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Codon–anticodon pairing

A model for interacting codon–anticodon duplexes located at the ribosomal A- and P-sites

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The interaction between two codon–anticodon duplexes of the ribosomal A- and P-site-bound tRNAs is the key feature of the proposed model. This interaction prohibits non-canonical base pairing at the first and second positions of the codon and controls base pairing at the third position (wobbling rules ensuing from the model are in good accord with those generated from experiments). The model is capable of predicting codon context effects. It follows from the model that modifications of the first anticodon residue of the P-site tRNA can affect the stability of the A-site duplex, and that the translation of a DNA single chain analogue of mRNA should be accompanied by non-canonical base pairing at all three positions of the codon. These predictions of the model can be subjected to experimental tests.

Codon–anticodon pairing; Wobbling rule, Context effect

1. INTRODUCTION

Codon–anticodon pairing is not merely a simple process controlled by hydrogen bonding between two anti-parallel trinucleotides, namely the mRNA codon and the tRNA anticodon. For example, peculiarities of the codon–anticodon interaction such as the absence of non-canonical base pairing at the first two positions of the codon cannot be explained just by the internal stability of the codon–anticodon mini helix and the influence of the tRNA anticodon loop. It is known that a wide variety of non-canonical base pairs is observed in different regions of the double helices of RNA molecules [1–4], and even in different positions of the anticodon–anticodon mini helices [3,5]. There is also a series of indications that the translation of the codon can depend on adjacent codons (codon context effects [6–8]). All these facts indicate that 'outside' factors must affect the formation of the ribosomal A-site codon–anticodon duplex. In this work it is shown (using the 'FRODO' program on an Evans and Sutherland PS 390 computer graphics system) that the wobble pair (the first base of the anticodon paired with the third base of the codon) of the duplex located at the P-site can play the role of the main 'outside' factor in binding the A-site tRNA.

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2. MODEL OF INTERACTING CODON–ANTICODON DUPLEXES

2.1. *Stacking between the backbone of the A-site codon and the P-site wobble pair constrains the A-site codon into an A-form conformation*

Stereochemical modeling of ribosomal transpeptidation [9] and translocation [10], as well as the results of topographical studies with the ribosome [11] favour the Rich-type (R) orientation [12] of two tRNA molecules located at the ribosomal A- and P-sites (Fig. 1). In a previous study [10] it was concluded that the angle ω in the structure shown in Fig. 1 should be equal to 100°. This value of ω is supported by the data of Smith and Yarus [13] who have shown that there is a strong influence of specific point mutations in the anticodon loop of the P-site tRNA on the codon–anticodon binding properties of the A-site tRNA. The authors concluded that the 5′-side of the anticodon loop of the P-site tRNA is in direct physical contact with the A-site tRNA. Our analysis has shown that at values of the angle ω greater than 110° this contact is absent, and that steric overlaps take place when $\omega < 90°$. Hence the angle ω should be equal to $100° \pm 10°$.

At these values of ω the interaction between the A-site codon– anticodon duplex and the wobble base pair of the P-site codon–anticodon duplex is observed (Figs. 1 and 2). It can be seen from Fig. 2 that the sugar–phos-

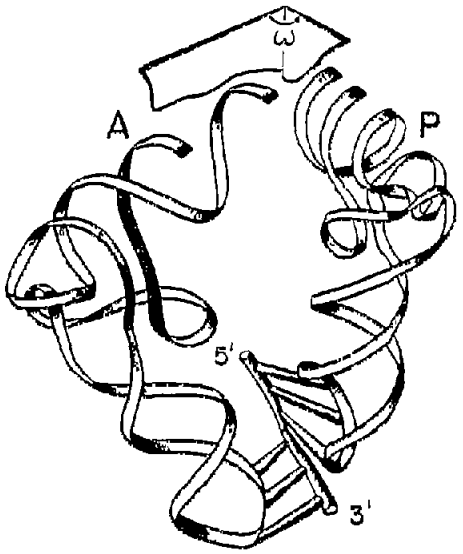

Fig. 1. R-orientation of the A- and P-site bound tRNAs. ω is the dihedral angle formed by the planes of tRNA molecules. The codon–anticodon duplexes are shown in the lower part of the drawing. The curved tube is the sugar–phosphate backbone of two adjacent mRNA codons. The rods are the codon–anticodon base pairs. The dotted rod is the wobble pair of the P-site duplex. This wobble pair interacts with the A-site codon-anticodon duplex.

