SELF-ASSEMBLY OF TRANSFER RNA FRAGMENTS

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Received 7 January 1970
(Revised version received 31 January 1970)

1. Introduction

This report is concerned with the results of our study of the association of halves and quarters of yeast $tRNA_1^{Val}$ molecules investigated by electrophoresis in polyacrylamide gel. The results provide evidence in favour of the "clover leaf" model of tRNA. The formation of the associated hybrid molecules composed of the 3′-half of yeast $tRNA_1^{Val}$ and 5′-halves isolated from total rat liver or *E. coli* tRNA or yeast tRNA after the preliminary removal of $tRNA^{Val}$ is also described. The restoration of acceptor activity in mixtures of the two halves and the four quarters of $tRNA_1^{Val}$ [1, 2] as well as that of 3′-half of yeast $tRNA_1^{Val}$ and of 5′-halves of total rat liver tRNA [3] has been reported previously.

2. Methods

The halves and quarters of $tRNA_1^{Val}$ were obtained by partial digestion with guanylo- and pyrimidylo-RNAases. Individual fragments were isolated by chromatography of the digests on DEAE-cellulose at pH 8.0 and pH 3.3 in 7 M urea [4]. Samples of 5′-halves were isolated from total rat liver tRNA and *E. coli* after incomplete hydrolysis with guanylo-RNAase [3]. Preparation of ^{14}C-valyl-3′-half of $tRNA_1^{Val}$ has been described [5]. For tritium label incorporation, the dephosphorylated fragments were oxidized with $NaIO_4$ and reduced with ^{3}H-$NaBH_4$ [6]. Electrophoresis in polyacrylamide gel (12% acrylamide and 2.5% methylene bis-acrylamide) was done in buffer containing 0.04 M tris acetate, 0.02 M sodium acetate, 0.002 M EDTA at pH 6.0 [7]. tRNA fragments were mixed in the electrophoresis buffer containing 5% of sucrose, incubated for 2 hr at 4° and applied to the surface of the polyacrylamide gel. Electrophoresis was carried out for 1.5 hr at +4° – +10°. Gel slabs, extracted from the siliconised glass tubes, were cut into 1 mm thick discs and the ^{14}C- or ^{3}H-radioactivity counted [7, 8].

3. Results

When $tRNA_1^{Val}$ is split at I_{35} of the anticodon (fig. 1) two halves are formed (3′-H and 5′-H). An additional splitting at G_{57} (T) and removal of the dinucleotide $C_{17}G_p$ (D) results in formation of four quarters (Q_1, Q_2, Q_3 and Q_4).

Fig. 2 (A) shows the positions of ^{14}C-valyl-tRNA, ^{14}C-valyl-3′-H and $Q_4(^3H)$ of $tRNA_1^{Val}$ in polyacrylamide gel and their complete electrophoretic separation. Fig. 2. also shows the electrophoresis of mixtures of all four quarters (B), of the two quarters $Q_1 + Q_4$ (C), and of yeast $tRNA_1^{Val}$ 3′-H and total rat liver tRNA 5′-halves (D). The association of other fragment combinations was also studied (table 1). Their electrophoretic patterns are not essentially different from those shown in fig. 2. It follows from the data in table 1 that the mixing of four quarters leads to the quantitative formation of completely associated "dissected" molecules of $tRNA_1^{Val}$. The contribution of each of the quarters $Q_2(^3H)$, $Q_3(^3H)$ or $Q_4(^3H)$ to the formation of completely associated

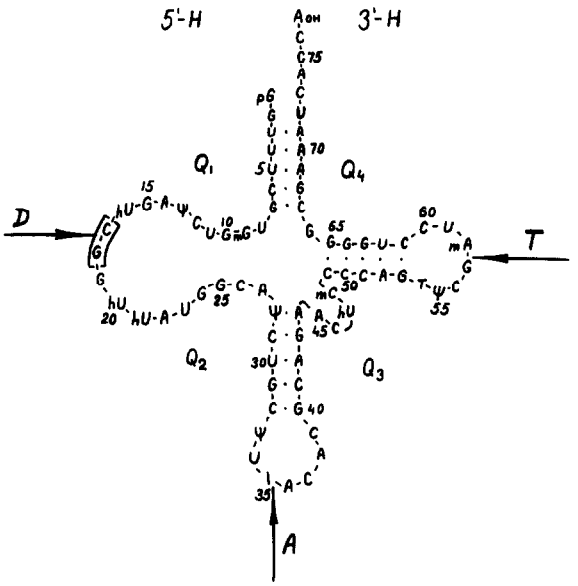

Fig. 1. Yeast $tRNA_1^{Val}$ ("clover leaf" model) [12].

