
Correlation between three-dimensional structure and chemical reactivity of transfer RNA

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Received 28 June, 1974

ABSTRACT

The bases of yeast tRNA^{Phe} which react with carbodiimide and methoxyamine have been determined and this information has been combined with chemical modification studies of other workers to produce a composite picture of base accessibility in this tRNA. The results are compared with the three-dimensional structure which we have recently determined. The bases which react chemically lie in exposed positions in the three-dimensional model and those which do not are either in the double helical stem regions or else are involved in maintaining the tertiary structure through pairing or stacking interactions.

INTRODUCTION

We have recently determined the three-dimensional structure of yeast tRNA^{Phe} at 3 Å resolution by an X-ray crystallographic analysis¹. Our structure has a similar overall shape to the structure recently described by a group at MIT², but there are significant differences in the density and in its interpretation. Above all we have been able to give a detailed description of the various base interactions involved in maintaining the tertiary structure, none of which are present in the MIT model. Our model is derived by interpreting the electron density map produced by the X-ray crystallographic analysis using the nucleotide sequence and stereochemical knowledge about the residues.

To supplement the crystallographic results and to clarify possible ambiguities in the structure determination we also embarked on chemical and enzymatic studies of yeast tRNA^{Phe} further to those already in the literature. In particular we have studied the effects of methoxyamine which reacts with cytosine bases and also with the carbodiimide reagent which reacts with uridine, guanosine and pseudo-uridine bases. In this paper we combine these results together with the results of chemical modification studies by other workers using different reagents to obtain a picture of the accessibility of the bases of yeast tRNA^{Phe} to chemical attack. The pattern of chemical modification is compared with the three-dimensional structure obtained by

X-ray analysis and the correlation between the exposed regions of the model and the regions of chemical reactivity is everywhere consistent.

MOLECULAR STRUCTURE

The main features of the model are sketched in figure 1 which also shows the topology of connections made in forming the tertiary structure. All but seven of the bases are represented in the diagram. Base pairs additional to those in the double helical stems of the clover leaf formula are shown as dotted lines. The stacking and interpolation of unpaired bases is also indicated. The region shown dashed is the TΨC loop - D loop corner where we have not been able to determine the structure unambiguously.

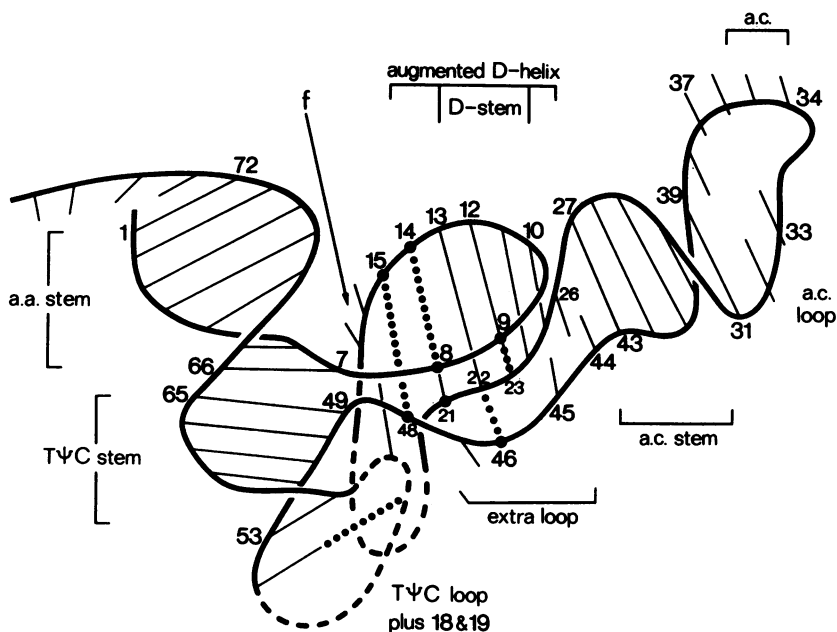


Figure 1

A schematic diagram of the tertiary structure of yeast tRNA^{Phe}. The ribose-phosphate backbone is represented by a continuous line, except where there is ambiguity when it is shown dashed. Base pairs in the double helical stems are represented by long light lines, and non-paired bases by shorter lines. Many of the latter stack as indicated; those which do not are drawn at an angle to the backbone. Base pairs additional to those in the clover leaf formula are indicated by dotted lines. f denotes residues D16 and D17.