phate moiety, of the A-site codon is stacked with the wobble base pair of the P-site duplex. Such a stacking interaction creates steric limitations **preventing** hydrogen bonding between the ribose 2′ OH groups of the first two codon residues and any outside hydrogen bond donors or acceptors, including water molecules. There is only the possibility of forming the two inter-ribose hydrogen bonds 2′ OH···O4′ (Fig. 2). Such hydrogen bonds are observed in RNA double helices [1–3,14], and in the present case (as no other hydrogen bonds can be formed) their formation will result in a large energetic gain (~30–40 kJ/mol). This suggests that the two shielded 2′ OH groups in the A-site codon–anticodon duplex should organize the inter-ribose hydrogen bonds, **constraining all three codon residues into the A-form.**

The first (wobble) base of the P-site tRNA anticodon can be cross- linked to the ribosomal base C1400 [15]. The only sterically permitted variant of the four possible cross-linked configurations is shown in Fig. 2. It is evident that in the absence of cross-links, the base C1400 should be similarly placed, and together with the wobble base would shield more reliable inter-ribose hydrogen bonding between the second and third codon residues.

X-ray diffraction studies have shown that replacement of a canonical base pair in a double helix by the non-canonical pair GU (in which the mutual arrangement of the glycosyl bonds differs minimally from the canonical one) is accompanied by shifts of 1.2 Å in the glycosyl bonds [16]. This leads to complete disruption of the inter-ribose hydrogen bonds. Consequently, to prevent energetic losses, departures from the A-form required for the formation of non-canonical base pairs in the A-site duplex should basically occur in the anticodon.

2.2. *Maintenance of the codon in the A-form and the structure of the anticodon loop counteract the non-canonical base pairing in the first and second positions of the codon*

With regard to the anticodon bases, the crystallographic data [1–3] indicate that the anticodon conformation is close to the A-form and the mobility of the second and third bases is strongly restricted by the interactions in the anticodon loop. These interactions are (see Fig. 2): hydrogen bonding between the NH group of conserved base U-33 and the phosphate group of the third anticodon residue (residue-36), stacking interactions of base-33 with base-32 and phosphate-35, steric contacts of base-33 with the second and third anticodon

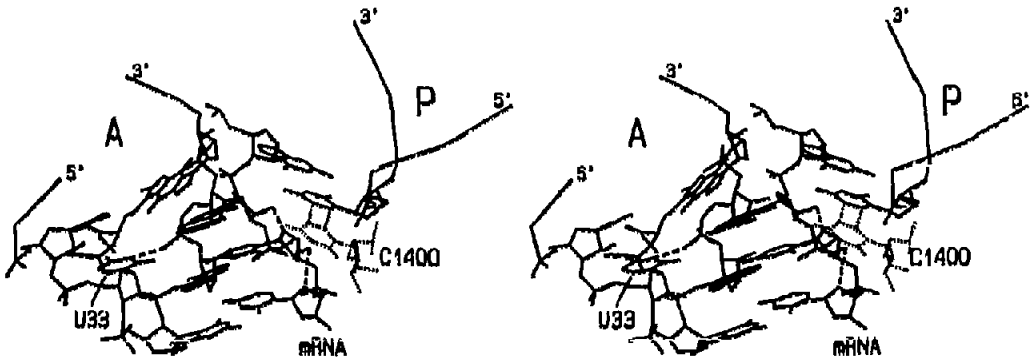

Fig. 2. Details of the interaction between the A- and P-site duplexes at ω = 100° (stereo views). In the P-site anticodon loop (right) only the wobble base is shown. This wobble base is paired with the codon base and cross-linked to base C1400 (dotted lines). In the A-site anticodon loop (left) the residues 32–37 are shown. The anticodon (residues 34–36) is paired with the codon. Dashed lines show the two inter-ribose hydrogen bonds in the codon and hydrogen bonding between U-33 and phosphate-36. The loops are drawn in accordance with the crystallographic coordinates of yeast $tRNA^{Phe}$ [1].

