THE NEW APPROACH TO tRNA PRIMARY STRUCTURE DETERMINATION

THE PRIMARY STRUCTURE OF $tRNA^{Val}_{2b}$

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SUMMARY

The new combination of TLC and high voltage electrophoresis on cooling plate is described.We have applied this technique to study of primary structure of tRNA.Preliminary sequence of baker's yeast $tRNA^{Val}_{2b}$ is described.

New approach to preparation of large tRNA fragments is demonstrated.

There are some problems when the investigation of sequence of the tRNA or its long fragments is carried out by UV-methods.A rapid separation of oligonucleotides is necessary for this.Twe two dimensional electrophoresis by Sanger et.al. /1965 / is commonly used to separate the oligonucleotides.This method has excellent resolution and is used successfully in studing of the primary structure of nucleic acids. However,becavse of the low capacity of acetate cellulose and using of UV-absorbing buffers it may be applied only to the analysis of labelled material.

Murray /1970 / improved the Sanger's method by using the two-dimension electrophoresis on DEAE paper.This combination was found to be very excellent both in resolution / mainly for the short oligomers/ and in capacity.The high pH of the first dimension makes this method unapplicable to the separation of oligonucleotides of tRNA containing minor components e.g. m^1A, m^7G.

The attempt was made by Gangloff et.al /1972/ to overcome these

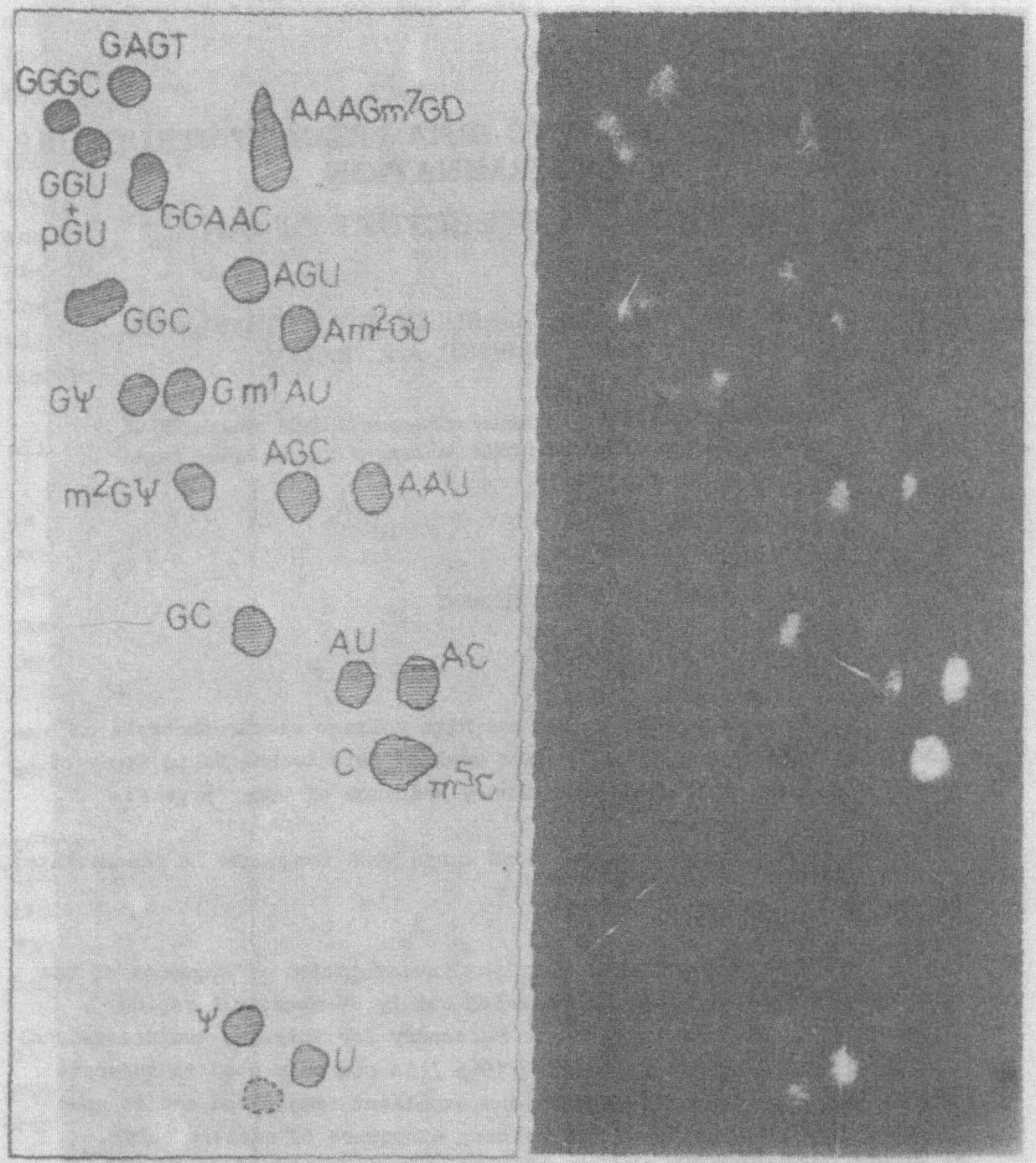

Fig.2 The fingerprint of pyrimidyl RNase digest of $tRNA^{Val}_{2b}$ from baker's yeast.

The isolated oligonucleotides were hydrolysed by ribonucleases and phosphdieesterase, analysed by TLC and as the result their sequences were determined. These procedures will be described in details elsewhere.

From the data obtained from this analysis and assuming that baker's yeast tRNA $^{Val}_{2b}$ satisfies the requirement of the maximum base pairing and the modified nucleosides occupy the constant positions as in the other tRNAs we reconstructed the primary structure of tRNA $^{Val}_{2b}$ /Fig.3/. It differs from tRNA $^{Val}_{2a}$ baker's yeast in 18 positions and from tRNA $^{Val}_{1}$ / baker's yeast / in 28 positions. Up to now more than 100 sequences of tRNAs were determined and it is possible to reconstruct the total sequence from the oligonucleotides of guanyl and pyrimidyl RNase digests.