The amino acid stem and the T ψ C stem are stacked together to form a continuous double helix. The anticodon stem and the D stem are also stacked although the two helical axes are not colinear: to a low order of approximation they form a single double helix which is roughly perpendicular to the first, amino acid plus T ψ C helix and joins the latter near its centre, so that the backbone structure of the tRNA molecule may be described as a T. A number of specific interactions responsible for maintaining the structure have been found. It is noteworthy that none of the additional base pairs so far found are of the Watson-Crick type. The base pairs U8-A14 and G15-C48 stack on the end of the D stem to produce an extended helix. The D stem is also swollen laterally by interactions with the short connecting sequence 8-9 and the extra loop III. A23 pairs with A9 resulting in a base triple 12-9-23. Similarly G46 pairs with G22 to give a second base triple 46-22-13. A21 interacts with 8, so that there is a third triple interaction in this region.

The single unpaired base 26 (which occurs in the equivalent position in all tRNAs) is stacked on the other end of the D stem. This base also partially intercalates between the bases 44 and 45, which are in turn stacked on the end of the anticodon stem. The result is that the anticodon stem is tilted relative to the D helix by an angle of 20°.

The anticodon loop is an ordered structure with a high degree of base stacking. On the 3' side A38 through G34 are stacked in a more or less helical manner. The sugar phosphate chain is kinked a little to allow a sort of gradual transition in the stacking. This places A38 on the anticodon helix and allows the remaining bases to move smoothly in a pseudo-helical configuration. Bases 32 and 33 on the 5' side are then stacked on each other, and may stack on the anticodon stem as well, but the overlap is not particularly strong.

The electron density corresponding to much of the T ψ C and D loops is difficult to interpret. It is broken in character and its unscrambling is complicated by the fact that both loops interact around the crystallographic screw axis with other molecules. Despite this difficulty some features of the loops seem reasonably certain. It appears for example that T54 continues stacking on the T ψ C helix and is also base paired to another residue, probably A58. The rest of the T ψ C loop is tightly folded and appears to interact with residues G18 and G19 of the D loop. It is also apparent that the variable residues D16, D17 and G20 are near each other on the molecular surface.

The variable loop III runs a rather straight path between the anticodon and T ψ C stems, with most of the bases involved in intramolecular contacts as already described. Only U47 appears to be exposed and it may serve some role in enzyme discrimination.

CHEMICAL MODIFICATION STUDIES

Yeast RNA was labelled by growing the yeast in a synthetic medium containing ^{32}P phosphate according to Rubin³. After isolating the tRNA by phenol extraction, the mixed tRNAs were separated by BD cellulose and RPC-5 column chromatography. The methods used for modifying tRNA₁^{Leu} with the reagents methoxyamine and 1-cyclohexyl-3-[2-morpholine-(4)-ethyl] carbodiimide methosylate in the presence of 10mM-magnesium ions were those described by Brown and his colleagues^{4,5,6}. The modified RNA samples were digested by T1 and pancreatic ribonucleases and analysed by two-dimensional electrophoretic fingerprinting techniques as described in references 4-7.

The extent and position of modification of yeast tRNA^{Phe} after treatment with carbodiimide or methoxyamine are marked in the cloverleaf diagram in figure 2. The figure also incorporates the results of other workers using different reagents⁸⁻¹¹. In general terms the CCA end and the anticodon loop are exposed or available to the chemical reactants while the T ψ C loop is not. Only the distal part of the D loop is available and only one of the bases of the extra loop III.

Of the six non-hydrogen bonded cytosine residues in the cloverleaf formula only two are reactive, C74 and C75 at the amino acid terminus. C33 in the anticodon loop shows only a trace reaction and C56 and C60 in the T ψ C loop do not react.

The only bases completely modified by the carbodiimide are U47, G20 and G34. There is some reaction at G18, which has not yet been quantitated. U33 is partially modified, a result which is consistent with studies on other tRNAs which indicate a gradient of chemical reactivity on the 5' side of the anticodon¹².

G4 and U69 in the amino acid stem are not affected by chemical modification. This is consistent with the electron density map obtained from the X-ray analysis in which no break in the double helix can be detected. It therefore appears likely that G and U form a base pair.

DISCUSSION

It is noteworthy that none of the bases which are involved in the tertiary interactions described in the last section and indicated in figure

