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The Role of Sugar-backbone Heterogeneity and Chimeras in the Simultaneous Emergence of RNA and DNA

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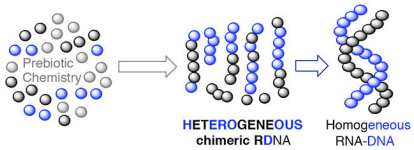
Abstract

Hypotheses of the origins of RNA and DNA are generally centered on the prebiotic synthesis of a pristine system (pre-RNA or RNA), which gives rise to its descendent. However, a lack of specificity in the synthesis of genetic polymers would likely result in chimeric sequences; the roles and fate of such sequences are unknown. Here we show that chimeras, exemplified by mixed TNA-RNA and RNA-DNA oligonucleotides, preferentially bind to, and act as templates for, homogeneous TNA-, RNA- and DNA-ligands. The chimeric-templates can act as a catalyst, mediating the ligation of oligomers to give homogenous-backbone sequences, and the regeneration of the chimeric templates potentiates a scenario for possible cross-catalytic cycle with amplification. This process provides a proof-of-principle demonstration of a heterogeneity-to-homogeneity scenario while giving credence to the idea that DNA could appear concurrently with RNA instead of being its later descendent.

Graphical Abstract



The RNA World hypothesis proposes the emergence of self-replicating and catalytic RNA giving rise later to proteins and DNA (Fig. 1b, middle).[1,2] Models posit the existence of a genetic polymer – whether RNA or its precursor – with a homogeneous backbone that

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Author Contributions
R.K. conceived the project. R.K. and S.B. designed the experiments. S.B. performed all the experiments. R.K. wrote the paper with inputs from S.B. All authors discussed the results and commented on the manuscript.

Data Availability Statement
Full experimental details and data are provided in the Supplementary Information. The raw data that support the findings of this study are available from the corresponding author upon reasonable request. Supplementary information and chemical compound information are available in the online version of the paper. Reprints and permissions information is available online at www.nature.com/reprints.

Competing interests
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Supplementary Information
Supplementary Methods, Supplementary Figures, Supplementary Tables and Supplementary References.

transitions to its homogenous-backbone successor[1,3–10] This transition is proposed to occur despite the difficulties[2,11–14] associated with generation of the pristine oligomers using prebiotic chemistry,[15,16] and the challenge of replacing one genetic polymer with another[2,17–21] in the absence of any sophisticated discrimination mechanism during the transition in a pre-biological world.[13,22] However, there is a growing realization[23–25] that most prebiotic pathways[26,27] would lead to nucleic acid oligomers consisting of mixed backbone units.[14,17,19,28] In this context, RNA containing a mixture of 2′,5′-and 3′,5′-linkages,[29,18,19] and chimeric RNA-DNA systems,[17,21] have been investigated (and it has been shown that these types of backbone-heterogeneity compromise aptamer function[17–19]), and we have shown that RNA-DNA chimeras consistently form weaker duplexes.[14] Though chimeric RNA-DNA genomes are known in extant biology[30] and such chimeras containing nonheritable-backbone-heterogeneity have been postulated to be useful in the emergence of functional nucleic acids,[17,19] questions have been raised about their role as enhanced-templates for replication[17,31] generating polymers with homogeneous-backbones[14]. For pre-RNA to RNA transitions, Orgel has speculated two extreme possibilities using TNA (Fig. 1a)[32] as an example: (a) an all TNA-organism converting to all RNA-organism, and (b) a gradual replacement of TNA residues by RNA residues within the oligomeric system.[33] The second scenario leads to a continuous-pathway from TNA to RNA, via chimeric sequences.[33] We have proposed a heterogeneity-to-homogeneity scenario[34] for the emergence of RNA and DNA[13,14], and argued that based on certain criteria such as the stability and functional advantages inherent to homogeneous-backbone-polymers, their emergence would be a natural consequence even when starting from a mixture of its constituent building blocks (Fig. 1b, top and bottom).[13] A demonstration that chimeric TNA-RNA sequences (TRNA, Fig. 1b, top) or RNA-DNA sequences (RDNA, Fig. 1b, bottom) can enable the non-enzymatic emergence of homogeneous backbone oligonucleotide (RNA or DNA) starting from mixtures of chimeric sequences would provide support to the heterogeneity-to-homogeneity scenario.[13]

Results

TNA-RNA chimeric sequences function as templates for RNA ligands.

We selected TNA[32] – a Watson-Crick base-pairing system able to cross-pair with RNA[32,35]– as a model pre-RNA polymer,[13] based on the prebiotic availability of the sugars[27,36–39] (Fig. 1a). We investigated TNA-RNA chimeric sequences (TRNA) which exhibited peculiar base-pairing properties even though TNA formed strong and stable duplexes with complementary RNA strands (Supplementary Table 1 & 2).[32] First, in general TRNA formed weaker duplexes compared to the unmodified strands. Second, based on which sugar (threose or ribose) unit contained a purine (A) or pyrimidine (T), TRNA demonstrated unpredictable duplex stabilities (Fig. 2a). Unexpectedly, TRNA non-self-complementary strands which showed weak affinity for each other (Fig. 2a, entry 7), formed stronger duplexes with the corresponding complementary RNA (or TNA) sequences (Fig. 2a, entries 6 and 8), a behavior which was general for sequences containing all four nucleobases (Supplementary Table 3 and Figs. 7–13).

The preferential association of chimeric TRNA sequences with homogeneous RNA (or TNA) sequences (Fig. 2a, entries 6, 8) implied that chimeric-sequences could act selectively as templates for the non-enzymatic ligation of homogeneous-sugar-backbone ligands, and thereby facilitate the emergence of homogeneous-backbone-oligomer (e.g. RNA), starting from a mixture of oligonucleotides. To test this proof-of-concept, we employed the widely used water soluble 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) mediated ligation conditions[40] of homogeneous-RNA ligands templated by TRNA-chimeric- and RNA-templates, and compared it with ligation of the chimeric TRNA ligands (Fig. 2b). The 3′-NH_2 modified TNA-ligand[41] and 3′-NH_2-deoxynucleotide (T^{NH2}) terminated RNA-ligand[42] was used to conduct the ligation-reaction within reasonable time-frame, since the corresponding TNA-3′-OH and RNA-3′-OH residues react very slowly (Supplementary Figs. 14–17). The single phosphoramidate linkage was shown to have no special effect on duplex stability (Supplementary Fig. 12). The reactions were monitored by anion-exchange chromatography (AEC), and products were confirmed by comparison with standards and matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (Supplementary Figs. 18–28). As expected from a previous study[41], the efficiency and the rate of ligation reactions paralleled the affinity (and thermal stability) of the templates for ligands, in the following order: RNA template with TNA ligands ≈ RNA template with RNA ligands ≥ TRNA chimeric template with RNA ligands >> RNA template with TRNA chimeric ligands >>>> TRNA chimeric template with TRNA chimeric ligands (Supplementary Figs. 18–23). Control reactions lacking the template(s) showed no product formation (Supplementary Figs. 25–28). We then examined the ligation behavior of the mixture of all four ligands in the presence of the chimeric TRNA template (Fig. 2c) and observed only the formation and growth of the RNA-product from homogeneous RNA-ligands, with no discernible chimeric-TRNA product from heterogeneous TRNA ligands by AEC (Fig. 2d; Supplementary Fig. 24). However, MALDI-TOF analysis of the reaction of chimeric TRNA ligands with chimeric TRNA template at 24 h did show traces of the chimeric TRNA-product (Supplementary Fig. 18). We have not investigated intensively a parallel scenario for the emergence of homogeneous TNA sequences[43] (due to the investment in synthesizing the various TNA 3′-NH_2-phosphoramidites), though we expect a similar propensity[32] based on the observation that homogeneous-TNA ligands also were preferentially ligated by the chimeric TRNA template (Supplementary Fig. 20).

