Mutually stabilizing interactions between proto-peptides and RNA

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The close synergy between peptides and nucleic acids in current biology is suggestive of a functional co-evolution between the two polymers. Here we show that cationic proto-peptides (depsipeptides and polyesters), either produced as mixtures from plausibly prebiotic dry-down reactions or synthetically prepared in pure form, can engage in direct interactions with RNA resulting in mutual stabilization. Cationic proto-peptides significantly increase the thermal stability of folded RNA structures. In turn, RNA increases the lifetime of a depsipeptide by >30-fold. Proto-peptides containing the proteinaceous amino acids Lys, Arg, or His adjacent to backbone ester bonds generally promote RNA duplex thermal stability to a greater magnitude than do analogous sequences containing non-proteinaceous residues. Our findings support a model in which tightly-intertwined biological dependencies of RNA and protein reflect a long co-evolutionary history that began with rudimentary, mutually-stabilizing interactions at early stages of polypeptide and nucleic acid co-existence.
C ooperative relationships between different classes of biomolecules are a hallmark of biology. RNA makes protein in the ribosome and protein makes RNA in polymerases. Rudimentary forerunners of such interactions must have influenced prebiotic chemical evolution. Interactions between diverse classes of molecules might have attenuated rates of degradation, promoted folding or solubility, supported ligand binding, or promoted catalysis. Cooperative interactions between molecules also could have expanded the possible mechanisms for the buildup and persistence of certain molecules in a prebiotic environment. For example, in an environment of periodic, random (non-coded) oligomer synthesis via condensation processes fueled by condensing agents or wet–dry cycles, intermolecular interactions that imparted reduced rates of hydrolysis for one or more of the oligomers involved could naturally lead to a buildup of those sequences. Understanding the nature and genesis of productive molecular interactions among prebiotic molecules is a central issue in exploring the origin of life.

A prevailing idea in origins-of-life research is that there was once an RNA World in which RNA served dual roles as genetic polymer and catalyst. Over time the pure RNA World incrementally gave rise to proteins and DNA on the path to the current RNA/DNA/protein biological system. An alternative theory is that the evolution of nucleic acids and polypeptides was intimately connected from the beginning (i.e., a Ribonucleo- or proteinoid World). In either scenario, it appears that productive relationships between nucleic acids and peptides (or between proto-nucleic acids and proto-peptides) would be required during maturation of the extant RNA/DNA/protein system. A few studies have investigated how peptides could promote folding or functions of RNA, or vice versa, but there are few examples of interactions that mutually benefit both partners. Given the importance that molecular cooperation may have played in the origins of peptides and RNA, and recent descriptions of prebiotic pathways by which amino acids, peptides, and nucleotides can simultaneously be produced, we aimed to evaluate the possibility of mutually stabilizing interactions between RNA and proto-peptides (polyesters and depsipeptides).

Polyesters and depsipeptides, which contain backbone ester linkages in place of some or all amide bonds, have been proposed as the chemical ancestors of present-day proteins (i.e., as proto-peptides). Polyesters and depsipeptides are prebiotically plausible. Not only can α-hydroxy acids be incorporated ribosomally during translation to generate depsipeptide and polyester, but these proto-peptides also form more readily than pure peptides under mild dry-heat conditions. Esters enable the formation of amide bonds through a process of ester-amide exchange. Hydroxy acids are produced together with amino acids in model prebiotic reactions, are found together in some meteorites, and can combine to form oligomers >20 residues in length in mild dry-down reaction conditions. We recently demonstrated that cationic depsipeptides form robustly in dry-down reactions of mixtures of α-hydroxy acids and cationic α-amino acids.

Given that electrostatics are key elements of protein–nucleic acid interactions in extant life, we hypothesized that cationic proto-peptides might functionally interact with nucleic acids. Here we show that cationic polyesters and depsipeptides, generated either as heterogeneous mixtures from dry-down reactions of α-hydroxy acid monomers alone or in combination with α-amino acids, or synthetically prepared in homogeneous pure form, can interact directly with RNA in mutually stabilizing partnerships. These interactions prolong the lifetimes of proto-peptides due to lower rates of backbone ester bond hydrolysis, and render RNA duplexes more stable against thermal denaturation. Proto-peptides containing Arg, His, or Lys adjacent to backbone ester bonds generally increase RNA duplex thermal stability to a greater extent than do analogous sequences containing non-proteinaceous ornithine (Orn), 2,4-diaminobutyric acid (Dab), or 2,3-diaminopropionic acid (Dpr). Thus, mutually stabilizing interactions appear to be a natural outcome when cationic proto-peptides and RNA co-exist in mixtures. The results of our study support the idea that the intermolecular interactions between RNA and peptides in extant biology have ancient origins and reflect a long co-evolutionary history.

Results

Proto-peptides produced in dry-downs increase the RNA $T_m$

We recently reported the formation of cationic depsipeptides generated from binary mixtures of an α-hydroxy acid (glycic acid, glc; or lactic acid, lac) and a cationic α-amino acid (lysine, Lys; arginine, Arg; histidine, His; ornithine, Orn; 2,4-diaminobutyric acid, Dab; or 2,3-diaminopropionic acid, Dpr) after one week of drying at 85 °C under unbuffered, acidic conditions, as indicated by nuclear magnetic resonance (NMR) and liquid chromatography–mass spectrometry (LC–MS) analysis. Analysis by NMR indicated that isr condensed less efficiently than hab and hah, whereas hab readily cyclized into lactams (Supplementary Figs. 2 and 4–8). Analysis by NMR analysis of the hah product mixture indicated that the majority of hah incorporated into products contained free ε-aminos, indicative of a linear, protein-like backbone topology with esters in place of the peptide bonds.

