DNA) may have been a natural outcome starting from a heterogeneous prebiotic scenario,[14–16] a scenario that can be extended to the emergence of homochiral backbones as well.[44,49] Instead of starting from a homogeneous/chiral RNA world, there could have been a heterogeneous/chiral mixture of RNA and DNA that could have led to the accumulation of the thermally and thermodynamically more stable homochiral homogeneous RNA and DNA sequences/duplexes/structures capable of fulfilling the informational and catalytic roles[50] necessary for Darwinian co-evolution (Scheme 2), avoiding the "prebiological necessity" for RNA to invent catalysts to give rise to DNA and the subsequent genetic takeover.[14] Such a heterogeneous-to-homogeneous scenario[19,45,51] would imply a co-



Scheme 2. Heterogeneity-to-homogeneity model: homogeneous backbone RNA and DNA systems could accumulate and emerge from a heterogeneous pool of compounds and intermediates based on increasing thermal and thermodynamic duplex stability. The model can be extended to include other heterogeneity such as configuration (α, β), chirality (D, L) and other sugars (e.g. pentoses).

formation, co-existence and co-evolution of RNA and DNA, likely to be aided by the presence of other classes of molecules such as proto-peptides and proto-lipids.[52–54]

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