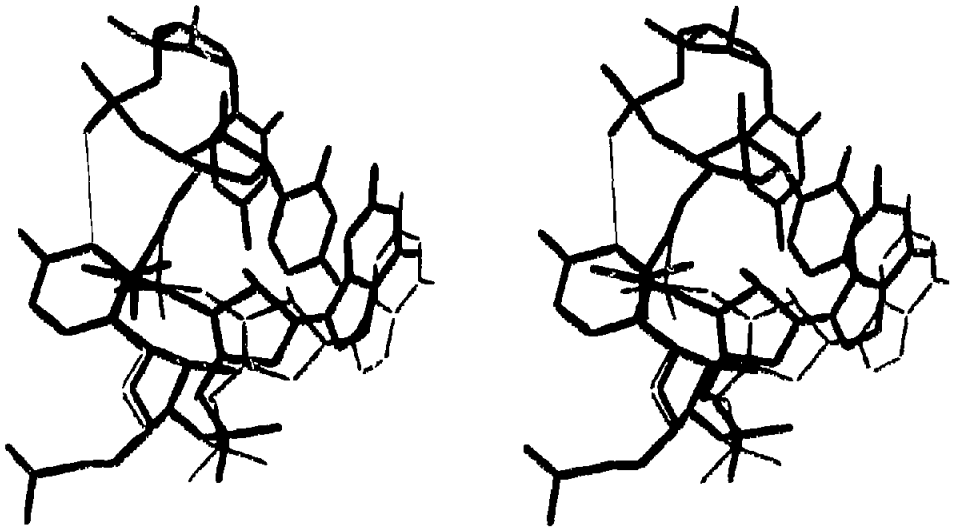

Fig 3 Stereo view of the anticodon loop section (residues 33–36) of $tRNA^{Asp}$ [3]. The anticodon triplet (right) and conserved U33 (left) are shown. The first anticodon residue (residue-34) and phosphate-35 are shown in two different positions.

bases, and steric contacts of ribose ring-33 with base-35. The above-mentioned interactions form a steric pocket for conserved base U-33, in which the base is tightly packed and hydrogen bonded to phosphate-36 located between the second and third anticodon bases. Such a system of interdependent interactions prevents shifts of 2–3 Å in the glycosyl bonds of the second and third anticodon residues (shifts are required in replacing a canonical base pair by a non-canonical one in the codon–anticodon mini helix, where the codon is fixed in the A-form). These shifts cannot be accomplished without leading to disallowed steric overlaps and the appearance of the unpaired hydrogen bond donor (HN3) and acceptor (O2) of base U-33. In other words, the second and third anticodon residues (like their codon partners) should be in the A-form, i.e. together with the codon bases they should organize only Watson–Crick base pairs.

2.3. *The rules for base pairing at the third position of the codon*

In contrast to the second and third bases, the first anticodon base is mobile. Its displacement only strongly affects the stacking between phosphate-35 and uracil-33, but in this case phosphate-35 slides over the surface of U-33 without any decrease in the stacking interaction (Fig. 3). This enables the first anticodon base to pair non-canonically with its fixed partner, in which only the glycosyl bond C1′-N was deviated by ±5° from its standard positions at the C1′ and N atoms. We have considered all the base pairs described in [17] in which the bases can be paired without dehydration of the polar atoms as well as the pyrimidine–pyrimidine pairs UU, UC containing a water bridge (Fig. 4). The UC pair with the water bridge (Fig. 4b) has been recently found experimentally [4]. In the UU pair containing the water bridge the bases were paired using the hydrogen bond 3NH···O4 and the water bridge 2O···H_2O···HN3. A possible CC-pairing was not considered because of dehydration of the polar atoms.

The A-form conformation of the third codon base was found to be incompatible with those pairs whose formation requires strong shifts (~3 Å) of the anticodon residue from its A-form position towards the codon partner or the major groove of the mini helix. In the first case, disallowed steric overlaps between the ribose ring of the first anticodon residue and the second anticodon base are observed (Figs. 2 and 3). In the second case a

Fig 4. The uracil-cytosine pair without (a) and with a water bridge (b).