We hypothesized that the mixtures of cationic proto-peptides now in hand might engage in interactions with nucleic acids. Accordingly, we determined the effect of cationic proto-peptides on RNA duplex stability by monitoring changes in the melting temperature ($T_m$) of a short 10mer RNA duplex in the presence or absence of individual proto-peptide samples. The complementary RNA strands were 5′- or 3′-labeled with either a fluorophore or a quencher so that the degree of RNA hybridization could be monitored by fluorescence in rtPCR instrumentation. As controls, we used (i) dry-down reactions containing the amino acid alone (no hydroxy acid), for which no oligomerization was observed for any amino acid (AA control); (ii) depsipeptide mixtures generated from a dry-down reaction of glc+Ala, which produced non-cationic oligomers; (iii) dry-down reactions of glc or lac alone (no amino acid), which produced non-cationic polyesters; and (iv) cationic α-hydroxy acids such as hah or hab that were not dried, and thus not oligomerized. For the $T_m$ measurements, the proto-peptide samples were diluted to a final concentration of 25 mM (based on the amount of cationic amino acid used at the start of the dry-down) and mixed with the RNA duplex (2.5 μM each strand). After measuring the thermal denaturation curve for each condition, the $T_m$ measurements showed a significant increase in the $T_m$ observed for the corresponding AA control, and plotted as change in $T_m$ (Fig. 2c).

Under these conditions, the presence of depsipeptide product mixtures that contained the amino acids Arg, His, and Lys,
generally caused increases in the $T_m$ of the RNA duplex, whereas product mixtures that contained Dab or Dpr did not affect the $T_m$ (Fig. 2c). The Orn product mixtures had variable effects depending on the hydroxy acid that was present in the dry-down; Orn + lac depsipeptides increased the RNA duplex $T_m$, whereas the Orn + glc depsipeptide mixture caused a slight reduction in the $T_m$ compared with the AA control. In general, the depsipeptide mixtures containing isr affected the $T_m$ less than the analogous lac or glc mixtures (Fig. 2c); this may be due to the possibility in these sequences for intramolecular O,N acyl transfer rearrangements that would reduce the cationic charge of the oligomer. We prepared two additional replicate series of dry-down reactions for the lac mixtures to confirm the reproducibility of the observed effects on $T_m$ (Supplementary Fig. 10). The observation that the presence of proteinaceous amino acids (Arg, His, and Lys) in the proto-peptides increased the $T_m$ of the RNA duplex more effectively than the non-proteinaceous analogs Dab and Dpr can be explained by our previous findings that the Arg, His, and Lys oligomers were longer and more cationic than the Dab and Dpr dry-down products, and thereby offer more stabilizing non-covalent points of contact with the RNA duplex. Moreover, depsipeptides containing Arg, His, and Lys are more stable than those containing Orn or Dab (vide infra).

We speculated that longer depsipeptides would be more effective than shorter ones in increasing the $T_m$ of the RNA duplex due to their higher charge. To confirm that the observed effects on RNA $T_m$ stemmed from the oligomeric products, we diazylated the lac- or glc-containing mixtures (Fig. 2a). Following the dialysis, the resulting mixtures were enriched in longer oligomers and largely free of monomeric amino acids and hydroxy acids (Supplementary Fig. 11). In general, similar trends on RNA duplex $T_m$ were observed for the diazylated samples as for the crude dry-down mixtures (Fig. 2d, Supplementary Fig. 12). The magnitude of effects on $T_m$ with the diazylated samples were greater than those for the crude samples even though the total amino acid concentrations of 200–600 μM was lower, consistent with the assumption that longer oligomers would have greater impact on $T_m$. Because neutral depsipeptides are not expected to interact strongly with RNAs, diazylated dry-down mixtures of glc...
Fig. 2 Cationic proto-peptide mixtures generated in dry-down reactions stabilize duplex RNA. Dry-down reaction products were dissolved in deionized water to give a 100 mM stock solution based on the amount of monomers used at the start of the dry-down. The mixtures contain diverse oligomers at varying abundances, so the concentration of a given oligomer in the mixture would be substantially lower. Control dry-down reactions contained the amino acid alone, with no hydroxy acid (AA control). a Schematic depicting the process used here to generate proto-peptide mixtures. RNA stability studies used either crude dry-down product mixtures, or dialyzed mixtures from which unreacted monomers and short oligomers had been removed with a 500-1000 Da cut-off membrane. For panels b–d, the experiments employed a 10-mer RNA duplex (5′-6-FAM-rCrGrCrUrArArUrCrG-3′, 2.5 μM strand) in buffer (100 mM MES-TEA, 2.5 mM NaCl, pH 7.5). The final pH of the samples was between 5.6 and 6.8, unless otherwise noted. b Thermal denaturation curves for the RNA duplex in the presence of various Arg-containing dry-down reaction mixtures or control mixtures. c Changes in RNA duplex Tm relative to the corresponding amino acid control (dry-down reaction of the amino acid without a hydroxy acid) for crude dry-down mixtures. d Changes in RNA duplex Tm upon addition of dialyzed depsipeptide oligomers. Data are shown as a scatter plot of duplicate experiments. The non-cationic glc + Ala oligomers were included as a control. For the RNA alone condition, three technical replicates from each mix were analyzed in all cases. e Changes in RNA duplex Tm values upon addition of crude polyester and depsipeptide mixtures obtained by drying hab or hah, either alone or with Gly or Ala (at a 1:1 molar ratio), or non-dried controls. Data are shown as a scatter plot of two independent experiments. f Structures of polyesters derived from drying hab or hah, showing potential routes of oligomer degradation via intramolecular O,N acyl transfer.

We found that cationic polyesters and depsipeptides containing hab, but not hab, increased RNA duplex stability in a concentration-dependent fashion (Fig. 2e, Supplementary Fig. 13). The striking difference in effects on Tm for hab vs. hab is consistent with the more facile potential routes of oligomer degradation available to hab via intramolecular O,N acyl transfer. Whereas hab could potentially degrade via intramolecular 5- or 6-membered ring, with alanine (Ala) served as a negative control for the dialysis experiments. Indeed, the non-cationic glc + Ala depsipeptides did not affect the Tm of the RNA duplex (Fig. 2d). Oligomers containing His increased the RNA duplex Tm at pH 5, but not at pH 7 (Fig. 2d), consistent with the expectation that His-containing oligomers would be cationic only at pH values below the His pKa of ~6.0.
6-membered ring transition states during the course of the RNA Tm measurements at neutral pH, the analogous intramolecular reactions in hah would require less favorable 7- or 8-membered ring transition states (Fig. 2f). As negative controls, we used samples of hah and hab that had not been subjected to the dry-down reaction (non-dried), and indeed these samples had no effect on the Tm of the RNA duplex (Fig. 2e).

