# YEAST $tRNA_1^{Lys}$ (ANTICODON CUU) TRANSLATES AAA CODON

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## 1. Introduction

We have shown [1] in an in vitro protein-synthesizing system dependent on added valine tRNA (valyl-$tRNA^{Val}$) and programmed with RNA from MS2 phage, that each of the isoaccepting valyl-$tRNAs^{Val}$ with anticodons UAC, GAC and IAC can recognize all four valine codons GUU, GUC, GUA and GUG. We therefore concluded that the genetic code, as far as the valine codons are concerned, is operationally a two letter code, i.e., the third codon nucleotide has no absolute discriminating function. These results are not according to the rules predicted by the wobble hypothesis [2]. The genetic code is made up of groups of four codons where the first two positions are the same for all four codons while the third position can be occupied by any of the nucleotides U, C, A and G. However, In the case of the asparagine (AAU, AAC) and lysine (AAA, AAG) codon group, a lysine tRNA does not recognize asparagine codons and vice versa. This is documented by the fact that there is a very low error frequency in protein synthesis and this clearly indicates that interactions which would lead to the incorporation of the wrong amino acid into protein are not allowed. However, it remains to be seen in the lysine codon group whether the rules of wobble are still strictly applicable or not. Results are presented here which show that a $tRNA^{Lys}$ with anticodon CUU can recognize both the lysine codons AAA and AAG. The recognition of AA codon cannot be explained by the wobble hypothesis.

## 2. Materials and methods

### 2.1. *Materials*

MS2 RNA was prepared from MS2 phage as in [1]. Crude tRNA from brewers yeast was obtained from Boehringer. Crude tRNA from *E. coli* was obtained from Bio-Cult Laboratories. Antibody against purified *E. coli* lysyl-tRNA synthetase was prepared according to the procedure in [3] and was provided by P. Elias of this department. Trinucleotides AAA and AAG were obtained from Miles Laboratory.

### 2.2. *Purification of $tRNA_1^{Lys}$ and $tRNA_2^{Lys}$ from yeast*

Highly purified $tRNA_1^{Lys}$ and $tRNA_2^{Lys}$ were prepared from crude yeast tRNA by chromatography on BD-cellulose columns before and after phenoxyacetylation as in [4,5]. The isoaccepting $tRNAs^{Lys}$ were esterified with [$^{14}$C]lysine as in [6].

### 2.3. *Cell-free extracts and protein synthesizing conditions*

The S30 fraction from *E. coli* B was prepared as in [1]. The conditions for in vitro coat protain synthesis were as in [1] with the difference that esterified [$^{14}$C]-lysyl-tRNAs were used. When antibody against the lysyl-tRNA synthetase was used, the S30 fraction was preincubated with a suitable amount of antibody before addition of MS2 RNA and lysyl-tRNAs. The hot acid precipitable radioactivity was taken to be a measure of more than 90% of coat protein synthesis under these conditions, as determined in [1] with valyl-$tRNAs^{Val}$.



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2.4. *Ribosome binding assay*

The ribosome binding assays with isoaccepting [$^{14}$C]lysyl-tRNAs$^{Lys}$ were carried out according to the procedure in [7].

## 3. Results

### 3.1. *Binding properties of esterified tRNA$_1^{Lys}$ and tRNA$_2^{Lys}$*

Binding experiments were carried out with ribosomes from *E. coli* in the presence and absence of AAA and AAG trinucleotides. The results are shown in table 1.

It is evident from the results that tRNA$_1^{Lys}$ is absolutely specific for AAG codon whereas tRNA$_1^{Lys}$ recognizes both lysine codons AAA and AAG to about the same extent. The nucleotide sequences of both tRNA$_1^{Lys}$ and tRNA$_2^{Lys}$ are known [8,9]. The anticodon of yeast tRNA$_1^{Lys}$ is CUU and according to the wobble hypothesis should only respond to the AAG codon, whereas yeast tRNA$_2^{Lys}$ has anticodon ZUU (where Z is a derivative of 2-thiouridine). If Z is functioning like U, it should respond to both AAA and AAG codon according to wobble theory. Although it was found that 2-thiouridine in the wobble position of the anticodon of tRNA$^{Glu}$ from yeast [10] is unable to base pair with G, tRNA$_2^{Lys}$ from yeast is able to base pair with both A and G. The reason for these differences is not known.

