ACCEPTOR ACTIVITY IN HOMOLOGOUS AND HETEROLOGOUS COMBINATIONS OF HALF MOLECULES FROM $tRNA^{Phe}_{yeast}$ AND $tRNA^{Phe}_{wheat}$.

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Summary: Homologous and heterologous combinations of half molecules from yeast and wheat germ $tRNA^{Phe}$ accept phenylalanine in the enzymatic charging reaction with Phe-tRNA synthetase from yeast. The charging of heterologous combinations is possible although in cloverleaf models several base pairs in the 3'-, 5'-terminal stem and in the stem of the anticodon loop are changed or can no longer be formed.

Introduction: The primary structures of $tRNA^{Phe}$ from yeast and wheat germ have been established by RajBhandary et al.[1] and Dudock et al.[2], respectively. The unknown bases Y of $tRNA^{Phe}_{yeast}$ and Y_w of $tRNA^{Phe}_{wheat}$, which are located in the anticodon regions of the tRNAs, can be excised under weakly acidic conditions without breaking the polynucleotide chains[3,4]. The modified tRNAs which are called $tRNA^{Phe}$(HCl) still contain the ribose moiety of the nucleoside Y or Y_w[3]. $tRNA^{Phe}_{yeast}$(HCl) chains can be split by chemical means next to this ribose and still accept phenylalanine[5]. An analogous chain scission was now found for $tRNA^{Phe}_{wheat}$(HCl). The availability of the half molecules allowed an investigation of certain structural requirements of the recognition of $tRNA^{Phe}$ by Phe-tRNA synthetase.

Materials and Methods: $tRNA^{Phe}_{yeast}$ and $tRNA^{Phe}_{yeast}$(HCl) were prepared as previously described[3,5] and had acceptor activities of 1.2 nmoles Phe/A_{260} unit. $tRNA_{wheat}$ was isolated from wheat germ of Bäcker-Kunstmühle, München, following the procedure of Moustafa and Lyttleton[6] with slight modifications: The phenol extraction was carried out at room temperature; after precipitation with ethanol the total RNA fraction was suspended with DEAE-cellulose and charged on to the column; tRNA was separated from the carbohydrates by gradient elution. $tRNA^{Phe}_{wheat}$ and $tRNA^{Phe}_{wheat}$(HCl) were prepared exactly as the corresponding tRNAs from yeast[5] and had acceptor activities of 0.95 nmoles Phe/A_{260} unit. A 300-fold purified preparation of Phe-tRNA synthetase from brewer's yeast, which was prepared similar to the procedure of Makman and Cantoni[7] was kindly supplied by Dr. R. Hirsch and Mr. J. van der Bosch. Polynucleotide kinase, which was prepared similar to the procedure of Richardson[8] was a gift of Dr. U.J. Hänggi. The half molecules were separated under conditions previously described for the fractionation of $tRNA^{Ser}_{yeast}$ fragments (column S-3 in ref. 9); gradients of 350 ml each of 0.1 and 0.4 M NaCl and 500 ml each of 0.1 and 0.5 M NaCl were used for the half molecules from $tRNA^{Phe}_{yeast}$ and $tRNA^{Phe}_{wheat}$, respectively.

Results: According to disc electrophoresis the splitting of $tRNA^{Phe}_{wheat}$(HCl) into half molecules was as selective as the analogous reaction with $tRNA^{Phe}_{yeast}$(HCl). Under the previously described conditions[5] the CCA-half of $tRNA^{Phe}_{yeast}$ moved faster than its pG-half; with the half molecules from $tRNA^{Phe}_{wheat}$ the order was reversed. On a preparative scale the half molecules were easily separated on DEAE-Sephadex columns at pH 3.0. The CCA- and pG-