Depsiteptide/RNA structure–function relationship. To facilitate a more controlled structure–function relationship study for depsiteptide interactions with RNA than is possible with the complex oligomer mixtures described above, we synthesized a library of cationic depsiteptides and peptides using solid-phase protocols. The sequences varied in the cationic side chains and in the number and location of ester linkages within the depsiteptide backbone (Fig. 3, Table 1, Supplementary Table 1). To incorporate the ester bonds during solid phase synthesis, Fmoc-protected depsiteptide building blocks 1a–1e were synthesized for each of the required amino acids (Fig. 3b)49.

We prepared two series of depsiteptides, 2–6 and 7–11 (Fig. 3a), to systematically study the effect of the cationic side chain on RNA duplex stabilization41,42. Sequences 2–6 each contained 8 ester bonds in the context of an oligo-depsiteptide repeat, whereas 7–11 contained a single ester bond directly adjacent to a variable cationic amino acid. For depsiteptides 2–6, we observed that the proteinaceous amino acids His (at pH 5, where the imidazole side chain tends to be protonated), Arg, and Lys significantly increased the duplex Tm, whereas Orn and Dab did not (Fig. 3c, Table 1, Supplementary Table 1). In contrast, Orn and Dab, in the context of the analogous all-peptide backbone, substantially increased the RNA duplex Tm (Fig. 3c, Table 1, Supplementary Table 1), indicating that Orn and Dab are not inherently deficient in stabilizing RNA duplexes. Similar results were observed with depsiteptides 7–11, where Arg, His, and Lys promoted greater increases in the duplex Tm than Orn or Dab (Fig. 3d). The ineffectiveness of sequences containing Orn and Dab adjacent to ester bonds can be explained by more facile intramolecular O,N-acyl transfer reactions in these structures compared to Arg, Lys, or His (Fig. 3e), leading to the degradation of Orn- and Dab-containing sequences during the course of the thermal denaturation experiment (Supplementary Fig. 14). In contrast to Orn and Dab, the presence of number of ester bonds did not impact the observed effect on RNA duplex Tm in depsiteptides containing Arg, His, or Lys adjacent to an ester bond (for example, compare Table 1 entries #5 vs. #6, #15 vs. #16, #27 vs. #28, or #30 vs. #31). Several lines of evidence support the importance of electrostatic interactions in the observed stabilizations of RNA duplexes. First, as would be expected, higher numbers of cationic residues present in the depsiteptide/peptide led to greater increases in RNA duplex Tm (Table 1, entries #1–7). Second, the effect of His-containing depsiteptide was pH-dependent (Figs. 2d and 3c, d), as expected based on the His pKa of ~6.0. Along the same lines, a Dpr-containing sequence did not increase the Tm of the RNA duplex (Table 1, entry #28). The ineffectiveness of this sequence is probably due to the low pKa of ~6.3 for the side chain β-amine of Dpr when incorporated into oligomeric sequences43. In addition, as a negative control, we verified that a negatively charged depsiteptide containing Asp and Glu residues did not affect the thermal stability of the RNA duplex (Table 1, entry #14). Furthermore, peptides composed of all d-amino acids or mixed n- and l-stereochemistry exerted the same effects on Tm values as an all l-peptide (Table 1, entries #32–34), implying that long-range electrostatics play the more dominant role than peptide stereochemistry/conformation.

To establish the generality of thermal stabilization of folded RNA structures by cationic depsiteptides, we measured the effect of a number of cationic peptides and depsiteptides on three additional RNA sequences (Table 1, Supplementary Table 1). Whereas RNA duplex 1 is composed of two complementary 10mer strands containing 50% GC-content, RNA duplex 2 consists of two 16mer strands with 0% GC-content, RNA duplex 3 consists of a 12mer palindromic strand with 33% GC-content, and H26a RNA is a 23mer hairpin with 35% GC-content. H26a RNA, which is derived from ribosomal RNA, adopts a more complex, hairpin-like fold compared to the duplexes. All RNAs showed similar Tm increases by a given peptide/depsiteptide sequence (Table 1, Supplementary Table 1), suggesting that RNA stabilization by cationic proto-peptides is a general characteristic of folded RNA, which would be modulated by proto-peptide sequence, side chains, and backbone composition.

Binding studies for cationic depsiteptides and RNA. To establish that depsiteptides were directly associating with the RNA duplex, we used a gel mobility shift assay and circular dichroism (CD) spectroscopy. Using 5-FAM-labeled peptide 12 (Table 1, entry #27) or analogous depsiteptide 13 (Table 1, entry #28) (Fig. 4a), we observed a concentration-dependent band shift for the single-stranded RNA 5′-Cy5-U10-3′ (Fig. 4b). The observed affinity for RNA inferred from the gel shift assay was similar for the depsiteptide vs. the peptide. Band shifts were also detected for other cationic peptides and depsiteptides (Supplementary Fig. 15). In agreement with the gel mobility shift assay, changes were observed in the CD spectra of the 10mer RNA duplex upon addition of the depsiteptide 14 or its corresponding peptide analog Ac-Tyr-Gly-(Ala-Dab-Lys)3-NH2, further supporting direct association between the cationic oligomers and RNA (Fig. 4c, d, Supplementary Fig. 16). Since the CD spectra were recorded at 5 °C, below the Tm of the RNA duplex (29.5 ± 0.6 °C), we attribute the changes in the spectra to alterations in RNA duplex conformation upon binding of the cationic depsipeptide/peptides. Fitting of the CD data using a one-site binding model yielded a Kd value of ~3 μM (Fig. 4d).