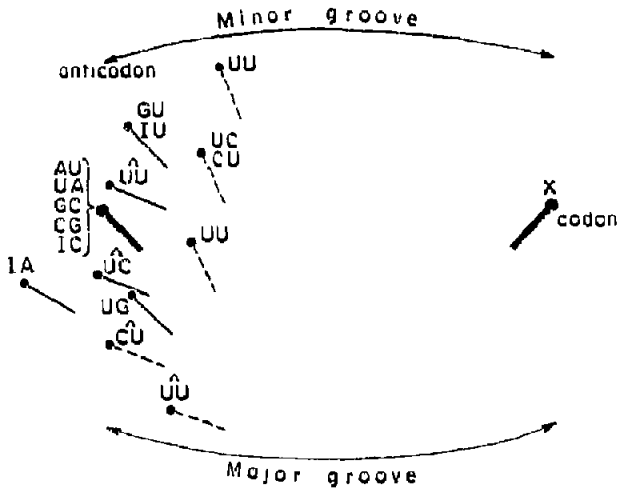

Fig. 5 The point X represents the position of the C1′ atom of the glycosyl bond in the third codon residue. The other points show where the C1′ atom and the glycosyl bond would lie for the various base pairs. The dashed lines are glycosyl bonds of sterically disallowed base pairs. U^U, U^C, C^U are the base pairs containing the water bridges. The length of the lines corresponds to 1.4 Å.

disallowed stretching of the sugar-phosphate backbone section between the first and second anticodon ribose rings takes place (Figs. 2 and 3). For instance, disallowed steric overlaps are caused by formation of the pairs UC and CU without a water bridge. In order to form these pairs, a considerable (~3 Å) displacement of the wobble base in the codon third base direction must be made (Fig. 5). Correspondingly, a disallowed stretching is observed by formation of the pair CU with a water bridge when the C-base is in the anticodon. In this case a considerable shift of the wobble base in the direction of the major groove should occur (Fig. 5).

Disallowed steric overlaps or stretching are not observed by formation of the purine–purine pair inosine–adenine (IA), but appreciable deformations (~10°) of the bond angles of the backbone section between the first and second anticodon residues take place. This suggests that inosine does not recognize adenine very well.

A major portion of the wobbling rules (Fig. 5) ensuing from the model coincide with those proposed by Crick [17]. In accordance with Crick's rules, **U** recognizes **A** and **G** (although according to our rules **U** can also recognize **U** and **C**); **C** recognizes **G**; **A** recognizes **U**; **G** recognizes **U** and **C**; **I** recognizes **U**, **C**, **A** (although according to our rules **I** should recognize **A** less well than **U** and **C**).

Our supplements to Crick's rules are supported experimentally. For example, the yeast $tRNA^{Ser}_{IGA}$ is incapable of effectively recognizing a UCA codon in the cell [18]. It is possible that a special isoacceptor tRNA may exist for codons ending with A [18,19]. With regard to uracil, the unmodified **U** recognizes all four bases. In cases where uracil is capable of recognizing only purine nucleotides in codons, the **U** in the first position of the anticodon is modified [19].

3. DISCUSSION – TESTING THE MODEL

The major key feature of our model is the interaction between the A- and P-site duplexes, which is observed only in the R-orientation of tRNA molecules (there are no interduplex interactions at all for values of ω greater than 120° [11]). In this article only stacking between the sugar–phosphate moiety of the A-site codon and the wobble base pair of the P-site duplex has been considered. The other component of the interduplex interaction is the interaction of the edge of the P-site wobble pair with the minor groove of the A-site mini helix and the purine base-37 of the A-site tRNA (Fig. 2). This interaction can equally stabilize or destabilize the A-site duplex, thus affecting for example the rate of ribosomal translation or the level of misreading. This stabilization–destabilization can be realized by selection of the appropriate isoacceptor tRNA species and/or synonymous codons for the A- and P-site duplexes. Thus, within the framework of a model of **interacting** codon–anticodon duplexes it is possible to attempt to understand codon context effects [6–8] at the molecular level in terms of concrete atomic interactions. The results of such a stereochemical analysis and the changes in the wobbling rules induced by modifications of the first anticodon residue will be published elsewhere.

The model can be subjected to experimental tests. Two obvious ones present themselves:

(1) The model (see Fig. 2) predicts that modifications of the P-site anticodon wobble base (especially modifications at the 5 position in uracil, which are most often observed) should affect the stability of the A-site duplex.

(2) Owing to the absence of inter-ribose hydrogen bonds, the translation of a deoxyribonucleic acid (DNA) single chain mRNA analogue should allow non-canonical base-pairing to occur at all three positions of the codon.

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