LEUCINE $tRNA_1$ FROM *HisT* MUTANT OF *SALMONELLA TYPHIMURIUM* LACKS TWO PSEUDOURIDINES

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1. Introduction

There is ample evidence which points to the involvement of tRNA in metabolic regulation [1]. Recently it became known that even minor alterations in tRNA structure such as a lack in base modification may render such a tRNA ineffective in repression. The *hisT* mutant of *Salmonella typhimurium*, a mutant defective in proper regulation of the histidine operon, appears to have a defect in enzyme activity which converts uridine into pseudouridine in tRNA. Thus, the $tRNA^{His}$ in this mutant lacks two pseudouridines which are present in the anticodon region of the wild type $tRNA^{His}$ [2, 3]. The enzymes of the histidine operon are derepressed [4]. Following our earlier sequence studies of $tRNA_1^{Leu}$ which showed the presence of two pseudouridines in the anticodon region [5] and our studies on pseudouridine biosynthesis in tRNA [6] we investigated the structure of the major $tRNA^{Leu}$ in the wild type as well as the *hisT* mutant of *S. typhimurium*. Additional interest came from the observation (H.S. Allaudeen, unpublished results) that the leucine biosynthetic enzymes in the mutant are derepressed.

This paper describes the total nucleotide sequence of the major $tRNA^{Leu}$ species of *S. typhimurium*. Its primary structure is identical to that of the major $tRNA^{Leu}$ species of *E. coli* [5, 7]. A comparison of the nucleotide sequence of this tRNA with that from the *hisT* mutant reveals that the mutant tRNA lacks two pseudouridines in the anticodon region and contains instead two uridines. The T–ψ–C sequence is unaltered. Using cell-free extracts from *E. coli* an *in vitro* conversion of the two uridines in the anticodon region of the tRNA to pseudouridines was achieved.

2. Materials and methods

Wild type *S. typhimurium* (LT2) and the mutant *hisT* 1504 were kindly given by Dr. B.N. Ames. The growth of cells and extraction of ^{32}P-labelled tRNA were performed as described earlier for *E. coli* [5]. Electrophoresis on polyacrylamide slab gel was done as described by De Wachter and Fiers [8] and in the legend to fig. 1. After electrophoresis the gel was autoradiographed and the bands of interest were excised. The nucleic acid material of these bands was eluted electrophoretically. The tRNA was subsequently chromatographed on benzoylated DEAE-cellulose as described earlier [9]. The aminoacylation of *Salmonella* tRNA was carried out with pure *E. coli* leucyl-tRNA synthetase [10]. The radioactive $tRNA^{Leu}$ was sequenced according to the methods described by Barrell [11]. An undialyzed S-100 preparation of *E. coli* Q13 was used for the *in vitro* modification of uridines to pseudouridines in the mutant tRNA.

3. Results

$tRNA^{Leu}$ from *S. typhimurium* was purified by electrophoresis in a 10% polyacrylamide gel (fig. 1). The band 1 in fig 1 was cut out. The electrophoretically eluted material was about 60% pure $tRNA^{Leu}$. Subsequent benzoylated DEAE-cellulose chromatography yielded pure $tRNA_1^{Leu}$. Complete digestion of this material with pancreatic and T_1 ribonucleases

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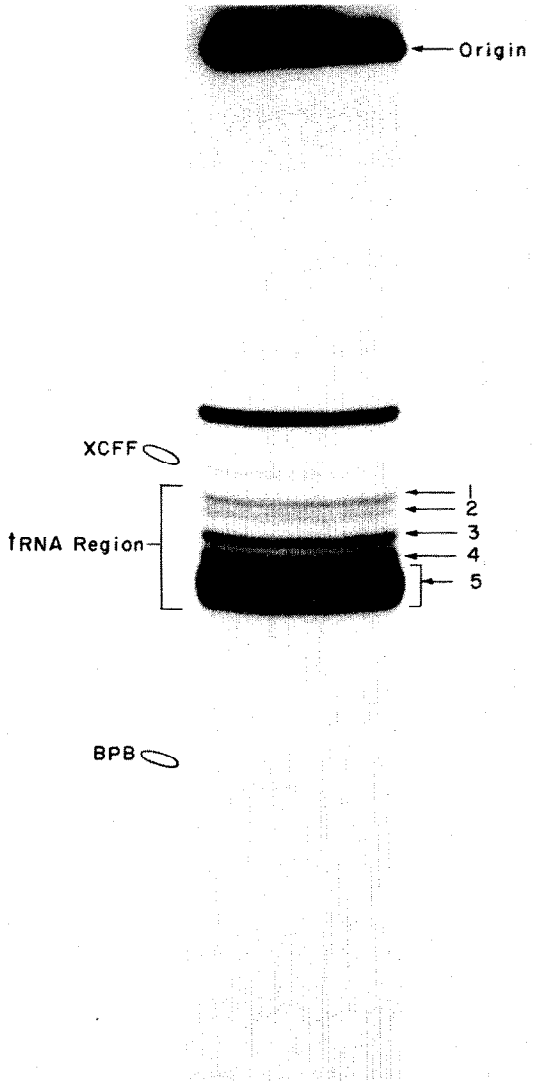