RNA prolongs the lifetime of cationic depsiteptides. We suspected that the formation of RNA-depsiteptide complexes could slow the hydrolysis rate of backbone ester bonds by a number of possible mechanisms. For instance, formation of a complex could sterically restrict access of water to the ester bond, might alter the bond geometries and conformational flexibility of the depsipeptide, or could reduce the extent of general base catalysis of ester hydrolysis by engaging the cationic side chains in electrostatic interactions with the RNA. To test the hypothesis that protopeptide backbone hydrolysis would be slowed in the presence of RNA, we incubated depsiteptide 9 in the absence or presence of varying concentrations of RNA duplex 1 or single-stranded RNA at 37 °C in pH 7.3 buffer (Fig. 5). High-performance liquid chromatography (HPLC) was used to monitor the concentrations of intact depsiteptide and its degradation products over time (Fig. 5b). Indeed, the presence of the RNA duplex increased the observed lifetime of the depsiteptide by up to ~30-fold (Fig. 5c, d). At all tested RNA duplex concentrations equal to or greater than 25 μM (equivalent to the initial concentration of depsiteptide 9), the observed kinetics of depsiteptide hydrolysis were similar to that of the single-stranded 10mer RNA (5′-rCrGrArUrUrUrArGrGrG-3′, 100 μM) increased the depsiteptide lifetime, but to a lesser extent (lifetime increased by roughly fivefold) compared to duplex RNA (Fig. 5e).
Structures of the Fmoc-didepsipeptide building blocks used for solid-phase synthesis of depsipeptide sequences. Fmoc stability. Lactic acid residues are highlighted. Ac acetyl, Aba acetamidobenzoic acid, which was appended to the N-terminus to increase UV absorbance.

Measurements:

- 10 mM acetate, 100 mM NaCl, pH 5.0.
- 37°C.

Fig. 3 Structure-function studies of cationic depsipeptides in stabilizing an RNA duplex. Each sample contained RNA duplex 1 (5'-rCrGrUrArArUrGrG-3' and 5'-rCrGrUrUrArGrGrG-3', 2.5 μM strand) and peptide/depsipeptide (100 μM) in buffered solution (10 mM phosphate, 100 mM NaCl, pH 7.0 or 10 mM acetate, 100 mM NaCl, pH 5.0).

- Structures of depsipeptides used to systematically characterize cationic side chain effects on RNA duplex stability. Lactic acid residues are highlighted.
- Ac acetyl, Aba acetamidobenzoic acid, which was appended to the N-terminus to increase UV absorbance.

C cationic side chains
- Arg, His, Lys, Orn, Dab

Comparative effects of oligo-didesipeptide sequences and on the Tm of the RNA duplex (number of independent measurements: n = 3 for Arg and Lys sequences; n = 2 for His and Dab sequences; n = 4 for Orn sequences). The shaded areas correspond to the mean ± SD. Tm measured for the RNA duplex alone (n = 16 independent measurements). Data are shown as a scatter plot with mean ± SD.

- Comparative effects on RNA duplex Tm of depsipeptide sequences containing a single backbone ester (number of independent measurements: n = 3 for Arg; n = 2 for His, Orn, and Dab; n = 4 for Lys). Relative to RNA duplex alone (n = 16 independent measurements). Data are shown as a scatter plot with mean ± SD.

- To assess the impact of different cationic side chains on depsipeptide degradation rates, depsipeptides 7-11 (40 μM) were incubated at 37°C in buffer (100 mM HEPES, 10 mM NaCl, pH 7.3). After 5 min, the reactions were quenched by the addition of 3% TFA, and samples were analyzed by HPLC. Consistent with the hypothesis that sequences containing Orn or Dab adjacent to a backbone ester bond would undergo facile intramolecular O,N acyl transfer, only 37-39% of the starting depsipeptide remained, predominantly due to the formation of lactam products. In contrast, intramolecular degradation products were not observed for the sequences containing Arg, His, or Lys adjacent to the ester bond, and >80% of the intact depsipeptide were observed in these cases.