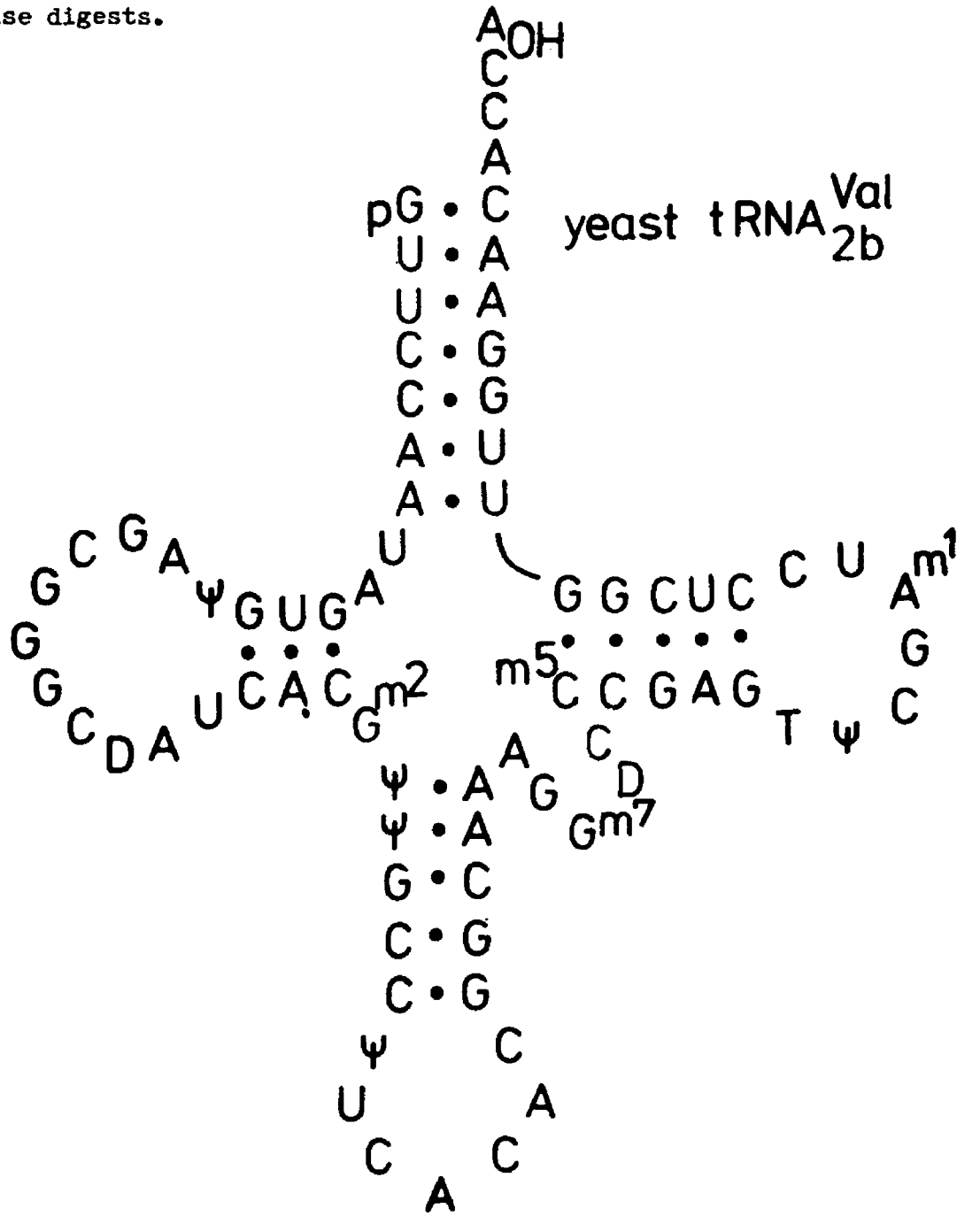

Fig.3 The preliminary primary structure of tRNA $^{Val}_{2b}$ from baker's yeast.

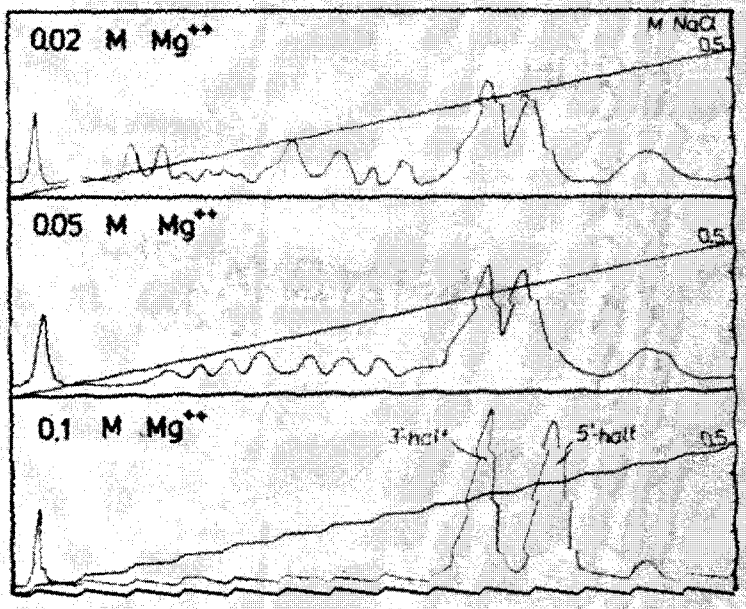

Fig.4 A The separation of pyrimidyl RNase digests of tRNA $^{Val}_{2a}$ from baker's yeast at different concentration of magnesium. Temperature hydrolysis is 0°C. The conditions of chromatography: column 1.4 mm × 140 mm. The load of 0.2 OD_{260} units. Gradient of NaCl from, 0 to 0.6 M in 7 M urea pH 3.5 / total volume 6 ml /. Flow 0.2 ml/hour.

Fig.4 B The electrophoresis on DE 81 paper of guanyl RNase digests of 5' and 3'- halves tRNA $^{Val}_{2a}$.