RNA-DNA chimeric templates ligate complementary RNA and DNA ligands.

The above results inspired us to investigate mixed DNA and RNA chimeric sequences based on (a) our previous studies of RNA-DNA (RDNA) chimeras[14] and the plausible coexistence and coevolution of RNA and DNA in prebiotic scenarios[17,21,28,44], and (b) the ease of commercial/synthetic availability of diverse RDNA chimeric sequences. We studied a series of RDNA chimeric sequences, (Supplementary Table 4), which, again, formed stronger duplexes with complementary homogeneous RNA over corresponding complementary chimeric RDNA (Supplementary Table 5 and Supplementary Figs. 29–35). To test whether the preferential association of RDNA with RNA would also translate to selective ligation of RNA ligands (as seen in the TRNA system), we investigated the ligation behavior of a hexadecamer chimeric RDNA template (C_{T2}, Fig. 3a) with RNA and RDNA ligands containing 3′-NH_2 deoxynucleotide units. Ligation of RNA sequences (R_{L3} and R_{L4}) on the

chimeric RDNA template (C_{T2}) was not only faster than the corresponding ligation of chimeric RDNA ligands (C_{L3} and C_{L4} on C_{T2}; Fig. 3b), but was almost equal to the efficiency of RNA ligands R_{L3} and R_{L4} (or chimeric C_{L3} and C_{L4} ligands; Supplementary Fig. 39) on an RNA template, R_{T2} (Supplementary Figs. 36–46).

The duplex formation in octameric homogeneous and chimeric sequences containing all five canonical nucleosides again showed preferential association of homogeneous-backbone sequences with complementary chimeric templates (Supplementary Table 5). Based on this, we investigated the ligation reaction mediated by the chimeric template (C_{T4}) with RNA and chimeric ligands shown in Fig. 3c. The results revealed a temperature-dependent ligation behavior that was not observed in the hexadecameric-AU system (Supplementary Figs. 50–52). While at lower temperatures (4°C) there was little difference between the rate of ligation between the two systems, the rate of ligation of chimeric ligands and the amounts of products formed at higher temperature (10 and 16°C) differed considerably with preference for the ligation product from homogeneous-ligands on the chimeric template (Fig. 3d and Supplementary Fig. 52). This indicates that temperature could also control and modulate the overall dynamics and distribution of the end-products.

The trend of preferential association correlating with ligation capacity of C_{T2} also extended to DNA ligands (D_{L1}, D_{L2}), in place of RNA ligands, giving rise to the homogeneous DNA product D_{P1} (Fig. 3b), and was valid even when starting from a pool of mixed R_{L3}+R_{L4}+C_{L3}+C_{L4} ligands or D_{L1}+D_{L2}+C_{L3}+C_{L4} ligands (Fig. 3b, Supplementary Figs. 47–49). When all ligands (R_{L3}, R_{L4}, D_{L1}, D_{L2}, C_{L3} and C_{L4}) were added to chimeric RDNA template C_{T2} in a single pot, three major ligation products, R_{P2} (38%), D_{P1} (20%) and an RNA-DNA cross ligation product (RD_{P1}, 75%) were formed at 24h; no chimeric product from C_{L3}+C_{L4} was detected (Supplementary Figs. 53–54). The nature of cross-ligation product was confirmed with appropriate control experiments and shown to be the result of D_{L1}-R_{L4} ligation (Supplementary Figs. 55–59). Replacing the chimeric template with RNA template, under otherwise identical conditions, gave R_{P2} (65%), D_{P1} (12%) and 62% of RD_{P1} and RD_{P2} (R_{L3}-D_{L2}), indicating that RNA template also gives rise to significant cross-ligation products (Supplementary Figs. 60–61). Changing the ratios of RNA ligand (R_{L3}+R_{L4}) to DNA ligands (D_{L1}+D_{L2}) affected the product distribution (Supplementary Fig. 59) implying that generation of chimeric oligomers (along with homogeneous backbone oligomeric products) have to be reckoned with; these chimeric oligomer products should, in turn, help in the formation of homogeneous RNA and DNA ligation products. While this hypothesis is reinforced by the results in Fig. 3, it was demonstrated to be so by isolating RD_{P1} and using it as a template with RNA ligands producing R_{P3} efficiently in 108% yield (Supplementary Fig. 62). The above results show that from a mixed system with two different oligonucleotides (e.g. RDNA) there is indeed the possibility of the *simultaneous emergence* of the two respective homogeneous-nucleotide polymers (e.g. RNA and DNA).

RNA-DNA chimeric-templates are better in overcoming template-product inhibition.

The above observations suggest that chimeric-templates could provide a solution to the problem of product inhibition (Fig. 4a), where the continuous production of the product is curtailed due to the strong association of the initially formed template-product complex.

[45–48] For instance, RNA-ligands R_{L3} and R_{L4} in the presence of the R_{P2}-R_{P3} RNA duplex under the EDC-activation conditions showed no production of R_{P2} even after 24 hours, indicative of classic product inhibition behavior; but the addition of chimeric template C_{T2} led to the formation of more R_{P2} within a matter of few hours (Supplementary Figs. 67–68). As outlined in Fig. 4b, if there was the adventitious presence/formation of a complementary RNA partner (R_{P3}, from its corresponding ligands R_{L5} and R_{L6}) in the mixture containing the chimeric duplex (R_{P2}-C_{T2}), it would induce the formation of the stronger RNA (R_{P2}-R_{P3}) duplex. This should release the original chimeric RDNA template for another round of ligation of R_{L3} and R_{L4} forming more of R_{P2}, and result in a continuous accumulation of duplex R_{P2}-R_{P3}, with the chimeric template C_{T2} taking the role of a catalyst producing more of R_{P2} from its respective ligands. To test this scenario, we first conducted a step-wise addition of RNA ligands R_{L3} and R_{L4} to RDNA chimeric template C_{T2}, leading to the formation of the product R_{P2} (97% in 20 h, Fig. 4c). Then, ligands R_{L5} and R_{L6} were added to this mixture. The formation of the second ligation product R_{P3} (21% in 1 h increasing to 77% in 24 h, Fig. 4c), indicated that the *in situ* generated first ligation product R_{P2} was indeed acting as a template (Supplementary Figs. 64–66). More encouragingly, with higher ligand ratios of R_{L3} and R_{L4}, an increased amount of the first ligation product R_{P2} (251% with respect to C_{T2}) and of the second ligation product R_{P3} (204%) was observed after 24 hours (Fig. 4c, Supplementary Fig. 65 and Table). This indicates that the chimeric template C_{T2} was indeed being released to take part in a turn-over, which in turn leads to formation of more R_{P2}. Pertinent control experiments confirmed the need for all components to be present for this system to operate; importantly, C_{T2} itself did not serve as a template to ligate R_{L5} and R_{L6} and did not produce R_{P3} (Supplementary Figs. 45, 66). Encouraged by these results, we set up a one-pot experiment where all components, C_{T2}+R_{L3}+R_{L4}+R_{L5}+R_{L6}, were mixed from the beginning and observed the concomitant production of the two RNA ligation products R_{P2} and R_{P3} (as efficiently as the step-wise addition experiment) (Fig. 4c). The presence of chimeric template C_{T2} in a mixed-one-pot-system not only initiated the ligation process, but also acted as a turn-over intermediary down-stream, potentially enabling continuous production of R_{P2} and R_{P3} by mitigating the inhibition by template-product complex. This process was mainly driven by the preference of a thermodynamically stable homogeneous-backbone duplex R_{P2}:R_{P3}. Control reactions for R_{L3}+R_{L4} or R_{L5}+R_{L6} ligands without C_{T2} template showed no observable background ligation reactions. However, when all four ligands (absent C_{T2}) were mixed together, 33% R_{P2}, 20% R_{P3} and 13% cross-ligation products (probably from R_{L3}+R_{L6} and/or R_{L5}+R_{L4}) were formed but more slowly at 24 h (Supplementary Fig. 70), as opposed to 259% of R_{P2} and 191% of R_{P3} with no cross-ligation products in the presence of chimeric template C_{T2} (Supplementary Fig. 69). The background ligation-reactions were eliminated when ligand concentrations were lowered from 200 μM to 20 μM each; and only in the presence of 10 μM chimeric template C_{T2}, the formation R_{P2} (83%) and R_{P3} (18%) in 24 h was observed (Supplementary Figs. 73–76). Furthermore, we tested whether the presence of the complementary ligands (C_{L3}+C_{L4}, Fig. 3a) leading to the C_{T2}-C_{P2} duplex would prevent further copying of the first two RNA ligands R_{L4}+R_{L3} and also impact the next round of copying when all four RNA ligands R_{L4}+R_{L3}+R_{L5}+R_{L6} are present. In both cases, in 24 h at 4°C, corresponding RNA products formed in good yields; 92% of R_{P2} (with 30% of C_{P2}) for the first experiment and in the