Data from the nine independent depsipeptide hydrolysis reactions carried out with different concentrations of RNA duplex 1 (Fig. 5c) were simultaneously fit using SimFit49 to the reaction model shown in Fig. 5a. In the model, the free depsipeptide is subject to hydrolysis at a pseudo-first order rate governed by kaq, hydrolysis of the depsipeptide to RNA according to an equilibrium determined by kassoc/kdissoc, yields a depsipeptide-RNA complex, for which the pseudo-first order rate of ester hydrolysis is described by khyd-complex. Under conditions where depsipeptide-RNA complex formation is favorable and khyd-RNA < khyd-free, the presence of RNA will reduce the observed rate of depsipeptide hydrolysis. During fitting, we fixed kassoc = 1 × 10^5 M^-1 s^-1, on the lower end of rates observed and expected for protein-RNA association45. Therefore, these parameters were variable during the fitting, and the final rate constants determined by the fitting were: khyd-free = 1.1 × 10^-4 s^-1, khyd-complex = 3.2 × 10^-6 s^-1, and kassoc = 8.3 × 10^-3 s^-1. Based on these values, the rate constant for backbone ester hydrolysis in the depsipeptide-RNA complex (khyd-complex) was ~34-fold lower than
| Entry | Sequence | # Cationic residues | RNA duplex 1 $\Delta T_m$ (°C) | RNA duplex 2 $\Delta T_m$ (°C) | RNA duplex 3 $\Delta T_m$ (°C) | H26a RNA $\Delta T_m$ (°C) |
|-------|----------|---------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| 1     | Ac-Lys-NH$_2$ (1 mM) | +1 | 0.0 | -0.1 | -1.1 | 1.3 |
| 2     | Ac-Tyr-Gly-Ala-Dab-Lys-NH$_2$ (400 μM) | +2 | -0.7 | -0.2 | 1.3 |
| 3     | Ac-Tyr-Gly-Ala-Dab-Lys-Ala-Dab-Lys-NH$_2$ (200 μM) | +4 | 0.7 | 0.3 | 1.3 |
| 4     | Ac-Tyr-Gly-Ala-Dab-Lys-Ala-Dab-Lys-Ala-Dab-Lys-NH$_2$ (133 μM) | +6 | 3.4 | 0.3 | 1.3 |
| 5     | Ac-Tyr-Gly-Ala-Dab-Lys-Ala-Dab-Lys-Ala-Dab-Lys-NH$_2$ (100 μM) | +8 | -0.8 | -0.8 | 1.3 |
| 6     | Ac-Tyr-Gly-Ala-Dab-Lys-lac-Dab-Lys-lac-Dab-Lys-lac-Dab-Lys-lac-NH$_2$ (80 μM) | +8 | 10.0 | 9.1 | 10.4 |
| 7     | Ac-Tyr-Gly-Ala-Dab-Lys-Ala-Dab-Lys-Ala-Dab-Lys-NH$_2$ (80 μM) | +10 | 9.6 | 9.6 | 10.4 |
| 8     | Aba-Gly-Ala-Dab-Lys-Ala-Dab-Arg-lac-Dab-Lys-Ala-Dab-Lys-NH$_2$ (7) | +8 | 10.4 | 8.2 | 9.9 | 11.5 |
| 9     | Aba-Gly-Ala-Dab-Lys-Ala-Dab-His-lac-Dab-Lys-Ala-Dab-Lys-NH$_2$ (8) (pH 5.0) | +8 | 9.8 | 8.7 | 2.5 | 3.5 |
| 10    | Aba-Gly-Ala-Dab-Lys-Ala-Dab-His-lac-Dab-Lys-Ala-Dab-Lys-NH$_2$ (8) (pH 7.0) | +8 | 9.8 | 8.2 | 2.5 | 3.5 |
| 11    | Aba-Gly-Ala-Dab-Lys-Ala-Dab-Lys-lac-Dab-Lys-Ala-Dab-Lys-NH$_2$ (9) | +8 | 9.8 | 8.2 | 2.5 | 3.5 |
| 12    | Aba-Gly-Ala-Dab-Lys-Ala-Dab-Orn-lac-Dab-Lys-Ala-Dab-Lys-NH$_2$ (8) (pH 5.0) | +8 | 9.8 | 8.2 | 2.5 | 3.5 |
| 13    | Aba-Gly-Ala-Dab-Lys-Ala-Dab-Orn-lac-Dab-Lys-Ala-Dab-Lys-NH$_2$ (10) | +8 | 9.8 | 8.2 | 2.5 | 3.5 |
| 14    | Aba-Gly-Ala-Asp-Glu-Ala-Asp-lac-Asp-Glu-Ala-Asp-Glu-NH$_2$ | -6 | 0.3 | 0.7 | -1.5 | 1.1 |
| 15    | Aba-Arg-Ala-Arg-Ala-Arg-Ala-Arg-Ala-Arg-Ala-Arg-Ala-NH$_2$ | +8 | 5.9 | 5.9 | 5.9 | 5.9 |
| 16    | Aba-Arg-lac-Arg-lac-Arg-lac-Arg-lac-Arg-lac-Arg-lac-NH$_2$ | +8 | 5.9 | 5.9 | 5.9 | 5.9 |
| 17    | Aba-His-Ala-His-Ala-His-Ala-His-Ala-His-Ala-His-Ala-His-Ala-His-Ala-NH$_2$ (pH 5.0) | +8 | 4.2 | 4.2 | 4.2 | 4.2 |
| 18    | Aba-His-lac-His-lac-His-lac-His-lac-His-lac-His-lac-NH$_2$ (3) (pH 5.0) | +8 | 4.6 | 4.6 | 4.6 | 4.6 |
| 19    | Aba-His-lac-His-lac-His-lac-His-lac-His-lac-His-lac-NH$_2$ (3) (pH 7.0) | +8 | 0.5 | 0.5 | 0.5 | 0.5 |
| 20    | Ac-Tyr-Gly-Lys-Ala-Lys-Ala-Lys-Ala-Lys-Ala-Lys-Ala-Lys-Ala-Lys-Ala-Lys-Ala-NH$_2$ | +8 | 3.1 | 3.0 | 4.5 | 4.1 |
| 21    | Aba-lac-lac-lac-lac-lac-lac-lac-NH$_2$ (4) | +8 | 2.6 | 2.5 | 3.9 | 6.3 |
| 22    | Aba-Tyr-Gly-Orn-Ala-Orn-Ala-Orn-Ala-Orn-Ala-Orn-NH$_2$ | +8 | 4.5 | 4.5 | 4.5 | 4.5 |
| 23    | Aba-Orn-lac-Om-Orn-Ala-Orn-Om-Orn-Ala-Orn-NH$_2$ (5) | +8 | 0.0 | 0.0 | 0.5 | -1.1 |
| 24    | Aba-Orn-lac-Om-Orn-Om-Orn-NH$_2$ (6) | +8 | 6.9 | 8.0 | 11.0 | 10.3 |
| Entry | Sequence | # Cationic residues | RNA duplex 1 ΔT_m (°C) | RNA duplex 2 ΔT_m (°C) | RNA duplex 3 ΔT_m (°C) | H26a RNA ΔT_m (°C) |
|-------|----------|---------------------|------------------------|------------------------|------------------------|-------------------|
| 25    | Ac-Tyr-Gly-Dab-Ala-Dab-Ala-Dab-Ala-Dab-Ala-Dab-Ala-Dab-Ala-NH₂ | +8 | 0.4 | 2.6 | −0.4 | 0.5 |
| 26    | Ac-Tyr-Gly-Dpr-Ala-Dpr-Ala-Dpr-Ala-Dpr-Ala-Dpr-Ala-Ala-Dpr-Ala-Ala-Ala-NH₂ | +8 | 0.9 | | | |
| 27    | 5-FAM-Gly-Ala-Dab-Lys-Ala-Dab-Lys-Ala-Dab-Lys-Ala-Dab-Lys-NH₂ (12) (80 μM) | +10 | 10.6 | | | |
| 28    | 5-FAM-Gly-Ala-Dab-Lys-Ala-Dab-Lys-Ala-Dab-Lys-Ala-Dab-Lys-NH₂ (13) (80 μM) | +10 | 12.2 | | | |
| 29    | Ac-Tyr-Gly-Lys-Ala-Lys-Ala-Lys-Ala-Lys-Ala-Lys-Ala-Lys-Ala-NH₂ | +8 | 3.4 | | | |
| 30    | Ac-Tyr-Gly-Ala-Dab-Ala-Dab-Ala-Dab-Ala-Dab-Ala-Dab-Ala-Dab-Ala-NH₂ | +8 | 6.8 | 8.9 | 9.9 | 8.4 |
| 31    | Ac-Tyr-Gly-Ala-Dab-Ala-Dab-Ala-Dab-Ala-Dab-Ala-Dab-Ala-Dab-Ala-NH₂ | +8 | 7.1 | 7.2 | 10.6 | 10.9 |
| 32    | Ac-Tyr-Gly-Lys-Lys-Gly-Lys-Lys-Gly-Lys-Lys-Lys-Gly-Lys-NH₂ | +8 | 5.8 | | | |
| 33    | Ac-Tyr-Gly-Lys-Gly-Lys-Gly-Lys-Gly-Lys-Gly-Lys-Gly-Lys-NH₂ | +8 | 5.8 | | | |
| 34    | Ac-Tyr-Gly-Lys-Gly-Lys-Gly-Lys-Gly-Lys-Gly-Lys-Gly-Lys-NH₂ | +8 | 6.3 | | | |
for the free depsipeptide ($k_{\text{hyd-free}}$). Given the fixed value of $k_{\text{assoc}}$ and the calculated value of $K_{\text{eq-RNA}}$, the predicted dissociation constant of the depsipeptide 9-RNA complex is 0.1 μM, somewhat lower than the $K_{d}$ of ~3 μM measured by CD for depsipeptide sequence 14 binding to the same RNA duplex.