Fig. 1. Separation of $tRNA^{Leu}$ by electrophoresis on polyacrylamide slab gel. The gel (9.7% acrylamide, 0.3% bisacrylamide) was prepared as described by De Wachter and Fiers [8]; 0.09 M Tris-boric acid buffer containing 0.025 M EDTA was used. RNA labelled with ^{32}P was isolated by phenol extraction from *S. typhimurium* grown in the presence of [^{32}P] phosphoric acid. Electrophoresis was carried out at 6° for 20 hr at a constant current of 30 mA and 300–500 V. XCFF and BPB indicate the positions of the marker dyes Xylene cyanol FF and bromophenol blue, respectively.

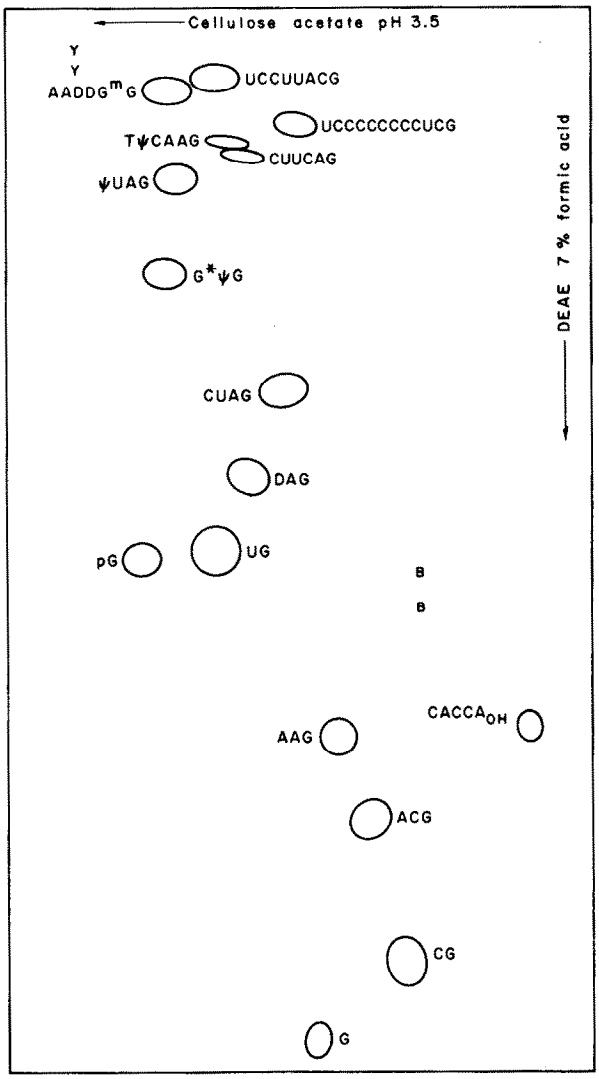

Fig. 2. A two-dimensional fractionation of T_1-RNAase digest of $tRNA_1^{Leu}$ from *S. typhimurium* LT2. B and Y refer to the positions of the blue and yellow markers, respectively.

yielded oligonucleotides listed in table 1. It was immediately obvious from the fingerprint (fig. 2) that the nucleotide sequence of *Salmonella* $tRNA_1^{Leu}$ is identical to that of *E. coli* $tRNA_1^{Leu}$ [5, 7]. Analysis of the large fragments obtained by partial digestion by T_1 ribonuclease of the tRNA confirmed this and established unambiguously the sequence shown in fig. 3.