second scenario, 83% R_{P2} and 16% R_{P3}, with no discernible peak for C_{P2} in the chromatogram trace (Supplementary Figs. 47 and 77).

In order to assess the efficiency of chimeric template C_{T2} versus that of the corresponding homogeneous backbone RNA counterpart R_{T2}, the all-in-one-pot reaction was repeated but with RNA template R_{T2} in place of C_{T2}. In this case, as expected, the production of R_{P2} at 48 h was comparable (99% for R_{T2} versus 109% for C_{T2}); however, R_{P3} formation dropped by almost half to 18% (for R_{T2}) when compared to 30% (for C_{T2}) indicating template-product inhibition by the stronger R_{T2}:R_{P2} complex meant that R_{P2} was less available for ligating R_{L5}+R_{L6} (Fig. 4d). The advantage of C_{T2} over R_{T2} was more apparent when the ratio of ligands was changed to 5(R_{L3}+R_{L4}):2(R_{L5}+R_{L6}) with C_{T2} producing 178% of R_{P2} and 77% of R_{P3} when compared to 119% of R_{P2} and 43% of R_{P3} with RNA template, R_{T2} (Fig. 4d). This strongly suggests that C_{T2} is better able to dissociate from the C_{T2}:R_{P2} template-product complex while the RNA template R_{T2} is limited by the classic R_{T2}:R_{P2} template-product inhibition and is, therefore, unable to recycle to produce more R_{P2} and R_{P3}. In fact, C_{T2} consistently outperformed R_{T2} in the production of R_{P3} for all other combinations of ligand ratios (Fig 4d, Supplementary Fig. 78), indicative of the beneficial role played by chimeric templates in moving towards the emergence of homogeneous-backbone sequences. But for this to be possible, this phenomenon must hold good for other strands in terms of length and sequence diversity. Given the limitations imposed by the EDC-ligation-chemistries and analysis of the chimeric sequences involved, we set up a proof-of-principle experiment as in Fig. 4b but with octameric AUGC containing chimeric template C_{T4} (Supplementary Fig. 79), since it also showed a preference for the complementary homogeneous ligands over the chimeric counterparts as seen in Fig. 3d. As expected, the chimeric template C_{T4} was efficient in producing the homogeneous product R_{P4} and R_{P5} (Supplementary Fig. 80) overcoming the template-product inhibition even in the presence of all four ligands (R_{L7}+R_{L8}+R_{L9}+R_{L10}), paralleling the observations for the AU-based system. Thus, the ability of the chimeric template to give rise to homogeneous backbones (the heterogeneity-to-homogeneity paradigm) seems to be still operative in this RDNA-chimeric system even when shortening the length of the template and expanding the sequence diversity.

We then examined the effect of step-wise dilution (as a selection pressure) on the efficiency of the templates in overcoming the template-product inhibition, asking the question – which of the templates, chimeric-RDNA or the homogeneous-RNA would produce the ligation products more efficiently as the step-wise dilution was continued? Using the AU-system outlined in Fig. 4a we conducted a step-wise dilution experiment in parallel with templates C_{T2} and R_{T2} containing the complementary RNA-ligands (R_{L3}, R_{L4}, R_{L5} and R_{L6}) where, every 24 h a portion of the reaction mixture was removed and fresh ligands and EDC were added, such that the concentrations of the ligands remained constant, but the template concentration decreased with each dilution-step (Supplementary Figs. 91–93). As seen from Fig. 5a, as the step-wise dilution was implemented at 24 h intervals, the formation of R_{P2} and R_{P3} was observed in both cases; while there was a concomitant drop in the product concentration (by 2 μM) at each dilution step, the amount of R_{P2} and R_{P3} increased to level greater than the previous value with progress of time. The amount of the first ligation product R_{P2} was almost the same between the chimeric-(C_{T2}) and homogeneous-(R_{T2})

template containing vials over the first two-steps (48 h) of dilution, with C_{T2} performing slightly better than R_{T2} as the dilution steps were continued (72–96 h, Fig. 5b). However, there was a remarkable difference in the production of the second ligation product R_{P3} with increasing step-wise dilutions; the chimeric template C_{T2} outperformed the homogeneous template R_{T2} in producing R_{P3} by ca 250% (Fig. 5c), even as the concentration of the templates were going down with each step of dilution. A comparison of the chromatogram traces at 96 h (Fig. 5d) shows the dramatic difference and highlights the ability of C_{T2} to be a superior template[17] for the production of homogeneous product R_{P3}, demonstrating the ability of chimeric template C_{T2} to better bypass the template-product inhibition and turn-over even under dilute conditions when compared to R_{T2}. Appropriate controls without the template showed no product formation (Fig. 5d).

RNA-DNA chimeric templates harbor the potential for cross-catalytic self-replication.