To demonstrate the simultaneous mutual stabilization that RNA and cationic depsipeptides gain through their non-covalent interactions, three parallel samples were prepared: one sample containing only RNA duplex 1 (25 μM each complementary strand), one containing only depsipeptide 9 (25 μM), and the last sample containing both duplex 1 and depsipeptide 9 (Fig. 5f). After incubation at 37 °C for 12 h, the extent of depsipeptide hydrolysis was measured by HPLC and/or the degree of RNA hybridization was separately measured by UV absorbance. The reaction containing both RNA and depsipeptide exhibited substantially higher levels of both RNA hybridization (due to increased $T_{m}$) and intact depsipeptide 9 (due to reduced backbone ester hydrolysis) (Fig. 5f). Thus, non-covalent interactions between the RNA and the depsipeptide had increased the lifetime of the covalent depsipeptide backbone and increased the stability of the duplex form of RNA relative to single strands.

**Discussion**

We are exploring the hypothesis that the relationship between RNA and peptide extends back into early chemical evolution, to an era when proto-peptides with heterogeneous backbones (polymesters and/or depsipeptides) interacted with proto-nucleic acids. Here, we have studied the potential for cationic protoprotein mixtures generated in plausibly prebiotic dry-down reactions to engage in direct interactions with RNA. We found that such interactions can increase the lifetimes of the proteo-peptides by decreasing their rate of degradation, and can stabilize the RNA by increasing the melting temperature of folds and assemblies. Cationic depsipeptides and polymesters can form robustly via simple dry-down reactions in the presence of condensing agents (Fig. 1). We also synthesized a library of model cationic depsipeptides containing one to eight ester bonds to facilitate mechanistic and structure–function relationship studies (Table 1). Proto-peptides containing proteinaceous amino acids Arg, Lys, and His adjacent to ester bonds generally promoted RNA duplex thermal stability to a greater magnitude than did analogous sequences containing the non-proteinaceous residues Orn, Dab, or Dpr (Figs. 2 and 3 and Table 1). In turn, interactions
between RNA and a depsipeptide could dramatically increase the hydrolytic stability of the depsipeptide backbone (Fig. 5). Thus, RNA–depsipeptide interactions can mutually prolong the lifetime of the depsipeptide backbone and stabilize the fold adopted by the RNA.

Undoubtedly, many factors would have influenced the selection processes that eventually led to the set of proteinaceous amino acids. These factors would include availabilities of various building blocks on the ancient Earth, efficiencies of non-enzymatic incorporation into growing oligomers, and the relative abilities of different side chains to impart functions within the context of the oligomer.

It is generally believed that the cationic amino acids found in proteins (Lys, Arg, and His) were not abundant on prebiotic Earth, although Lys has been observed in meteorites, and potentially-prebiotic routes for both Arg and His have been proposed.

Amino acids with shorter cationic side chains, such as Orn, Dab, and Dpr, have also been observed in meteorites and model prebiotic reactions, and are thought to have been more abundant on prebiotic Earth. It is intriguing that proto-peptide oligomers containing Arg, His, and Lys generally promoted greater increases in RNA duplex thermal stability than those containing Orn, Dab, or Dpr, both as product mixtures from dry-down reactions (Fig. 2) and as pure, synthetic molecules (Fig. 3, Table 1, Supplementary Table 1). We recently showed that the proteinaceous cationic amino acids Arg, His, and Lys also oligomerized with higher efficiencies and regioselectivities than non-proteinaceous analogs Orn, Dab, and Dpr. Thus, not only are the proteinaceous cationic amino acids chemically predisposed to produce longer linear oligomers than Orn, Dab, or Dpr, they can also generate depsipeptides that are superior in terms of stabilizing RNA structures, even in otherwise identical sequences differing only in the cationic side chain. Mechanistically, it appears that the same underlying factors lead to both the different efficiencies of oligomerization and the different degrees of stabilization of RNA duplexes. The side chains of Arg, His, and Lys have an inherently lower likelihood of undergoing intramolecular reactions via O-N acyl transfer compared to Orn, Dab, or Dpr, reducing the chances of chain-terminating side reactions during oligomerization.

