EXPERIENTIA

Vol. 27 – Fasc. 1 Pag. 1–120 15. 1. 1971

The Primary Structure of Transfer Ribonucleic Acid

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At a recent meeting on tRNA[1] three new primary structures have been reported. This might be an appropriate time to ask the questions: what information has been obtained so far from the known primary sequences of tRNAs and what can we deduce from them about the general structure of these molecules?

When tRNAs were discovered and their role in accepting amino acids and transferring them to ribosomes was elucidated, a total of 20 different tRNAs would have been sufficient to account for all their functions. This was still so when the first code was published in 1962[2]. But it was soon recognized that the genetic code was degenerate[3] and therefore it was thought that 64 tRNAs would be required. Fortunately, in 1966 CRICK's wobble hypothesis[4] was put forward, which again reduced the number of required tRNAs by one half. Up to the year 1968 only tRNA sequences from yeast were known, i.e. alanine, serine, tyrosine, phenylalanine and valine tRNA[5–10]. But in that year sequences of similar tRNAs from other sources were reported, namely from *E. coli*[11–14] and from rat liver[15]. These tRNAs showed marked differences from their yeast counterparts. This indicated that the primary structure of the tRNA might vary from species to species and opened the way to a vast number of different tRNA primary sequences. Further structures of wheat germ and Torula yeast tRNAs have already extended this point[16–18]. While on the one hand all tRNAs have many features in common, on the other there is good evidence that the number of tRNAs with different primary sequences, even those specific for the same amino acid, is probably extremely large. This variety of structures is due to 3 types of variations.

1. Variations in primary structures between species.

2. Variations of isoaccepting tRNAs within a given species. These are of two kinds: a) tRNAs with different coding properties; b) tRNAs with different primary structures recognizing the same coding triplet.

That large species differences exist in the primary structures of tRNAs is apparent from a comparison of the structures of valine, tyrosine and phenylalanine-tRNA in *E. coli* and yeast, or of serine-tRNA in yeast and rat liver. One of the first instances in which the heterogeneity of isoaccepting tRNAs within one species was recognized is that of leucine-tRNA from *E. coli*, where 5 fractions, some with different but also some with similar coding properties, were found by countercurrent distribution[19,20]. Studies on sequence determination have also indicated that isolated tRNAs may consist of mixtures of tRNAs with very similar structures. Thus, in several instances in highly purified tRNAs minor variations of 1–3 nucleotides in the extra arm and the TΨC arm have been reported[10,11,14].

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Table I. Major and minor fragments obtained from rat liver serine tRNA highly purified on partition chromatography columns

I	Major fragments																						
	Pancreas RNase	m^2_2G	A	Ψ	/	G	G	A	m^3C	U	/	I	G	A	i^6A	A	Ψm	C				UmG	G	G	G	U
	RNase T_1		A	Ψ	G			A	m^3C	U	I			A	i^6A	A	Ψm	C	C	A	U	UmG				
II	Minor fragments																								
a)	Pancreas RNase	m^2_2G	Ψ					A	m^3C	U			G	A	i^6A	A	Ψm	C		A	A	UmG	G	G	G	U
b)	RNase T_1		Ψ	Ψ	G			A	m^3C	U	C	G	/	A	i^6A	A	Ψm	C	C	A	A	UmG				
								A	m^3C	U	'Ψ'	G														

With improved methods of separation in combination with sequence analysis the nature of this heterogeneity of isoaccepting tRNAs has become more apparent. Recent studies on rat liver tRNA[21] have shown that by partition chromatography 2 fractions of serine-tRNA can be separated. Enzymatic digests of highly purified serine-tRNA from peak A yielded a series of closely similar fragments, some of which are indicated in Table I. That this tRNA fraction actually contained different serine-tRNAs became apparent when it was examined by reversed-phase chromatography, where 2 species with different coding properties could partially be separated. These (I = UCU, UCC and UCA, II = UCG) together with the coding properties of serine-tRNA III from peak B (AGU and AGC) account for all 6 known serine codons. But sequence analysis of serine-tRNA III indicated that it contained at least 2 serine-tRNAs differing in the amino acid arm. Serine-tRNA II also seemed to contain 2 species since in enzymatic digests even 2 fragments containing different sequences in the anticodon were found (Table I, IIa and b). Only in another reversed-phase chromatographic system could these 2 species be separated.

Thus, at least 5 different species of serine tRNA can be detected in rat liver. Only 3 serine tRNAs would be required to recognize all 6 coding triplets, but multiple tRNA species exist for the recognition of the same codon.

The most important information resulting from sequence determinations is that all these species of serine-tRNA differ not only within the anticodon and some minor secondary modifications but that they also differ at many sites in the primary structure of the molecule. Some sites where the various other species differ from serine-tRNA I are indicated in Figure 1. This should serve to illustrate that a great many tRNAs, all capable of accepting the same amino acid but differing in their primary structure, may be present in a cell and that they often are very difficult to separate from one another.

A tabulation of the various tRNA sequences that have been reported in the literature[22–28] has therefore to take into account that each tRNA may only represent one of many isoaccepting tRNAs present in a particular cell and that possibly the primary sequences may have been derived from mixtures of tRNAs with closely similar structures.