The promise of turnover of RNA ligation (Fig. 4b) when coupled with the observation that RDNA (C_{P2}) chimeric products can also be formed on the RNA template (Supplementary Fig. 39) suggested that the catalytic chimeric template (C_{T2}) could also be regenerated in the same reaction mixture if the corresponding chimeric ligands (C_{L7}+C_{L8}) are present (Fig. 6). If this would be possible, then the regeneration of the catalytic template C_{T2} could allow for a cross-catalytic cycle to be operative, which would be expected to lead to the amplification of the homogeneous RNA product R_{P2} (Fig. 6b). To test this possibility, we set up a one-pot EDC-ligation reaction with RNA ligands R_{L3}+R_{L4} along with chimeric ligands C_{L7}+C_{L8} in the presence of chimeric template C_{T2} (Fig. 6). We observed within 1–4 h the formation of expected product R_{P2} (90%), which now can act as the template for the chimeric ligands C_{L7}+C_{L8}. Indeed, by 24 h, formation of the phosphoramide-linked equivalent of C_{T2} (C_{T2}^{NH}, 16%) was clearly observed, and kept increasing with time to 36% in 48 h and to 48% in 72 h. And, in parallel, the amount of R_{P2} increased accordingly to 125% in 24 h, to 148% in 48 h and to 160% in 72h (Supplementary Fig. 94). This is well above the levels of R_{P2} produced in the ligation reaction mediated by C_{T2} in the presence of only R_{L4}+R_{L3} and lacking the chimeric ligands (Fig. 6c), where the amount of R_{P2} leveled at around 108% by 72 h. Thus, the chimeric template mediated ligation process shows potential for cross-catalytic self-replicating systems that can result in amplification of the down-stream product. Further systematic investigations are ongoing to understand the scope and limitation of this system. In all of the experiments described in this work no discernible degradation of the homogeneous or chimeric templates or products was observed (confirmed by comparing with an external standard of oligonucleotide dT_{24} added to the samples just before analysis).

Discussion

The results described in this work have confirmed experimentally the beneficial roles of chimeric sequences (backbone-heterogeneity) in nucleic acid replication, augmenting the evolution of functionality in mosaic nucleic acids[17]; and suggest that the nucleobase sequence-information encoded in heterogeneous-backbones can indeed be heritable for chemical evolution (similar to homogeneous-backbone systems). In these chimeric systems, there is the added advantage of (a) by-passing the template-product inhibition problem commonly encountered in the non-enzymatic replication of nucleic acids (unlike the

homogeneous-backbone systems), and (b) moving towards (cross-catalytic) self-replication of the chimeric-templates, that eventually are able to assist in the transition from heterogeneity-to-homogeneity in nucleic acid systems[13,14]. Whether the preference for homogeneous-backbone ligands by chimeric templates (dictated by the thermodynamic stability of duplex formation) could be a general phenomenon for oligonucleotides composed of other different sugar-backbones/nucleobases that are able to cross-pair needs further examples (such as chimeras of 2′,5′-RNA with 3′,5′-RNA)[19,49,50] to validate its scope and limitations.

For the work described here, however, there are some issues still to be addressed: firstly, the use of EDC-mediated-ligation combined with 3′-NH_2 modified deoxynucleotide in this proof-of-principle study is not considered to be plausibly prebiotic. To this end, we are exploring the use of other prebiotically plausible phosphorylation-activation combined with oligomerization and ligation/recombination chemistries that may be compatible with the replication conditions.[51–54] We briefly explored the use of enzymes (T4 DNA ligase and T4 RNA ligase 2) with canonical RNA, DNA and RDNA chimeric sequences, to check if ligases could be used to overcome the limitations of (a) the side reactions with chemical (EDC) activation[55] and (b) the need for synthesizing sequences with the 3′-deoxy-NH_2 modification – so that we may be able to push towards many rounds of replication and sequence analysis within a shorter time span, but have had limited success (Supplementary Figs. 95–103). We are exploring other ligases to expand the sequence-space and length parameters to overcome restrictions imposed by the EDC chemical-ligation methods.[55, 56]

Secondly, longer homogeneous products formed in the scenario described above are unlikely to work as continuous templates and may not provide the solution when moving towards sustained replication of longer homogeneous strands relying on thermodynamic-driven effects alone. One possible solution (alluded to in this work) is that chimeric-templates can facilitate indirect replication by catalysing the accumulation of homogeneous strands. The product homogenous strands can act as information storage, but cannot be directly replicated. Therefore, other mechanisms need to be invoked to allow the transfer of information stored in the homogeneous strands.[56] One straightforward pathway consistent with the above heterogeneity-to-homogeneity scenario would be for the homogeneous RNA strands to give rise to functional ribozymes (ligase or polymerase) with the capability to take over the replication the homogeneous strands.[57] Other pathways could involve the beneficial effects provided by different classes of molecules that have not been considered in this study. For example, two other components, primordial (depsi)peptides[58] and protocells[59] should be invoked, since they would have been an important part of any prebiotic scenario; they are as elementary as, if not more than, the nucleotide building blocks.[53,60] Including them would be the next logical step to test the idea whether they could have not only aided in the transition from heterogeneity-to-homogeneity,[34] but could also play a role in enabling the replication of information stored in the longer homogeneous RNA and DNA strands by overcoming the slower kinetics of strand exchange in replication of homogeneous RNA and DNA strands as strand lengths increase.[61,62]

Finally, in a prebiotic context, the possibility of oligomerizing on chimeric templates starting with monomeric building blocks has to be considered along-side the ligation chemistry

demonstrated in this study.[8] In our work, we were influenced by the duplex stabilities and reasoned that (a) the selectivity expressed at the ligand-template level may not translate to the level of weaker monomer-template associations and (b) based on earlier studies,[8,63] oligomerization of monomers would be biased towards G and C containing sequences (due to their stronger association) over A and U residues. Also, as argued by others,[64,65] the presence of dimers and trimers along with monomers in a prebiotic clutter may lead to selective incorporation of the higher order oligomers (dimers and trimers) over the monomers and, therefore, the ligation process may have an advantage over the oligomerization process. It would be necessary to test the limits of oligomerization with monomers in a chimeric scenario to observe what the preference is, both in terms of effect of the sugar and base residue (based on the nearest-neighboring nucleotide).[49,50]