Fig. 5 RNA increases the hydrolytic lifetime of a cationic depsipeptide. a A schematic of depsipeptide-RNA interactions leading to increased depsipeptide lifetimes. The free depsipeptide is hydrolyzed with a pseudo-first rate constant, $k_{\text{hyd-free}}$. Binding of the depsipeptide to RNA is governed at equilibrium by $K_{\text{ass}}/K_{\text{dissoc}}$. The pseudo-first rate constant for the hydrolysis of the depsipeptide within the depsipeptide–RNA complex is $k_{\text{hyd-complex}}$. Under conditions where complex formation is favorable and $k_{\text{hyd-complex}} < k_{\text{hyd-free}}$, the presence of RNA will increase the depsipeptide lifetime. b HPLC traces (270 nm) showing hydrolysis of depsipeptide 9 (25 μM) at various time points in the presence or absence of RNA duplex 1 at 37 °C in pH 7.3 buffer. The C-terminal fragment of the hydrolyzed depsipeptide is not observed because it lacks the Aba chromophore. (c) Time courses for hydrolysis of depsipeptide 9 (25 μM) with varying concentrations of the RNA duplex 1. The curves shown are from simultaneous fits of data to the model given in panel a using SimFit. During fitting, we fixed $k_{\text{dissoc}} = 1 \times 10^{-5} \text{M}^{-1}\text{s}^{-1}$. Therefore, three rate constants were fit, with values obtained by the fitting of: $k_{\text{hyd-free}} = 1.1 \times 10^{-4} \text{s}^{-1}$, $k_{\text{hyd-complex}} = 3.2 \times 10^{-6} \text{s}^{-1}$, and $K_{\text{ass}}/K_{\text{dissoc}} = 8.3 \times 10^{-3} \text{s}^{-1}$. d Kinetic profile of the hydrolysis reaction of depsipeptide 9 (25 μM) in the presence of 7 μM RNA duplex 1. Observed data are shown as filled circles, while the curves represent concentrations of molecular species predicted by SimFit modeling. e Comparison of depsipeptide hydrolysis in the absence of RNA, or in the presence of single-stranded RNA (100 μM 5′-rCrGrArUrUrArGrGr3′) or RNA duplex 1 (50 μM each strand). f To illustrate the mutually increased depsipeptide lifetime and RNA duplex $T_m$, three samples were prepared in parallel: one containing only RNA duplex 1 (25 μM each complementary strand), one containing only depsipeptide 9 (25 μM), and one containing both the RNA and 9 (at a 1:1 molar ratio). The extent of depsipeptide hydrolysis and RNA hybridization were then measured. Data are shown as a scatter plot of two independently repeated experiments.
degradation of the product oligomer in aqueous solution, which erodes the effectiveness of RNA binding/stabilization.

Mutually stabilizing interactions of the type described here might have provided important benefits to certain prebiotic systems. In a background of periodic, random (non-coded and non-templated) condensation processes, mutually stabilizing interactions could naturally lead to a buildup of those oligomers that possessed increased lifetimes due to the interactions. Shielding RNA against denaturation could increase its robustness and the range of environments in which it was folded and functional. Additional mechanisms have been put forth by which interactions between peptides and RNA could promote synthesis, stabilization, or localization of prebiotic oligomers. In one example, cationic poly(Leu-Lys) peptides were reported to encourage the oligomerization of activated mononucleotide diphosphates, and were especially effective in increasing the abundance of the longer RNA oligomers. RNA can template the native chemical ligation of cationic peptide fragments derived from a biological protein-binding partner. In the context of ribonucleotide- and cationic amino acid copolymers, the phosphoramidate and ester linkages within the co-oligomer backbone can be mutually stabilized against hydrolysis. Short, cationic, amphiphilic peptides can localize RNA to the surface of protocol membranes. Collectively, these findings suggest that interactions between RNA and cationic peptides/protopeptides could mutually support synthesis and stabilization of both classes of molecules.

In addition to the vast span of known beneficial interactions between RNA and cationic peptides, it is worth mentioning that certain interactions between cationic oligomers and RNA could have a reverse effect and could have negatively impacted the lifetimes or functions of molecules involved in the interaction. For example, it is known that interactions between cationic peptides and RNA can accelerate RNA degradation or cause RNA aggregation. For instance, Brack and colleagues studied a series of (Leu-Lys) or (Leu-Lys-Lys-Leu), peptides of varying lengths and degrees of stereochemical purity, and found that they exhibited RNA hydrolysis activities that correlated with the degree of β-sheet or α-helical character in the peptides. Considering that depsipeptides and polyesters should have less β-sheet or α-helical character compared to pure peptides (due to missing hydrogen bond donors and weakened hydrogen bond acceptors), it is possible that catalysis of RNA hydrolysis by protopeptides would be attenuated compared to peptides. Another consideration is that the formation of complexes between proto-peptides and RNA could impair chemical processes that required conformational changes or unfolding of RNA structures, such as RNA catalysis or replication. An important caveat when considering interactions based primarily on electrostatics is that the strength of the molecular interactions would be modulated by changes in the ionic strength of the solution.

Investigations of biopolymer origins from a co-evolutionary perspective might afford valuable insights into early chemical evolution. The origins and evolution of biopolymers would have occurred amongst significant molecular heterogeneity, and productive cooperative interactions between molecules were almost certainly involved in the emergence, selection, and persistence of certain sets of molecules out of the cluteter. In principle, primordial molecular partnerships could have increased the lifetimes of certain molecules by numerous mechanisms. Our findings demonstrate that proto-peptides and early nucleic acids could have interacted in mutually stabilizing ways, but the processes demonstrated here involving increased thermal stability of noncovalent assemblies and increased backbone ester hydrolytic stability are only two possibilities out of many. Mutually stabilizing interactions provide a potential avenue for chemical selection—in mixtures containing multiple proto-peptide and RNA sequences, molecules with higher binding affinities could selectively associate in solution, persist, and adopt better-folded or more stable structures. We are currently investigating this possibility. Further studies of chemical systems in which productive interactions between different types of molecules could occur will likely lead to important insights in prebiotic chemistry and will be necessary for solving some of the enduring problems in the origin of life.

