Peptidyl transferase: ancient and exiguous

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The finding that the universal ribosomal peptidyl transferase is an RNA enzyme casts new light on its ancient origins, on the use of transition state analogues for ribozymes, and on the role of selection-amplification in studies of molecular evolution.

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Introduction

Proteins are the everyday agents of biology, supporting the structures and catalyzing the reactions that make cells run. Therefore, the revelation in a recent publication [1] that the ribosomal peptidyl transferase, which forms the peptide bonds of all cellular proteins, is instead an RNA enzyme is a thought-provoking turnabout. However, this difference is probably a result of the history of the peptidyl transferase, that is, a result of its descent from an RNA world.

Defining an active center amongst 100 000 atoms

The identification of the built-in peptidyl transferase of the large ribosomal subunit, where peptide-forming chemistry occurs, relied on a transition state analogue [2]. A transition state analogue is a specific molecular pointer directed at an active site, whose chemistry is mimicked in the structure of the transition state analogue itself [3].

What chemistry to mimic was initially uncertain. Proteases insert the elements of water between amino acids, and peptidyl transferases effectively do the reverse. Thus peptidyl transferase might have either used a direct attack or a covalent intermediate, as do proteases catalyzing a reaction in the other direction. We now know the peptide-forming reaction is direct (Figure 1A). Thus the transition state analogue called CCdApPuro has a ribosomal P(peptidyl)-site-directed part and an A(aminoacyl)-site-directed part, and imitates the result of direct nucleophilic attack between these ribosomal sites as the peptidyl transferase mechanism (Figure 1B) [2]. As expected, CCdApPuro is an effective inhibitor of ribosomes, and binds simultaneously at the ribosomal A and P sites [2]. The specific binding of CCdApPuro to bacterial, eukaryal [4] and now archaeal ribosomes [1] shows that the direct attack mechanism is conserved in all three domains of extant life and, therefore, probably predates the last common ancestor of life on earth.

Partial reconstitution of the active center

However, there is an even more striking finding. When the transition state analogue is tethered to agarose beads and bound RNAs eluted with CCdApPuro, RNAs can be selected that also bind both A and P halves of the free reaction mimic [5]. These RNAs bind with affinities comparable to the peptidyl transferase itself. Most relevant is the fact that the selected RNAs exhibit up to 17 nucleotides identical to large subunit ribosomal RNA [5], in regions both conserved (Figure 1C) and implicated in peptide synthesis [6]. We now see [1] that the predominant CCdApPuro-binding RNAs [5] duplicate the sequence of a conserved, ancient octamer in the heart of the ribosomal peptidyl transferase, AUAACAGG (Figure 1, bottom). There are $4^8 = 65\,536$ possible octamers. Thus to find the same one in contact with CCdApPuro within the ribosome and in selected RNAs seems beyond coincidence. While the structure of selected CCdApPuro-RNAs are not yet known in detail, the entire octamer is required for binding, and chemical protection experiments suggest that CCdAp-

Puro is oriented similarly in both selected [5] and ribosomal complexes [1].

Therefore a selection for CCdApPuro-binding RNA yielded a part of the true ribosomal P site, a part of the true ribosomal A site, and the relation between them required for direct nucleophilic production of peptide bonds (Figure 1D). However, there is more.

An apparent acid–base center, too

Since shortly after peptidyl transferase could be directly assayed [7,8], it has been clear that there is a pH-sensitive group associated with the active center. This pK_a of about 7.5 was ironically thought to confirm that peptidyl transferase was proteinaceous, because nucleotides do not usually have a pK_a in this region. However, Muth et al. [9], using a biochemical nucleotide pK_a scanning technique [10], showed that there is one highly-shifted nucleotide base pK_a in the region of the peptidyl transferase. It is in fact the rRNA nucleotide A_{2451} (Figure 1D), pointed directly at a phosphoramide oxygen of CCdApPuro, which is the transition state analogue's stand-in for the 3′ carbonyl oxygen of a reacting peptidyl-tRNA [1]. Thus the proton(s) relocated during peptide formation (Figure 1A) could be transferred via an easily-protonated A_{2451}, apparently the peptidyl transferase's general acid–base catalyst. The nucleotide corresponding to A_{2451} is also conserved [5] and required for binding in newly-selected CCdApPuro binding RNAs. Thus, in vitro selection-amplification for binding CCdApPuro captured even A_{2451}, required for catalysis but not necessarily for binding reactants.