The results reported in this study have two-fold implications for the emergence of homogeneous-backbone nucleic acids. First, starting from a mixture of binary-chimeric-systems e.g. RDNA, (a possibility that is strengthened by the recent report[44] by Sutherland and co-workers on the plausibly prebiotic conversion of RNA nucleotides to DNA nucleos(t)ides), there is the potential for the simultaneous emergence of the two respective homogeneous-polymeric and communicating informational systems (RNA and DNA). This is opposed to the often-suggested sequential –RNA as the forerunner and DNA being the successor–paradigm. The successive replication cycles[42,66,67] are expected to lead, simultaneously, to the two respective strands containing the homogeneous sugar-backbone (RNA and DNA), as indicated by the results in Figs. 3–6. Therefore, if RNA and DNA could have appeared together, then there is no need for genetic takeover by the new informational system (DNA) from an older system (RNA), a suggestion that has been made implicitly and explicitly by others,[14,17,23–25,28,68,69] since there is neither a predecessor nor a successor in this scenario. This is also true for the supposed pre-RNA to RNA transition[33]; for example, there is no need for RNA being the descendent of TNA, when TRNA can simultaneously give rise to TNA and RNA. Second, the generality of this phenomenon–exemplified by RDNA and TRNA chimera systems–lends experimental credence to a point that is implied in Fig. 1, and one that has been discussed before;[13,21,28] namely, a clean and directed prebiotic synthesis of a nucleotide building block of a particular oligonucleotide (e.g. TNA or RNA or DNA) is not an absolute requisite for a homogeneous-backbone nucleic acid like RNA to emerge. In other words, as is suggested in Fig. 1, the appearance of system with homogeneous nucleotide-backbone repeat units can be achieved at the emergent level of a replicating polymer.[34] Therefore, a mixture of diverse nucleotides can, via the formation of mixtures of oligonucleotides and the ensuing emergent property of template-mediated ligation, tend towards homogeneous-nucleotide backbone systems.[13] This process can include alternative linker units and alternative nucleobases,[10,17,19,70] and chirality of the building blocks[71]. Which means, the appearance of a homogeneous-backbone homochiral polymer with a set of uniform building blocks from a prebiotic mixture is a natural outcome of chemical evolution,[14] without the need for invoking the predecessor-successor models of extant biology.[34,68,72]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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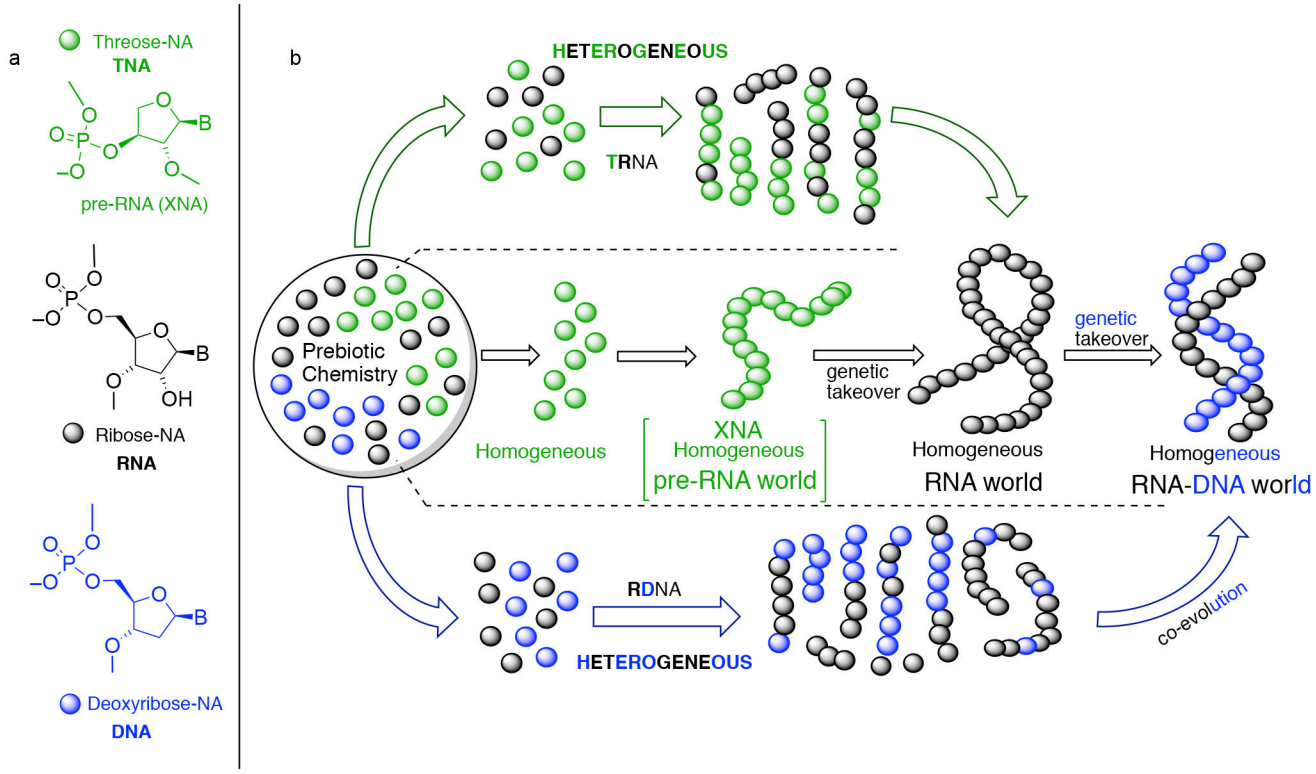
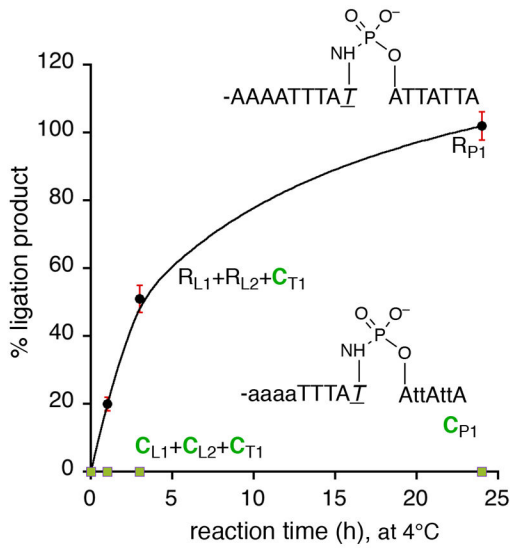


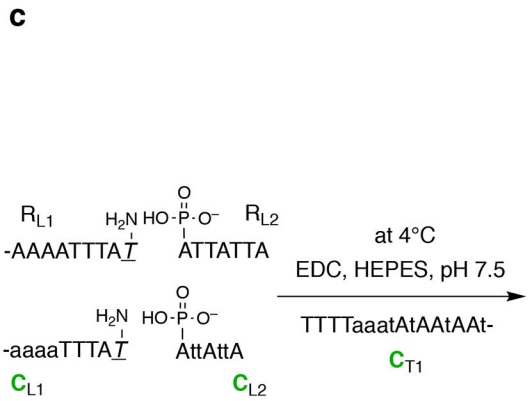
Figure 1. The prebiotic clutter generated heterogeneity-to-homogeneity scenario versus the biology inspired paradigm of replacing one homogeneous genetic system with its homogeneous genetic successor.

(a) Constitutional formula representation of the three oligonucleotide building blocks investigated in this study. (b) Three possible scenarios for the emergence of RNA and DNA from prebiotic chemistry. Middle: the classical RNA world concept where the formation of a pristine and homogeneous RNA (or pre-RNA) leads to its homogeneous-backbone successor DNA (or RNA). Top: a heterogeneous mixture of TNA (pre-RNA) and RNA forming chimeric TRNA sequences that transition to homogenous RNA, which then gives rise to DNA. Bottom: a heterogeneous RNA-DNA mixture progressing/co-evolving via chimeric RDNA sequences directly to homogeneous RNA and DNA simultaneously.

a

	Oligonucleotide	UV-T_m (°C)
1	aTaTaTaTaTaTaTaT	25.8[a]
2	AtAtAtAtAtAtAtAt	50.1[a]
3	aaaaaaaaTTTTTTTT	61.3[a]
4	AAAAAAAAtttttttt	< 5[a]
5	-AAAATTTATATTATTA TTTTAAATATAATAAT-	59.5[b]
6	-AAAATTTATATTATTA TTTTaaatAtAAtAAt-	40.5[b]
7	-aaaaTTTAtAttAttA TTTTaaatAtAAtAAt-	< 20[b,c]
8	-aaaatttatattatta TTTTaaatAtAAtAAt-	38.3[b]
9	-aaaatttatattatta ttttaaatataataat-	56.2[b]





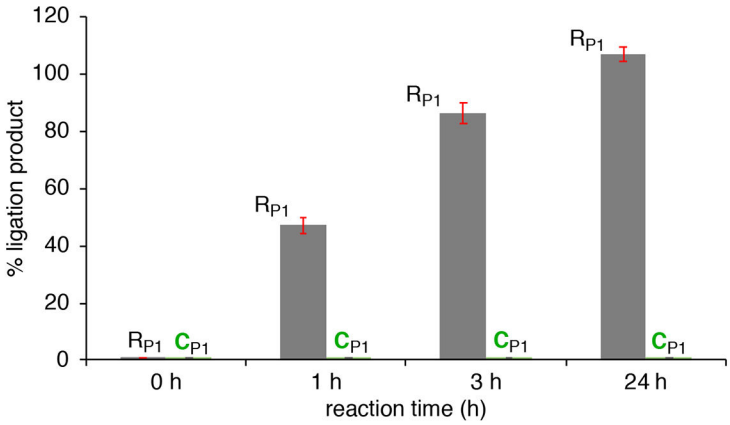


Figure 2. The preferential association with, and ligation of homogeneous ligands by, chimeric TRNA template over chimeric ligands.