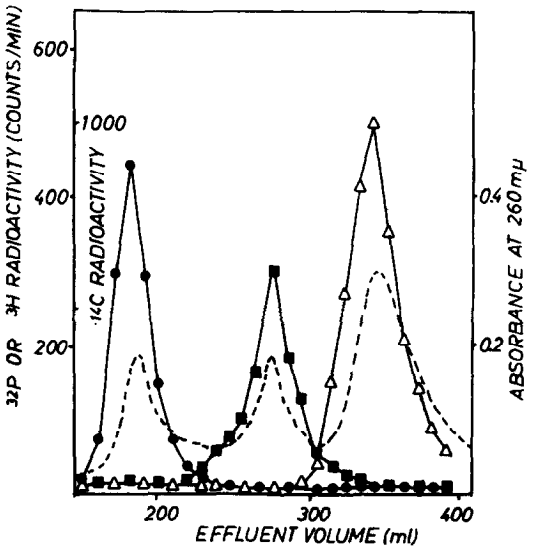

Fig. 1: Identification of labeled half molecules from $tRNA^{Phe}_{yeast}$ by cochromatography: 10 A_{260} units of $tRNA^{Phe}_{HCl}$ were charged with ^{3}H-Phe (180 C/mole) under previously described conditions[3]. A mixture of 2 A_{260} units each of the pG- and the CCA-halves was charged with ^{14}C-Phe (61 C/mole). 4 A_{260} units of $tRNA^{Phe}_{yeast}$(HCl) were dephosphorylated with 2 units bacterial alkaline phosphatase at 45° for 90 min. and rephosphorylated with polynucleotide kinase (3 units, 50 nmoles γ-^{32}P-ATP with ca. 25 C/mole, 1.6 μmoles Tris-HCl, pH 7.6, 0.8 μmoles $MgCl_2$, 0.4 μmoles mercaptoethanol, and 0.25 mg aldolase as carrier protein, in 0.25 ml). The ^{32}P-$tRNA^{Phe}_{yeast}$(HCl) was treated with aniline under the previously described conditions[5]. The charged tRNA and the half molecules were mixed after phenol treatment and acid precipitation and chromatographed on a DEAE-Sephadex A-25 column (0.4 x 140 cm, linear gradient of 350 ml each, 0.1 and 0.5 M NaCl in 7 M urea, adjusted with HCl to pH 3.0). 1.0 ml aliquots were counted in Bray's solution[10]. --- A_{260} absorption, Δ-Δ ^{3}H, ●-● ^{14}C, ■-■ ^{32}P.

halves were identified by charging with ^{14}C-Phe and by replacing the 5'-terminal phosphate by ^{32}P, respectively (Fig. 1). ^{3}H-Phe-$tRNA^{Phe}_{HCl}$ was cochromatographed with the half molecules in order to locate the position of the intact tRNA in the elution diagram and to determine the amount of unsplit tRNA in the halves. Since the ^{3}H-peak did not contain any ^{14}C-radioactivity it can be concluded that the half molecules were free from intact tRNA. The half molecules from $tRNA^{Phe}_{wheat}$ were identified by analogous chromatography experiments.

Absolute values for the acceptor activities of the half molecules and of the combinations of half molecules are shown in Fig. 2. If the acceptor activities of the untreated tRNAs (see Methods) are taken as 100%, the halves alone always accepted less than 2%. 25 - 70% acceptor activity were obtained in the homologous and heterologous combinations of half molecules (Fig. 2). Slightly lower values were obtained when the mixtures were not heated prior to the incubation, when the incubations were run at 37° instead of 25°, or when a crude synthetase preparation was used instead of the purified one. In homologous and heterologous combinations involving the pG-half of $tRNA^{Phe}_{wheat}$ acceptor activities of 40% were obtained shortly after the isolation of the fragment. The activities gradually dropped on storage of the fragment. This effect seems to be related to the poor solubility of the fragment in water (1.5 A_{260} units/ml; after a few days at -20° only 0.3 ~ 0.5 A_{260} units/ml) which may be due the clustering of G-residues. In salt-urea solution the solubility is greatly increased. It is likely that the acceptor activities of the homologous and heterologous combinations can still be increased by further varying the conditions of the pretreatment and the charging reaction.