### 3.2. *Codon–anticodon recognition in coat protein synthesis*

In order to study codon recognition with esterified tRNA$_1^{Lys}$ or tRNA$_2^{Lys}$ the in vitro protein synthesizing system should be strictly dependent on the esterified lysyl-tRNAs. To achieve this, the S30 protein synthesizing extract was preincubated with antibody against lysyl-tRNA synthetase to cut off the activity of the enzyme responsible for the esterification of lysine to tRNA$^{Lys}$ present in the crude extract and also in the mixture of crude *E. coli* tRNA, necessary for complete protein synthesis. Under this condition the incorporation of free lysine into coat protein in the presence of MS2 RNA was practically reduced to a negligible percent compared to the incorporation of lysine from pre-esterified lysyl-tRNA. The result of incorporation of [$^{14}$C]lysine into coat protein from individual pre-esterified isoaccepting species of tRNA$^{Lys}$ is shown in table 2. It is evident from the results presented in table 2 that both *E. coli* lysyl-tRNA$^{Lys}$ and yeast lysyl-tRNA$_2^{Lys}$ incorporated lysine into coat protein to about the same extent which is expected because both these tRNAs can recognize both lysine codons AAA and AAG. Although the rate and extent of incorporation of lysine from yeast tRNA$_1^{Lys}$ is low, it is very significant as no incorporation of lysine into coat protein was expected if the rule of wobble hypothesis was strictly followed.

Table 1
Binding properties of yeast lysyl-tRNAs$^{Lys}$ to trinucleotides

| Trinucleotide | None (pmol) | AAA (pmol) | AAG (pmol) |
|---|---|---|---|
| Yeast [$^{14}$C]lysyl tRNA$_1^{Lys}$ | 5.8 | 6.0 | 14.7 |
| Yeast [$^{14}$C]lysyl tRNA$_2^{Lys}$ | 3.7 | 9.7 | 10.1 |

The reaction mixture contained 0.18 $A_{260}$ unit of AAA and 0.15 $A_{260}$ unit of AAG, 2–3 $A_{260}$ units of ribosomes from *E. coli* $Q_{13}$ and 91 pmol [$^{14}$C]lysyl-tRNA$_1^{Lys}$ and 69 pmol [$^{14}$C]lysyl-tRNA$_2^{Lys}$

Table 2

| Reaction mixture containing | [$^{14}$C]Lysine incorporated (pmol) | | |
|---|---|---|---|
| | –MS2 | +MS | Δ |
| 1. Crude [$^{14}$C]lysyl-tRNA (*E. coli*) | 5.2 | 55.8 | 50.6 |
| 2. [$^{14}$C]Lysyl-tRNA$_1^{Lys}$ (yeast) | 1.1 | 17.7 | 16.6 |
| 3. [$^{14}$C]Lysyl-tRNA$_2^{Lys}$ (yeast) | 4.6 | 48.9 | 44.3 |
| 4. [$^{14}$C]Lysine | 1.2 | 2.3 | 1.1 |

Incorporation of [$^{14}$C]lysine from free and from pre-esterified [$^{14}$C]lysyl-tRNAs into hot acid-precipitable material. The S30 fraction was preincubated at 0°C for 20 min with a predetermined amount of antibody against the lysyl-tRNA synthetase. After the preincubation, 20 nmol each of 19 cold amino acids and 100 nmol cold lysine were added to experiments 1, 2 and 3. Experiment 4 received only 20 nmol 19 cold amino acids minus lysine. 4.0–5.0 $A_{260}$ units of MS2 RNA were then added (control experiment was run without MS2 RNA). 4.4 $A_{260}$ units of crude tRNA from *E. coli* were added to experiments 2, 3 and 4. The reaction was started with 800–900 pmol lysyl-tRNAs or 2000 pmol free [$^{14}$C]lysine. The reaction was carried out in total vol. 0.25 ml. Incubation was for 60 min at 37°C and the reaction was stopped and the radioactivity was determined as in [1]

## 4. Discussion

An examination of the sequence of the MS2 coat protein and coat protein cistron [11] shows that there are six lysine residues in the coat protein of 129 amino acids long. The six lysines with their corresponding codons are situated in position 43 (AAA), 57 (AAA), 61 (AAA), 66 (AAA), 106 (AAG) and 113 (AAA). Yeast $tRNA_1^{Lys}$ with anticodon CUU should be specific for AAG codon. This was also demonstrated by the result of the ribosome binding experiment shown in table 1. However, $tRNA_1^{Lys}$ incorporated a significant amount of lysine into coat protein whereas no incorporation was expected as the first four lysine codons in MS2 RNA correspond to AAA codons. The incorporation of [$^{14}$C]lysine from $tRNA_1^{Lys}$ into coat protein can only be explained if C in the first position of anticodon (wobble position) is also able to recognize A in the third position of codon. Thus this result is at variance with our present concept of how the genetic code is read, and also shows that the studies of ribosome binding of tRNAs do not always give a conclusive answer regarding their utilisation in protein synthesis.

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