(a) Thermal stability of TRNA chimeric duplexes in 1 M NaCl, 10 mM Na_2HPO_4, 100 μM EDTA, pH 7.2; a = [5μM], b = [2μM] duplex concentration; c = Entry 7, no clear sigmoidal transition in UV-thermal melt was observed. (b) Comparison of the rate of ligation reaction at 4°C of homogeneous-RNA (R_{L1}+R_{L2}) and heterogeneous TNA-RNA ligands (C_{L1}+C_{L2}) on heterogeneous TNA-RNA template, C_{T1}. (c) EDC-mediated ligation reaction at 4 °C of mixture of homogeneous-RNA (R_{L1}, R_{L2}) and chimeric TNA-RNA ligands (C_{L1}, C_{L2}) using TRNA chimeric sequence (C_{T1}) as template. (d) comparison of the amounts of products R_{P1} and C_{P1} produced in the reaction mixture in 2c; see supplementary Fig. 24, for conditions. A, T = RNA; $\underline{T}$ = DNA; a, t = TNA. Line in graph (2b) is drawn as guide to indicate the trend and is not a mathematical curve fitting. % yields are calculated with respect to the template C_{T1}. Experiments were run in triplicate and the error range is less than ± 5%; error bars represent standard deviation.

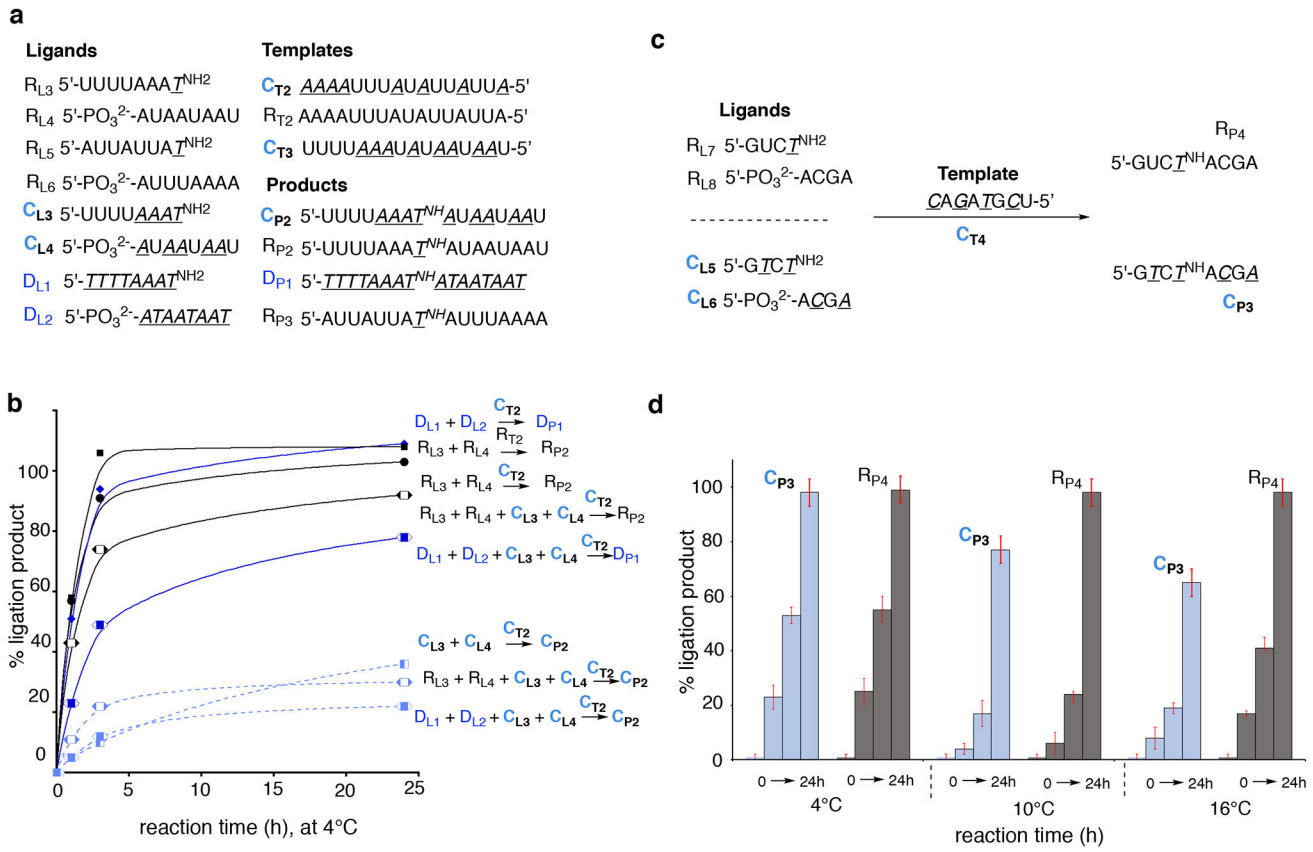


Figure 3. Chimeric RDNA templates preferentially associate and ligate homogeneous RNA and DNA ligands over chimeric ligands.

(a) The list of homogeneous and chimeric sequences used in this study. (b) Comparison of ligation efficiency by hexadecameric (AU)-RDNA template C_{T2}, with RNA (R_{L3}, R_{L4}), DNA (D_{L1}, D_{L2}) and chimeric RDNA (C_{L3}, C_{L4}) ligands, showing the consistent preferential formation of homogeneous ligation products, R_{P2} and D_{P1} over chimeric ligation products C_{P2}. (c and d) Comparison of ligation efficiency by octameric (A, U/T, G, C)-RDNA template C_{T4}, with RNA (R_{L7}, R_{L8}), and chimeric RDNA (C_{L5}, C_{L6}) ligands, showing the influence of temperature on the preferential formation of homogeneous ligation products, R_{P4} over chimeric ligation products C_{P3}. See supplementary figs. 50–52 for EDC-ligation reaction conditions. A, U, G, C = RNA; *A*, *T*, *G*, *C* = DNA. Lines in graph (3a) are drawn as guide indicating the trend and are not mathematical curve fittings. % yields were calculated with respect to the template C_{T2} or R_{T2} or C_{T4} respectively. Experiments were run in triplicate and the error range is less than ± 5%; error bars represent standard deviation.