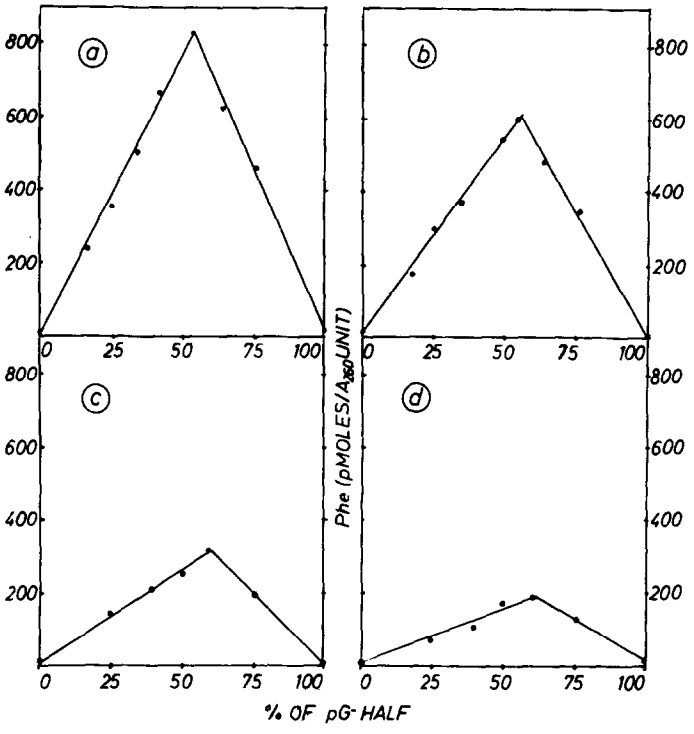

Fig. 2: Acceptor activities of combinations of half molecules from $tRNA^{Phe}$: In 0.1 ml 6 milliunits Phe-tRNA synthetase[7], 5 nmoles L-^{14}C-Phe, 1 µmole ATP, 1.5 µmoles $MgCl_2$, and 2.5 µmoles Tris-HCl, pH 7.5, were incubated for 45 min. at 25° with constant total amounts of the mixtures of half molecules (0.075 A_{260} units in a. and b., 0.02 A_{260} units in c. and d.). The mixtures of half molecules were heated for 1 min. at 75° in 0.05 ml buffer (150 mM KCl, 5 mM $MgCl_2$, 0.5 mM EDTA, 10 mM cacodylate, pH 7.0) prior to the incubation. a. pG- and CCA-halves of $tRNA^{Phe}_{yeast}$. b. pG-half of $tRNA^{Phe}_{yeast}$ and CCA-half of $tRNA^{Phe}_{wheat}$. c. pG- and CCA-halves of $tRNA^{Phe}_{wheat}$. d. pG-half of $tRNA^{Phe}_{wheat}$ and CCA-half of $tRNA^{Phe}_{yeast}$.

The highest phenylalanine incorporations were observed, when the pG-halves were 54-60% of the total A_{260} units in the incubation mixtures. From adding up the molar extinctions of the constituent

nucleotides of the fragments, equivalence points in the 47-50% range would be expected. The difference may be due to a higher hypochromicity in the CCA-halves compared to the pG-halves and to a number of secondary factors.

Discussion: tRNA molecules with chain scissions in a number of positions have been found to possess acceptor activity[5,11-16] In the present paper the first case of a successful recombination of fragments from tRNAs of different species is reported. $tRNA^{Phe}_{yeast}$ and $tRNA^{Phe}_{wheat}$, which differ in 16 of their 76 nucleotides, are both chargeable by Phe-tRNA synthetases from either source[2]. In

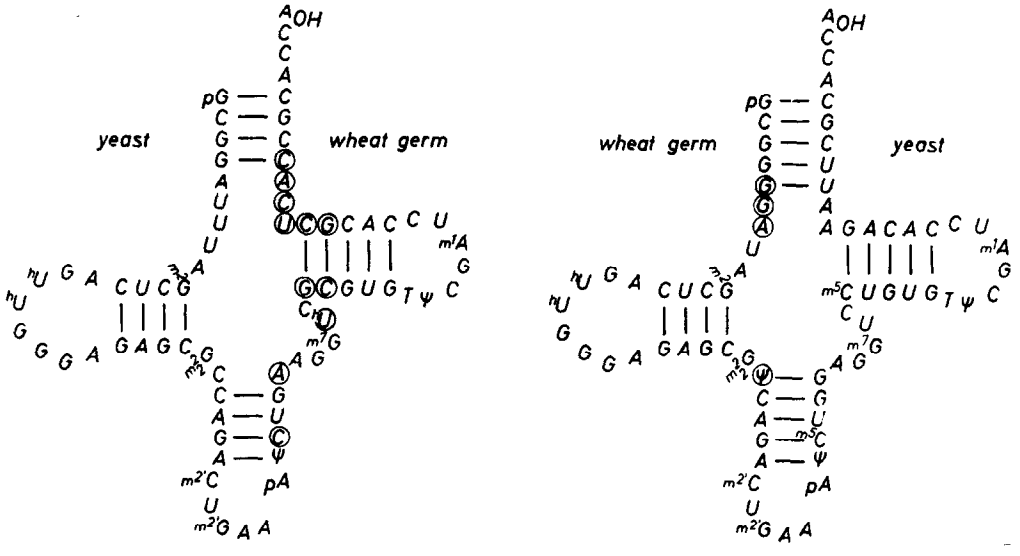

Fig. 3: Combination of half molecules from $tRNA^{Phe}_{yeast}$ and $tRNA^{Phe}_{wheat}$ in cloverleaf models. The nucleotide sequences are according to RajBhandary et al.[1] and Dudock et al.[2], respectively. Those nucleotides of $tRNA^{Phe}_{wheat}$ which differ from the corresponding nucleotides of $tRNA^{Phe}_{yeast}$ are encircled.

our experiments only the synthetase from yeast has been used up to now.

The finding of acceptor activity in heterologous combinations of half molecules indicates that certain base pairs are dispensable for synthetase recognition. As can be seen from Fig. 3, one base pair in the stem of the anticodon region and 3 or 4 base pairs in the 3'-, 5'-terminal part of the cloverleaf models are changed or eliminated in the combinations and, therefore, seem not to be essential for synthetase recognition. Detailed analyses of the various combinations of fragments by physical methods have to be performed and the kinetic data of the charging reaction should be determined before further conclusions with respect to the threedimensional structure and the synthetase recognition sites can be drawn.

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