molecules is shown in experiments 2–4. The electrophoresis of mixtures of two quarters $Q_1 + Q_4$, $Q_2 + Q_3$ and $Q_3 + Q_4$ leads to the shift of some of the radioactivity to the level of the halves, corresponding to the position of the associated dimers of the quarters (experiments 5–7). The extent of association of two quarters ranges from 30 to 80% but in no case is it quantitative. The absence of radioactivity in the gel between the positions of the halves and single quarters shows, however, that the associated dimers do not dissociate during electrophoresis (fig. 2C). No association is observed in the mixtures $Q_1 + Q_2$, $Q_1 + Q_3$ or $Q_2 + Q_4$ (experiments 8–10). The same results were obtained in the presence of Mg^{2+} ions (0.01 M) except that in experiments 5–7 the association of the mixture of two quarters became quantitative. Yeast $tRNA_1^{Val}$ 3'-half associates quantitatively with an equimolar amount of homologous 5'-valine half or with a tenfold excess of 5'-halves, isolated from total rat liver or *E. coli* tRNA, or yeast tRNA from which valine tRNA had been removed by countercurrent distribution.

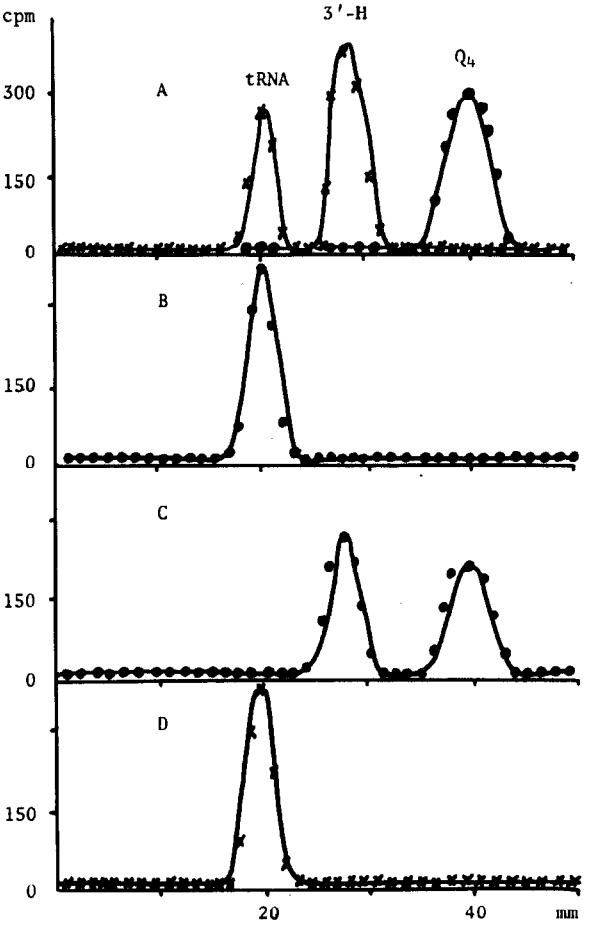

Fig. 2. Electrophoresis in polyacrylamide gel of a mixture of tRNA fragments.
A, ^{14}C-valyl-tRNA (A_{260} = 0.05) + ^{14}C-valyl-3'-H^{Val} (A_{260} = 0.05) + $Q_4(^3H)$ (A_{260} = 0.05);
B, $Q_1 + Q_2 + Q_3$ (A_{260} = 0.1 each) + $Q_4(^3H)$(A_{260} = 0.05);
C, Q_1 (A_{260} = 0.1) + $Q_4(^3H)$(A_{260} = 0.05);
D, ^{14}C-valyl-3-H^{Val}_{yeast} (A_{260} = 0.05) + 5'-$H^{total}_{rat\ liver}$ (A_{260} = 0.5).
x–x–x, ^{14}C cpm; •–•–•, ^{3}H cpm.

4. Discussion

The present data prove our earlier suggestion that the restoration of acceptor activity in mixtures of four $tRNA_1^{Val}$ quarters [2] as well as in mixtures of $tRNA_1^{Val}$ halves [1] is the result of their association and of the formation of a "dissected" molecule. On the other hand the data strongly suggest that the non-restoration of acceptor activity in any mixtures consisting of one half and one quarter (e.g. 5'-H + Q_4,

Table 1
Association of tRNA fragments.