Methods

Peptide and depsipeptide synthesis. Peptides and depsipeptides were synthesized using standard Fmoc chemistry with an Advanced Chemtech Apex 396 peptide synthesizer. A typical synthesis was performed on 0.09-mmol scale using Rink amide MBHA resin (0.6 mmol/g). Standard side chain protecting groups included Lys(Boc), Orn(Boc), diaminopropionic acid (Boc), diaminobutyric acid (Boc), His(Trr), Arg(Prh), Tyr(Ph), Glu(Obu), and Asp(Obu). Ester bonds were incorporated into the depsipeptides by coupling separately synthesized Fmoc-depsipeptide building blocks that were appropriately protected on the side chain. Chain elongations were carried out using 1,3-diisopropylcarbodiimide (DIC) and ethyl 2-cyano-2-(hydroxymino)acetate (oxzyma) in N,N-methylpyrrolidin-2-one (NMP) with 75-min couplings. Fmoc deprotection was achieved using 2 x 8 min treatments with 10% piperidine in NMP. Washing steps involved 6 x 1 min treatments with DMF. Sequences were cleaved from the resin with concomitant side chain deprotection by agitation in a solution of 95:2.5:2.5 TFA/triisopropylsilane (TIS):water for 3 h. The crude products were precipitated with diethyl ether, centrifuged, and washed three additional times with ether. Additional peptide sequences were purified by reverse-phase (RP)-HPLC on a Vydac 218TP C18 or Thermo BioBasic C18 column. Purity was confirmed by analytical RP-HPLC. Purified peptides were characterized by analytical HPLC and LC-MS. Analytical RP-HPLC was performed using a Zorbax 300-SC C-18 column connected to a Hitachi D-7000 HPLC system. Binary gradients of solvent A (99% H2O, 0.9% acetonitrile, 0.1% TFA) and solvent B (90% acetonitrile, 9.9% H2O, 0.07% TFA) were employed for HPLC.

In certain depsipeptide sequence contexts, such as ‑Dab-Ala-lac-Dab... extensive truncation/termination was observed during the solid-phase synthesis, presumably due to sequence-dependent DKP formation. Accordingly, in these problematic sequences, the Dab residue located at the second position following the N-terminus prior to the following coupling step was incorporated as a Bsmoc-protected amino acid rather than being Fmoc-protected, to enable its dehydration with lower concentrations of base. For instance, Brack and colleagues studied a series of (Leu-Lys) or (Leu-Lys-Lys-Leu), peptides of varying lengths and degrees of stereochemical purity, and found that they exhibited RNA hydrolysis activities that correlated with the degree of β-sheet or α-helical character in the peptides. Considering that depsipeptides and polyesters should have less β-sheet or α-helical character compared to pure peptides (due to missing hydrogen bond donors and weakened hydrogen bond acceptors), it is possible that catalysis of RNA hydrolysis by protopeptides would be attenuated compared to peptides. Another consideration is that the formation of complexes between proto-peptides and RNA could impair chemical processes that required conformational changes or unfolding of RNA structures, such as RNA catalysis or replication. An important caveat when considering interactions based primarily on electrostatics is that the strength of the molecular interactions would be modulated by changes in the ionic strength of the solution.

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isr with a single amino acid at a 5:1 molar ratio (in favor of the hydroxy acid) were allowed to dry at 85 °C under unbuffered, mildly acidic conditions (initial pH of ~3) for 1 week. The amino acids were all used in their HCl form, and no additional salt was added to the reactions. Control reactions contained either a hydroxy acid alone or an amino acid alone. For formation of cationic polyesters and depsipeptides from cationic hydroxy acid monomers, aqueous solutions of either isr, hab salt was added to the reactions. Control reactions contained either a hydroxy acid or a free amino acid at a 5:1 molar ratio (in favor of the hydroxy acid) were allowed to dry at 85 °C under unbuffered, mildly acidic conditions (initial pH of ~3) for 1 week, either alone or in a binary mixture with Gly, Ala, lac, or glc. Before analysis, dry-down reaction mixtures were resuspended in ultrapure water to 100 mM concentration (based on original cationic hydroxy acid concentration or on original cationic amino acid concentration for co-dry-downs). Ammonium acetate, sodium acetate, tetracycline in ice, and centrifuged at 15,250×g for 5 min. The supernatant was collected and diluted to the specified concentration. For dialysis, the lac- or glc-containing dry-down reaction mixtures were placed in a 500–1000 Da cut-off membrane (Micro Float-A-Lyzer, VWR #89219-388) and dialyzed against water.

NMR spectroscopy. NMR spectra were recorded on a Bruker Avance II-500. To ensure quantitative integration of the resonances, relaxation delay of 15 s was used for dry-down reaction mixtures. Data were processed and spectra were plotted with MestReNova software package. The overall conversion of monomers into products was estimated from integration of the free, non-reacted a-proton 1H-NMR resonances. The extent of amidation at side-chain amines was quantified by integration of the resonance corresponding to methylene protons adjacent to the side-chain amine.

LC-MS. LC-MS data were collected on an Agilent 1260 HPLC coupled to an Agilent 6130 single quadrupole mass spectrometer and an inline Agilent UV absorbance detector (210 nm) using a 3.0- or 3.5-kV electrospRAY ionization (ESI) capillary voltage. Samples were either directly infused into the mass spectrometer or separated using a Zorbax C18 column with a binary solvent system of water/acetonitrile/formic acid. Processing of MS data were conducted using a suite of MestReNova software package. The overall conversion of monomers into products present only as a duplex (ignores steps involving RNA strand hybridization to form the duplex). The final RMS error of the fit was 5.4%. The output file obtained from the fitting is provided in the Supplementary Note.

Statistics and reproducibility. The experiments described in this paper were repeated independently at least two times. All attempts at experimental replication were successful. Graphical data are shown as scatter plots with all data points included. No data were excluded from the analyses.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
All the data supporting the findings of this study are available within the main text and the Supplementary Information. Data are also available from the corresponding authors upon request.

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Author contributions

M.F.P., L.D.W., and L.J.L. conceived the experiments. M.F.P., L.D.W., L.J.L., A.S.P., R.K., and N.V.H designed the experiments. M.F.P., L.J.L., J.W.H., and A.M.M. carried out the experiments. M.F.P., L.D.W., and L.J.L. wrote the paper. M.F.P., L.D.W., L.J.L., M.C., A.B.S., A.S.P., R.K., and N.V.H. contributed to the data interpretation. L.D.W. and L.J.L. supervised the research. All authors reviewed the paper.

Competing interests

The authors declare no competing interests.