When the same experiments were carried out to isolate and sequence the $tRNA^{Leu}$ of the *hisT* mutant, all profiles and fingerprints were similar to those of wild type tRNA. When the content of modified nucleosides in this tRNA was examined it was clearly

Table 1
Products formed by complete degradation of $tRNA_1^{Leu}$ with:

Pancreatic RNAase	T_1 RNAase
14C	8G
9 U+ψ+D	3CG
	$CACCA_{OH}$
2AC	2ACG
3GC	AAG
AGC	pG
AGAC	3 UG
GGC	DAG
2 GU	CUAG
Gψ	G*ψG
pGC	ψUAG
AGU	CUUCAG
GGAC	TψCAAG
AAGU	UCCUUACG
G^mGD	UCCCCCCCCUCG
AGG*ψ	AADDG^mG
GGAAD	
GAAGGU	
GGGGGT	

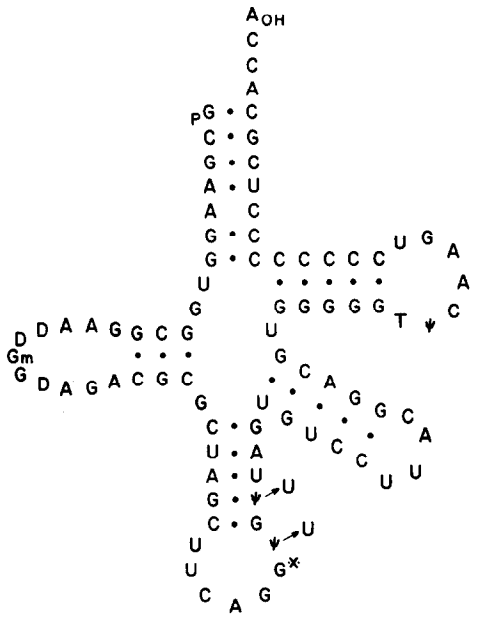

Fig. 3. Cloverleaf model of the nucleotide sequence of $tRNA_1^{Leu}$ from *S. typhimurium* LT2. The nucleotide sequence of $tRNA_1^{Leu}$ from the mutant *hisT* is the same except that it contains U in positions 39 and 41 instead of ψ as in the tRNA from the wild type.

seen that the dihydrouridine, ribothymidine, and G* positions were all normal as in the $tRNA_1^{Leu}$ of the wild type *Salmonella*. However, the pseudouridines in positions 39 and 41 were not present but were replaced by uridines (fig. 3 and table 2). The pseudouridine in position 66 (T−ψ−C loop) was present. Thus the situation is analogous to that in $tRNA^{His}$ of this mutant.

Previously we had reported that an enzyme preparation from *E. coli* brought about pseudouridine formation in the *in vitro* transcription product of tRNA genes and also in $tRNA^{His}$ from *hisT* mutant [6, 12]. When the $tRNA_1^{Leu}$ of this mutant was treated with the S-100 preparation of *E. coli*, the uridines in both positions 39 and 41 were converted to pseudouridines (table 2).

4. Discussion

The close analogy between *Salmonella* and *E. coli* found at the tRNA level tor $tRNA^{His}$ [3, 13] has also been observed for the major $tRNA^{Leu}$ species. More interestingly, the *hisT* mutant which is defective in the regulation of the histidine operon is also defective in the regulation of the leucine operon (H.S. Allaudeen, unpublished results). In both cases the cognate tRNA lacks two pseudouridines in the anticodon region. Thus, the enzyme or enzymes responsible for pseudouridine formation in this region of tRNA appears to be inactive in the *hisT* mutant. The specific recognition signal of this enzyme would be interesting to elucidate since the pseudouridines in the anticodon region of $tRNA^{His}$ and $tRNA^{Leu}$ are not in exactly the same position or nucleotide sequence The converion of the two uridines in the anticodon region of the mutant $tRNA^{Leu}$ to pseudouridines by *E. coli* extracts again shows the close homology between these organisms. It would be interesting to see whether one pseudouridine modification is a prerequisite for the other. We have not investigated the chromatographic behavior of the $tRNA^{Leu}$ and do not know whether the observed structural change in the mutant tRNA leads to the altered elution pattern seen

Abbreviation G* used above = unknown modified nucleoside.

Table 2
Oligonucleotides containing ψ found (%) $tRNA_1^{Leu}$.

tRNA from	G*ψG	GUG	ψUAG	UUAG	TψCAAG
LT2	100	--	100	--	100
hisT		100	--	100	100
hisT treated with *E. coli* extract	32	68	63	37	100

on reversed phase chromatography [2]. It will be interesting to learn whether the situation is similar in other cases where the tRNA's contain two pseudouridines in their anticodon region (e.g. $tRNA_2^{Glu}$ from *E. coli*).

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