Conclusions

What is the substance of all these observations? Remark-

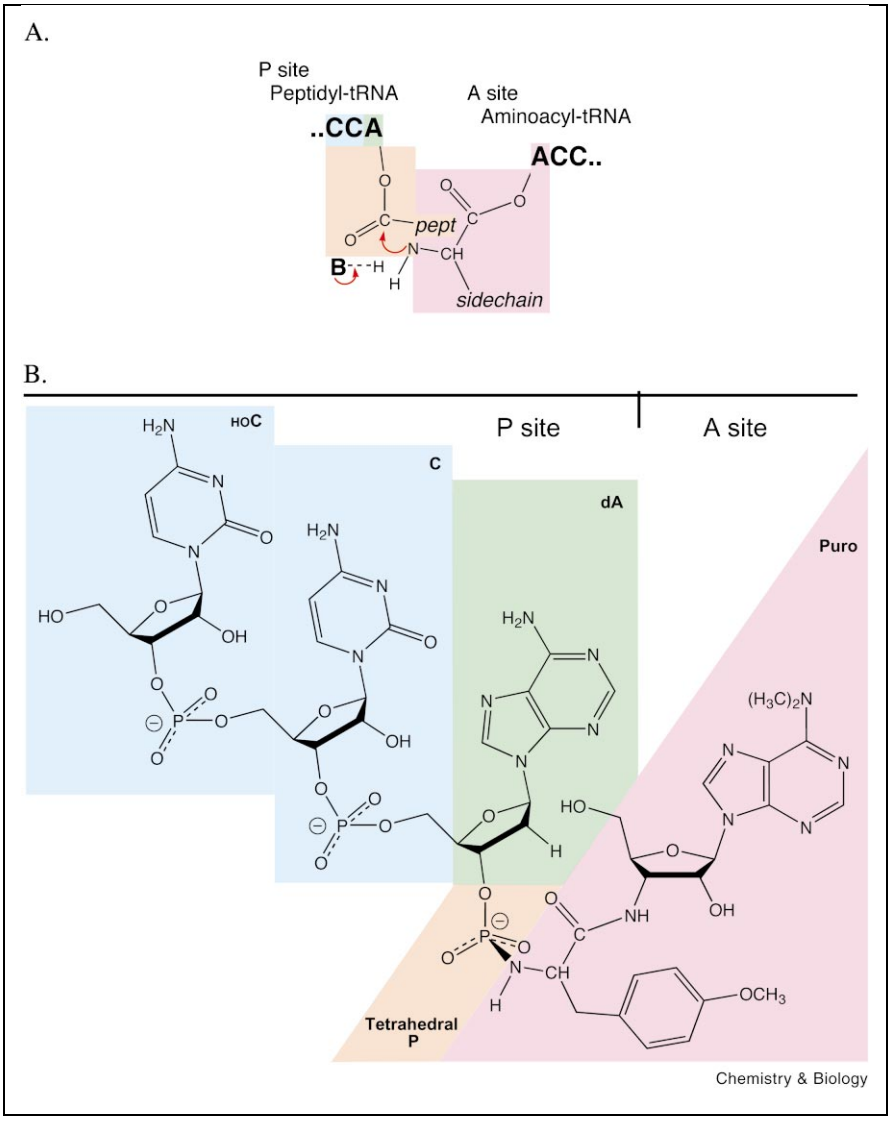

Figure 1.