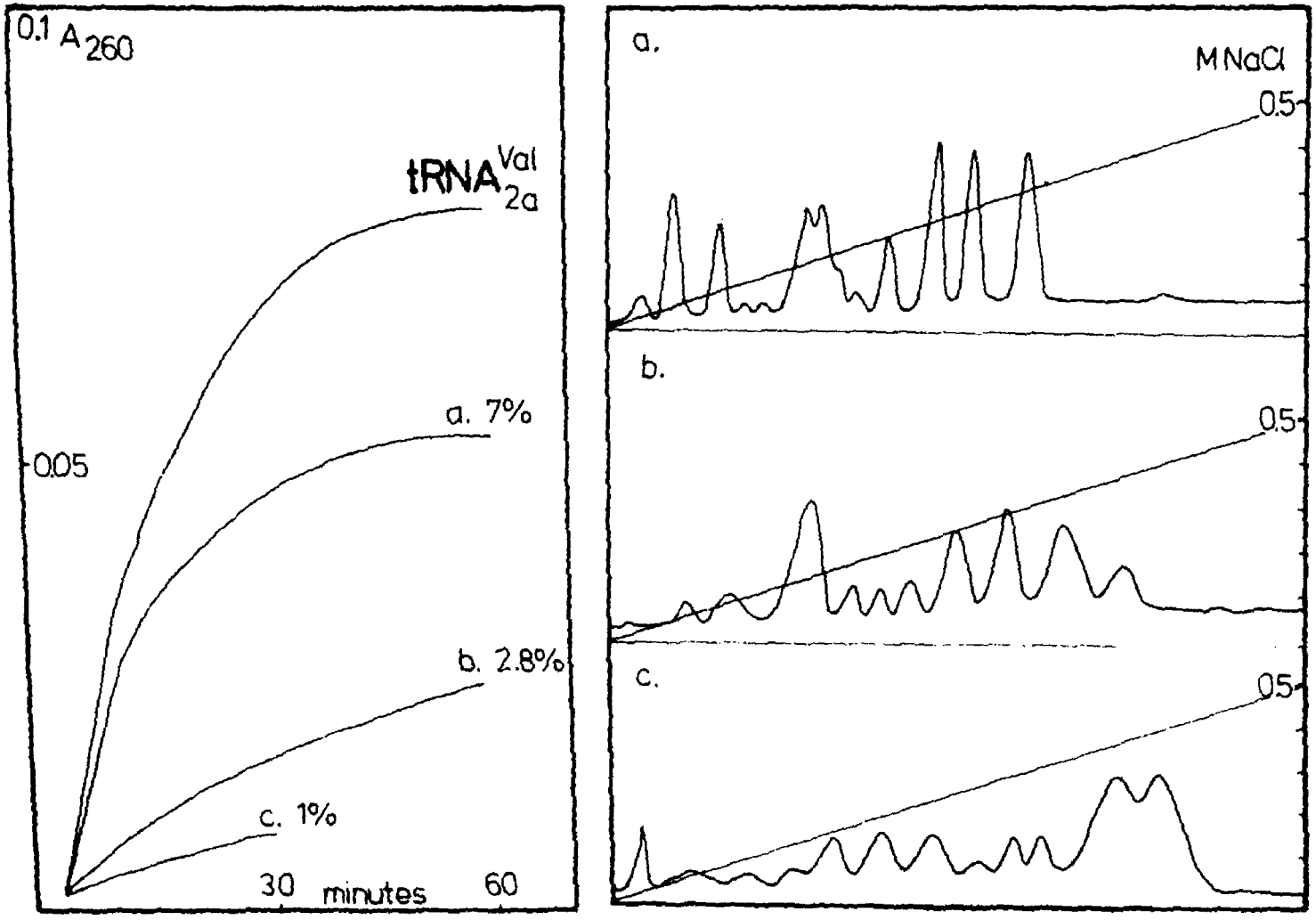

Fig.5 The hyperchromicity curves obtained during guanyl RNase digestion of 5'- half of baker's yeast tRNA $^{Val}_{2a}$ and analysis of digests by microcolumn chromatography.
Hydrolysis: 0.001 M EDTA, pH 7.5, t= +2°C.
The condition of column chromatography see in Fig.4 except: pH 7.5 .

However it is possible to make mistakes in this way.The new tRNA may have a unusual structure especially if modified nucleotides occupy unusual sites.The investigation of large fragments is necessary for the correct primary structure of tRNA.

We have studied the preparation of halves of tRNA becquse obtaining of the partial digests from halves is easier.The analysis of oligonucleotides in half molecule is easier too.The set of fragments obtained from tRNA by RNase digestion in presence of magnesium ions depends largely on the concentration of Mg^{2+}.The Fig.4 illustrates this relationship.When the concentration of Mg^{2+} is 0.1 M the halves and minimum of side products are formed reproducibly.The hydrolysis was stopped by addition of bentonite and EDTA.Digest was diluted ten times by water and applied to the column.The column was washed out with 0.001 M EDTA before sample application.

The set of fragments is obtained easier from half because the half have not as rigid in secondary structure as whole molecule.The partial digestion of the halfe of tRNA is more to carry out.The relationship between hyperchromicity and the degree of digestion of the half is shown on Fig.5 .It shows the curve hyperchromicity and the analysis products obtained at the different hyperchromicity values: a/ 7%, b/ 2.8% and c/1% .

We think,that technique of preparation and separation of the oligonucleotides and large fragments may be very useful for rapid analysis of nucleic acid sequences.

R E F E R E N C E S

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