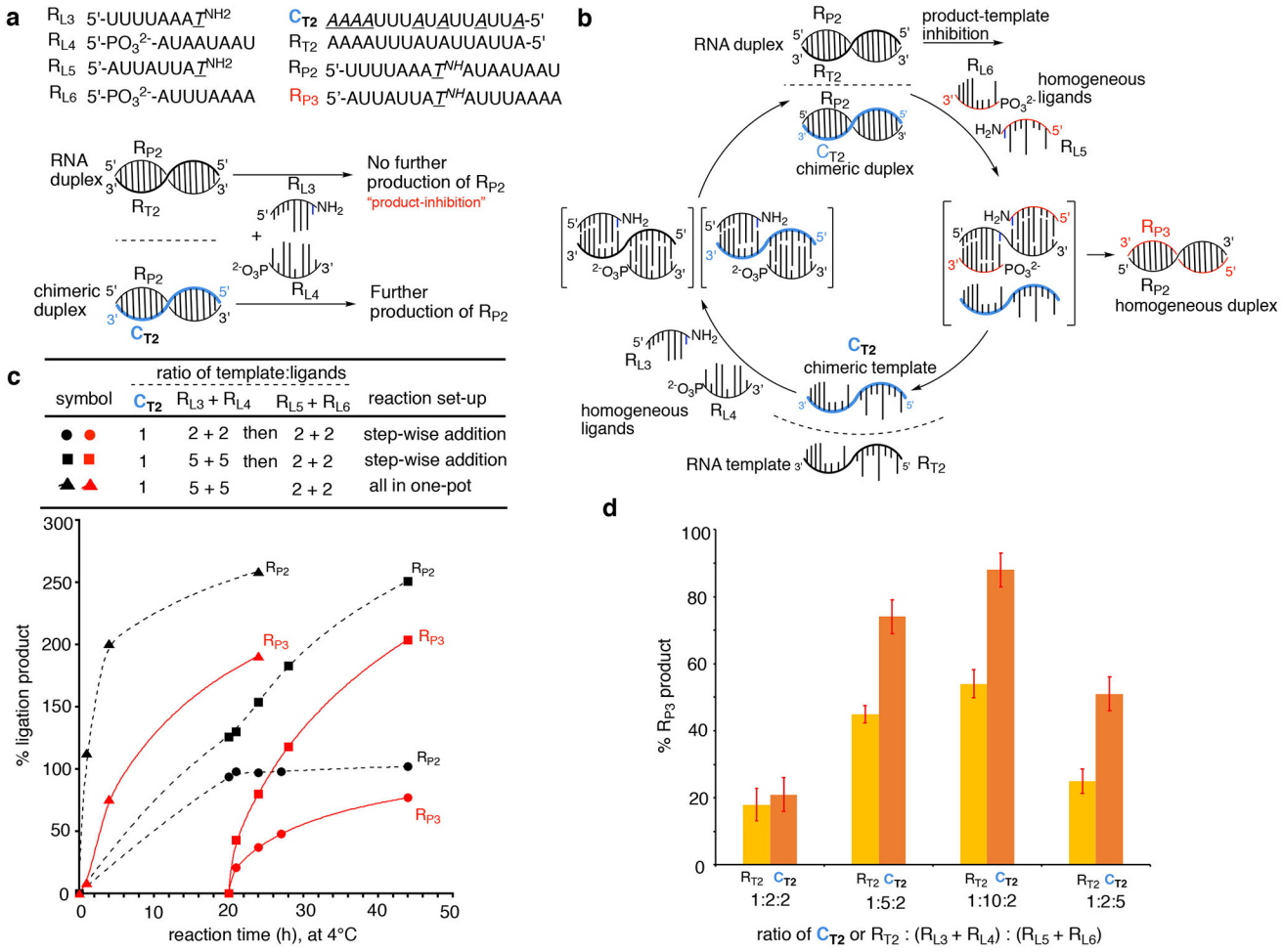


Figure 4. The beneficial role for chimeric RDNA template in overcoming the template-product inhibition based on thermodynamic stability of duplexes.

(a) The expected difference between chimeric RDNA-RNA duplex and the homogeneous RNA-RNA duplex in being able to overcome the template-product inhibition. (b) Schematic representation of the proposal that hexadecameric (AU)-RDNA template C_{T2} with RNA ligands R_{L3}+R_{L4} produces R_{P2}, which in the presence of R_{L5}, R_{L6} is expected to lead to R_{P3}, based on the greater thermodynamic stability of the R_{P2}:R_{P3} duplex over the R_{P2}:C_{T2} duplex, and release the C_{T2} for another round of ligation reaction. (c) Time course of the EDC-mediated-ligation experiments documenting the effect of change in ratio of ligands, and the sequential-addition of ligands R_{L5}+R_{L6} (0 h) followed by R_{L5}+R_{L6} (at 20 h) versus all-in-one-pot reaction on the production of R_{P2} and R_{P3}. (d) Comparison of the amount of R_{P3} formed by the homogeneous RNA template R_{T2} versus chimeric RDNA template C_{T2} (at 48 h) demonstrating the higher efficiency of C_{T2} in mediating the formation of R_{P3} by overcoming the template-product inhibition. See supplementary figs. 63–78 for EDC-ligation conditions. A, U = RNA; *A*, *T* = DNA. Lines in graph (4c) are drawn as guide indicating the trend and are not mathematical curve fittings. % yields were calculated with respect to the template C_{T2} or R_{T2} respectively. Experiments were run in triplicate and the error range is less than ± 5%; error bars represent standard deviation.