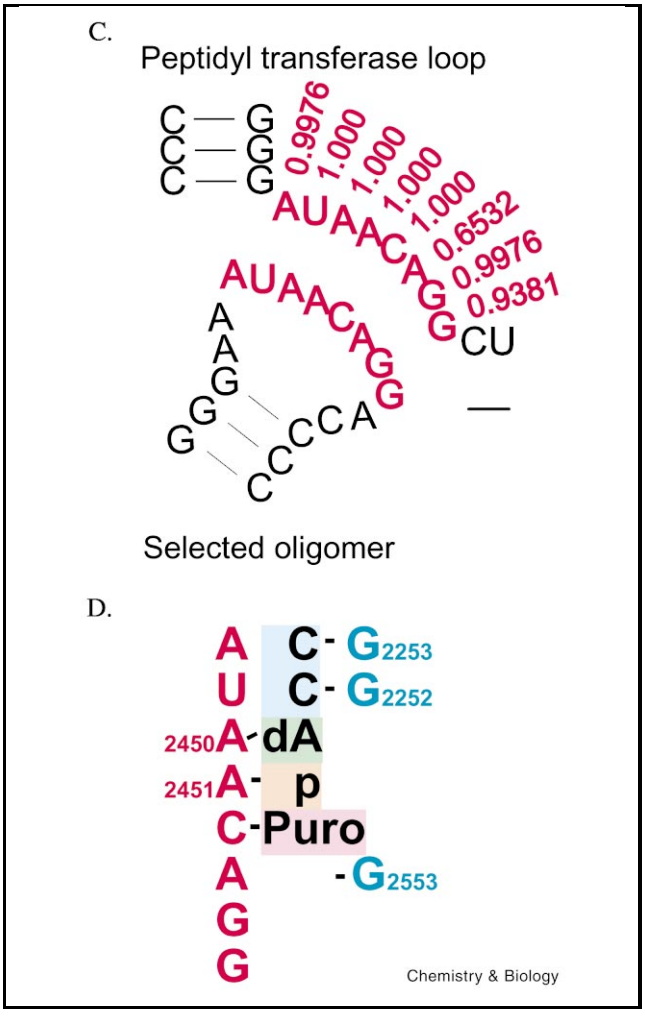

Figure 1. Colors divide structures into sections in a consistent way throughout. **A**: Direct nucleophilic attack to make a peptide bond. 'P site' is the ribosomal P site, *pept* is the growing peptidyl chain, and *sidechain* indicates the position of the characteristic group of the amino acid to be added. B is a general base to remove a proton from -NH_2. Only the conserved 3′ CCA sequences of the tRNAs are shown. **B**: CCdApPuro, the tetrahedral phosphoramide mimics a polar, tetrahedral carbonyl carbon, the expected transition intermediate during nucleophilic attack of an amine on the terminal carbonyl of a peptidyl-tRNA, as in **A**. **C**: Comparison of the conserved sequence at conventional 12–3 p.m. region of the ribosomal peptidyl transferase loop (top) with the selected CCdApPuro-binding loop (bottom). Red numbers show fractional conservation of the associated rRNA nucleotides over all three domains of life [6]. **D**: Structure of the ribosomal peptidyl transferase active site. CCdApPuro is black typeface with background colors as in **A**. Red, octamer sequence conserved in selected RNAs and in ribosomal peptidyl transferase: blue, P loop (top) and A loop (bottom) elements, also from rRNA domain V. Dashes indicate interactions of a varied kind. Numbering corresponds to *Escherichia coli* rRNA, rather than *Haloarcula marismortui* [1] or selected CCdApPuro binding sites.

ably, it appears that the core sequence of the universal ribosomal peptidyl transferase was identified by a modern selection (Figure 1D). As one consequence, recreation of many particulars of the active center required only a few nucleotides. Only 50 nucleotides were randomized in the starting RNAs, or more pointedly, only 16 nucleotides (AUAACAGG in a small loop; Figure 1C) were conserved among the most prevalent families of selected CCdApPuro binding RNAs [5]. Thus while the large rRNA now has about 3000 nucleotides bound to about 35 proteins, there is very good reason to think that the primordial peptidyl transferase could have been a very small RNA acting alone. Conservation of both peptidyl transferase rRNA sequences and affinity for CCdApPuro probably imply a recognizable modern peptidyl transferase prior to the divergence of the eubacteria, archaea and eukarya. However, the inference that only a very small RNA may be required takes us further, strongly supporting a considerably earlier origin in an RNA world.

These data also suggest that transition state analogues are worth a closer look for the derivation of RNA enzymes. That which binds a transition state analogue probably stabilizes a transition state, and may therefore be a catalyst [2]. RNAs selected to bind transition state analogues have been found to catalyze simple substrate conformational change like rotamer interconversion [11] and porphyrin metallation [12] (which requires a conformation change in the transition state). However, for a more complex reaction, carbon–carbon bond formation by Diels–Alder reaction, RNAs that bound a transition state analogue did not catalyze the reaction [13]. Nevertheless, this reaction was shown, using a different selection, to be within the catalytic repertoire of small RNAs containing only the standard four nucleotides [14]. Now the partial reconstruction of the peptidyl transferase active center suggests in particular that inclusion of easily-RNA-bound groups like nucleotides, bridging the point of reaction, may be good strategy. This is particularly so because the unsuccessful Diels–Alder transition state analogue bound relatively weakly to selected RNAs [13].

In fact, one final twist comes in noting that CCdApPuro is a tetranucleotide, though a slightly peculiar one (Figure 1B). One might therefore predict numerous RNA folds that would bind it. In spite of this probable multiplicity, the most prevalent selected RNA binding sites look like the peptidyl transferase (Figure 1C,D). This perhaps means that the choices available to biological evolution had an unexpected exiguity, meaning fewness or sparseness. Consequently, today's in vitro selections have a chance of rediscovering the options taken by earth's early biota. If we can understand how this artificial selection mimicked natural selection, we may presently find out more about the RNA world and early life than we might have thought.

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