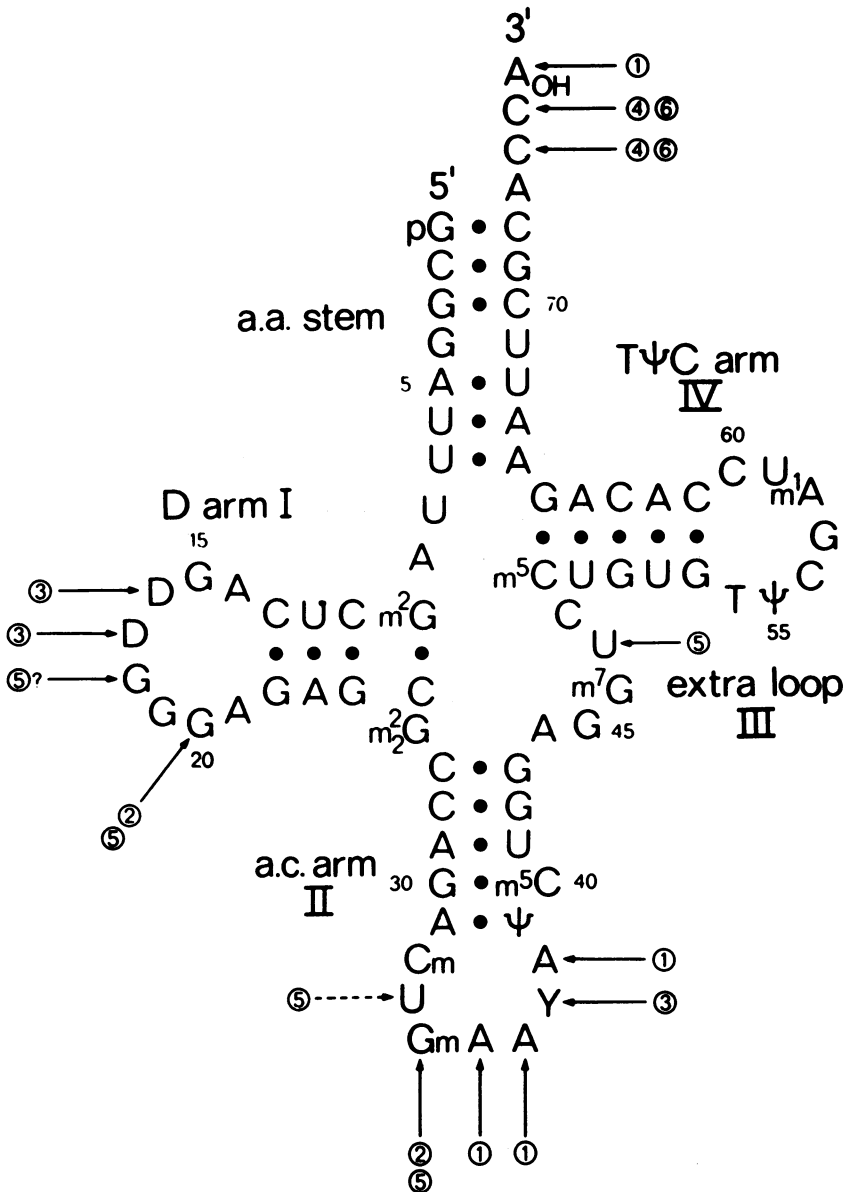


Figure 2

The full arrows indicate complete reactivity of bases towards the reagents listed below. The dotted arrow indicates a partial reaction and the question mark indicates a reaction to be confirmed.

- Reagents used (1) Perphthalic acid, specific for A-residues (8).
 (2) Kethoxal reacting with exposed G-residues (9).
 (3) NaBH₄ reduction (10, 11).
 (4) Methoxyamine)
 (5) Carbodiimide) (Rhodes, in preparation)
 (6) I₂/TlCl₃ (I. Batey unpublished results).

1 are modified by the methoxyamine and the carbodiimide and also that the bases which are strongly stacked or intercalated do not react as well. Of the residues which react, U47 is in an exposed position and so is G20. G34 is stacked but right at the tip of the molecule, and presumably therefore easily accessible. On the other hand U33 is partially protected by stacking on 32 and also by the proximity of its N3 to phosphate 36 on the other side of the anticodon loop.

All the results summarised in figure 2 are in good accord with the assumption behind chemical modification studies, as discussed for example by Cramer¹³, namely that a base residue which is not modified by a reagent specific for that base is inaccessible for one or more reasons. Bases involved in double helical stems of the molecule are unreactive and it is also assumed that the reaction will be diminished if a base is involved in maintaining the tertiary structure through stacking or base pairing. It will be seen that these assumptions are generally justified by a comparison of figures 1 and 2. However, a precise discussion requires examination of the environment of each potential site in the three dimensional model: thus A38, although stacked, has its N1 exposed, whereas C32 is not appropriately oriented.

We believe that our model can accommodate all tRNA sequences of the class I type¹⁴ and that many of its features would be retained in the other two classes. The pattern of chemical modification in figure 2 is very like that which has been obtained⁵ for formylmethionine tRNA which belongs to the same class I as yeast tRNA^{Phe}. None of the results of chemical modification studies on other tRNAs^{12,13} are inconsistent with our model, and indeed many of them confirm its essential correctness.

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