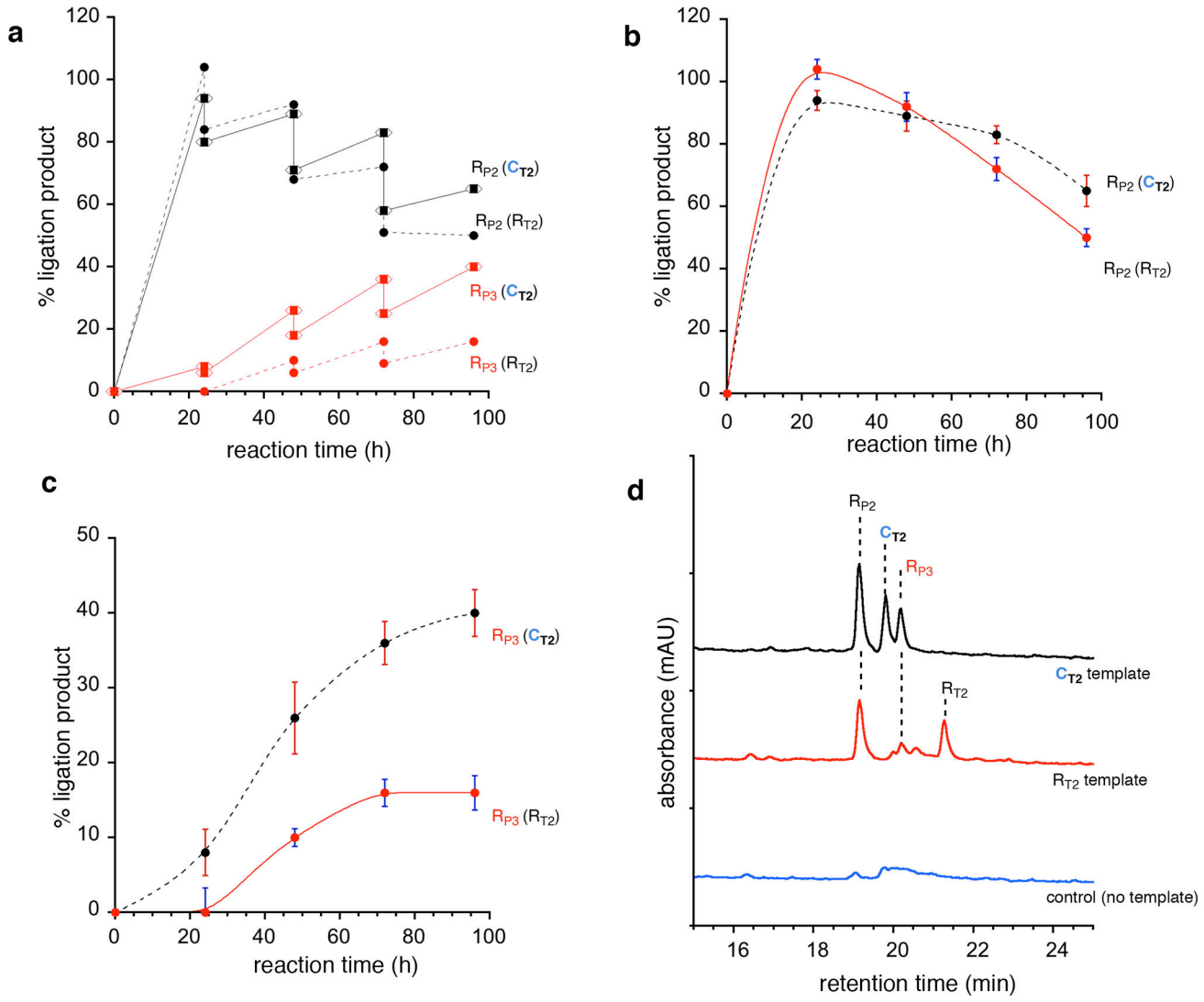


Figure 5. Comparison of the efficiency between chimeric RDNA (C_{T2}) and RNA (R_{T2}) templates in producing the final ligation product R_{P3} under step-wise dilution conditions, demonstrating the superior ability of C_{T2} to act as a template for ligation with turn over.

(a) Production of the ligation products R_{P2} and R_{P3} in the stepwise-dilution (in 24 h intervals) experiment with templates C_{T2} and R_{T2}, over a period of 96 h, containing all four ligands R_{L3}, R_{L4}, R_{L5} and R_{L6}; the drops at 24, 48, and 72 h indicate the dilution step. (b) Time course contrast between the templates C_{T2} and R_{T2} for the production of the first ligation product R_{P2} formed from R_{L3} and R_{L4}. (c) Comparison of the efficiency of production of the second ligation product R_{P3} (from R_{L5} and R_{L6}) between the templates C_{T2} and R_{T2}. (d) Chromatogram traces at 96 h after three stepwise-dilution juxtaposing the three parallel experiments in the presence of C_{T2} (top trace), R_{T2} (middle trace) and containing no template (bottom trace). See supplementary figs. 91–93 for EDC-ligation conditions (at 4 °C). For C_{T2}, R_{T2}, R_{P2}, R_{P3}, R_{L3}, R_{L4}, R_{L5} and R_{L6} see Fig. 4a. Lines in graphs (5a-c) are drawn as guide indicating the trend and are not mathematical curve fittings. % yields were calculated with respect to the template C_{T2}. Experiments were run in triplicate and the error range is less than ± 5%; error bars represent standard deviation.

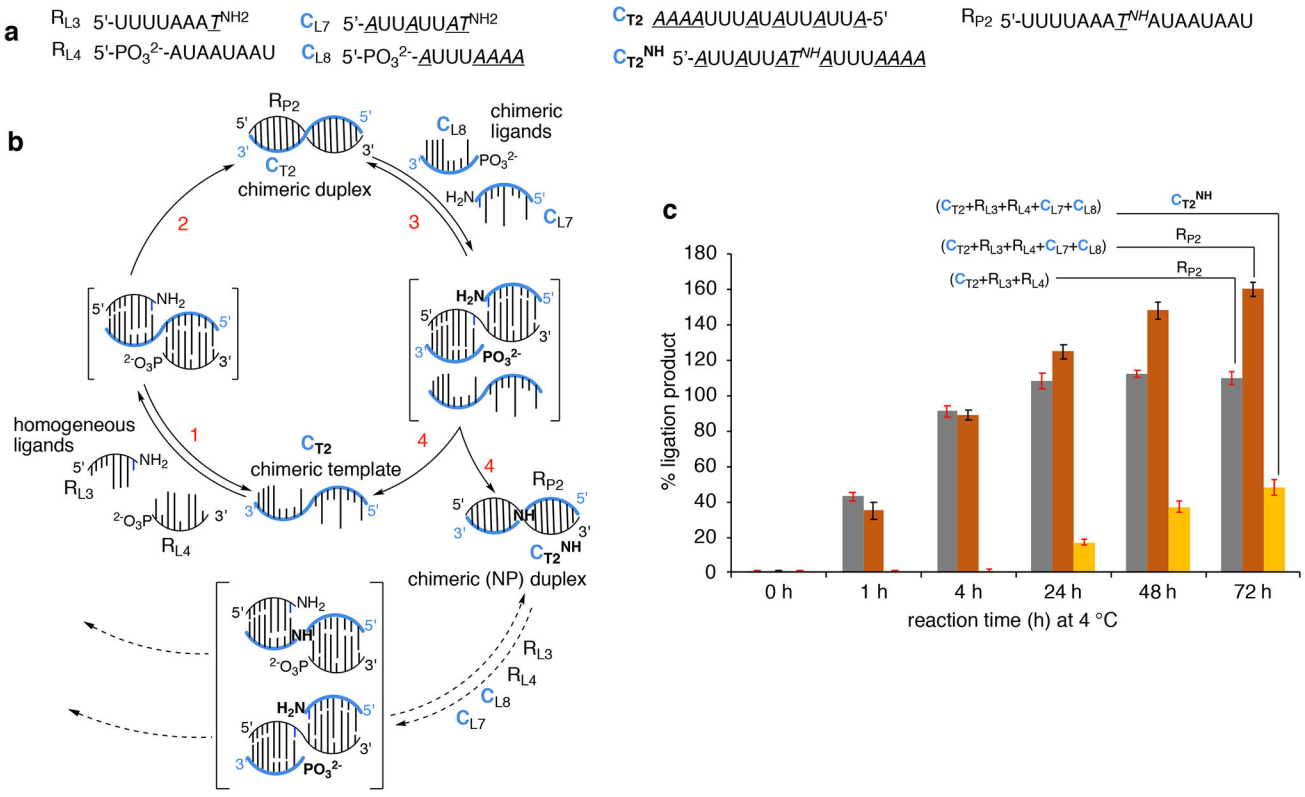


Figure 6. Experiment for testing the possibility of cross-catalytic amplification in oligonucleotide replication via regeneration of the chimeric RDNA (C_{T2}) template.

(a) The sequences of oligonucleotides used in this investigation; C_{T2}^{NH} is the same as chimeric template C_{T2} but with a single phosphoramidate (NP) link at the ligation junction. (b) Schematic representation of the hypothesis that the presence of chimeric ligands C_{L7} and C_{L8} (complementary to R_{P2}) could induce the regeneration of the chimeric template C_{T2}^{NH} leading to further production of R_{P2}. The concomitant release of C_{T2} also creates the potential for another round of ligation reaction. (c) Comparison of the amount of R_{P2} produced from the combination of C_{T2}+R_{L3}+R_{L4} (1:5:5) versus the combination of C_{T2}+R_{L3}+R_{L4}+C_{L7}+C_{L8} (1:5:5:2:2), demonstrating the regeneration of chimeric template C_{T2}^{NH} along with higher and increasing production of R_{P2} in the latter combination. See supplementary Fig. 94 for experimental conditions. % yields were calculated with respect to the template C_{T2}. Experiments were run in duplicate and the error range is less than ± 5%; error bars represent standard deviation.