Expt. No.	Composition of the mixture	Radioactivity (% of total)		
		Level of tRNA (18–22 mm)	Level of halves (28–31 mm)	Level of quarters (35–43 mm)
1	$Q_2(^3H)$, $Q_3(^3H)$ or $Q_4(^3H)$	0	0	100
2	$Q_1 + Q_2(^3H) + Q_3 + Q_4$	100	0	0
3	$Q_1 + Q_2 + Q_3(^3H) + Q_4$	100	0	0
4	$Q_1 + Q_2 + Q_3 + Q_4(^3H)$	100	0	0
5	$Q_1 + Q_4(^3H)$	0	54	46
6	$Q_2(^3H) + Q_3$	0	53	47
7	$Q_3 + Q_4(^3H)$	0	50	50
8	$Q_1 + Q_2(^3H)$	0	0	100
9	$Q_1 + Q_3(^3H)$	0	0	100
10	$Q_2 + Q_4(^3H)$	0	0	100
11	$^{14}C\text{-}3'\text{-}H^{Val}_{yeast}$	0	100	0
12	$^{14}C\text{-}3'\text{-}H^{Val}_{yeast} + 5'\text{-}H^{Val}_{yeast}$	100	0	0
13	$^{14}C\text{-}3'\text{-}H^{Val}_{yeast} + 5'\text{-}H^{total}_{rat\ liver}$	100	0	0
14	$^{14}C\text{-}3'\text{-}H^{Val}_{yeast} + 5'\text{-}H^{total}_{E.\ coli}$	100	0	0
15	$^{14}C\text{-}3'\text{-}H^{Val}_{yeast} + 5'\text{-}H^{-Val}_{yeast}$	100	0	0

The following components were mixed: $3'\text{-}H^{Val}_{yeast}$ (A_{260} = 0.05); 5′-half of yeast $tRNA^{Val}_1$ ($5'\text{-}H^{Val}_{yeast}$)($A_{260}$ = 0.05); 5′-halves from non-valine yeast tRNA($5'\text{-}H^{-Val}_{yeast}$), from total rat liver tRNA($5'\text{-}H^{total}_{rat\ liver}$) or *E. coli* tRNA ($5'\text{-}H^{total}_{E.\ coli}$) ($A_{260}$ = 0.5 each); tritium labelled quarters of yeast $tRNA^{Val}_1$(A_{260} = 0.05 each); non-radioactive quarters (A_{260} = 0.1 each). Electrophoresis was carried out in polyacrylamide gel (see Methods).

$3'\text{-H} + Q_1$, etc.) is not due to their inability to associate [2].

The association of $tRNA^{Val}_1$ quarters seems to be dependent on Watson-Crick complementary base pairing. The quarters $Q_1 + Q_4$ and $Q_3 + Q_4$ each have five neighbouring complementary base pairs (fig. 1), while $Q_2 + Q_3$ contains three additional complementary base pairs $(U_{23}GG) \cdot (C_{50}CA)$. The existence of complementary dinucleotides in fragments is of no account as these complexes are extremely unstable. The non-associating quarters $Q_1 + Q_2$ could only make two complementary pairs. Other non-associating mixtures of quarters contain either one cluster of three complementary base pairs $(U_{23}GG) \cdot (C_{75}CA)$ for $Q_2 + Q_4$ or two such clusters $(G_2UU) \cdot (A_{44}AC)$ and $(C_6GU) \cdot (A_{38}CG)$ for $Q_1 + Q_3$. These interactions appear, however, to be insufficient to stabilize associations.

The specificity of association of different combinations of quarter pairs indicates that the spatial structure of tRNA molecule is assured essentially by the interaction of an adequate number of adjacent complementary Watson-Crick pairs. For yeast $tRNA^{Val}_1$ one can propose two models involving a maximal number of complementary Watson-Crick pairs. One of them is the generally accepted "clover leaf" model (fig. 1), the other is the one-hairpin model [2]. Associations of the two quarters $Q_1 + Q_4$, $Q_2 + Q_3$ and $Q_3 + Q_4$ only or for all four quarters (table 1, experiments 2–7) substantiate exclusively the "clover leaf" model. The recently discovered

interactions of other fragments of tRNA is also in accord with this model [9, 10].

The 3′-half of yeast $tRNA_1^{Val}$ is able to form associations with 5′-halves isolated from non-valine yeast tRNA or from total rat liver or *E. coli* tRNA. In a tenfold excess these 5′-halves form associations with the 3′-half of yeast $tRNA_1^{Val}$ quantitatively; in a fivefold excess some part of the 3′-half radioactivity is found at the level of single halves. Hence only some of the different 5′-halves are able to associate with the 3′-valine half. The identity of heterologous halves that interact to form associations is as yet unknown. However one can postulate that the hybrid molecules are in some cases rather similar to native tRNA; for example the 3′-half of yeast $tRNA_1^{Val}$ in a mixture with 5′-halves of rat liver tRNA can be enzymatically aminoacylated by valine [3]. Active hybrid molecules consisting of halves of $tRNA^{Phe}$ isolated from yeast and wheat germ have been recently reported [11].

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