Among the known primary structures three classes of tRNAs can be distinguished as suggested by LEVITT[29]. The general structures of these 3 tRNAs are

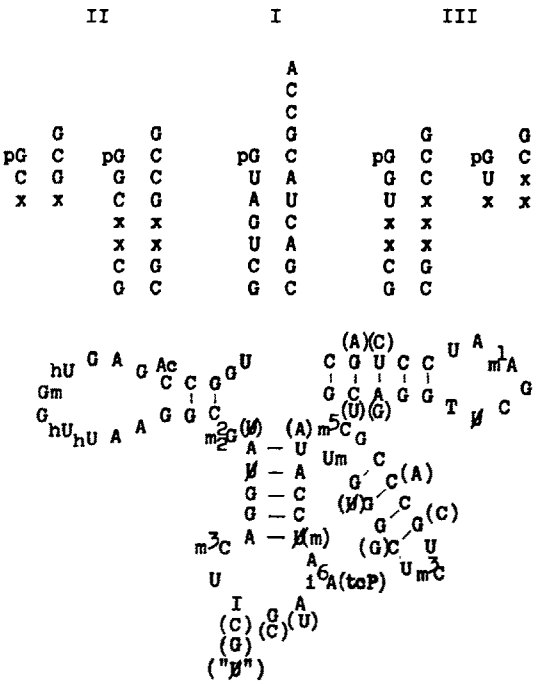

Fig. 1. Base sequences in different rat liver serine tRNAs. The nucleotides where serine tRNA II and III differ from serine tRNA I are indicated in brackets. On top the nucleotide sequence in the amino acid arm of tRNA I and tentative structural features of serine tRNA II and III subfractions are indicated.

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indicated in Figure 2: class I consists of tRNAs with 3 base pairs in the dihydroU arm and a short extra arm, class II of tRNAs containing three base pairs in the dihydroU arm and a long extra arm, and class III of tRNAs containing 4 base pairs in the dihydroU arm and a short extra arm.

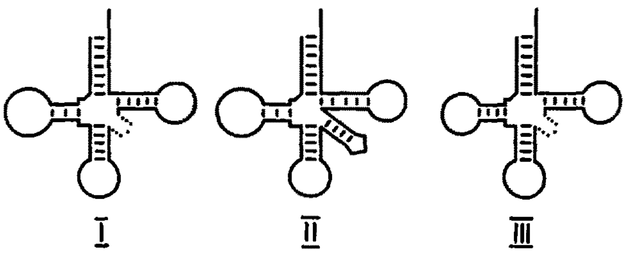

Fig. 2. Schematic representation of the 3 classes of tRNA.

The sequences of the tRNAs with known structures are indicated in Figures 3–7, according to the structure of the respective arms in the clover-leaf model. The underlined bases represent the base paired regions of the arm. Since the various tRNAs are of different length, the sequences have been so arranged that all sequences could be accommodated. The numbers, therefore, indicate not the actual length of the molecule but arbitrary positions in the direction from the 5′ end to the 3′ end of the primary structure for reference purposes. In several instances the tRNA used for sequence studies was reported to be a mixture of 2 tRNA species with identical amino acid acceptor and coding properties. These tRNAs differed apparently by only a few nucleotides, which are also indicated in Figures 4, 6, and 7.

The amino acid arm (Figure 3) shows 7 base pairs with the exception of *E. coli* tRNA Met F, which has

Fig. 3 AMINO ACID ARM

	1	2	3	4	5	6	7	......	82	83	84	85	86	87	88	89	90	91	92
Tyr yeast(B)	pC	U	C	/U/	C	G	G	......	C	C	G	/G/	G	A	G	A	C	C	A
Tyr yeast(T)	pC	U	C	U	C	G	G	......	C	C	G	A	G	A	G	A	C	C	A
Val yeast(B)	pG	/G/	U	U	U	C	G	......	C	G	A	A	A	/U/	C	A	C	C	A
Val yeast(T)	pG	/G/	U	U	U	C	G	......	C	G	A	A	A	/U/	C	A	C	C	A
Ile yeast(T)	pG	G	U	C	C	C	U	......	A	G	G	G	A	C	C	A	C	C	A
Ser yeast(B)	pG	/G/	C	A	A	C	U	......	A	G	U	U	G	/U/	C	G	C	C	A
Ser rat	pG	U	A	G	U	C	G	......	C	G	A	C	U	A	C	G	C	C	A
Tyr E.coli	pG	G	U	G	G	G	G	......	C	C	C	C	A	C	C	A	C	C	A
Leu E.coli	pG	C	G	A	/A/	G	G	......	C	C	/C/	U	C	G	C	A	C	C	A
Val E.coli	pG	G	G	U	G	A	U	......	A	U	C	A	C	C	C	A	C	C	A
Phe yeast(B)	pG	C	G	/G/	A	U	U	......	A	A	U	/U/	C	G	C	A	C	C	A
Phe wheat	pG	C	G	G	/G/	G	A	......	U	C	/A/	C	C	G	C	A	C	C	A
Phe E.coli	pG	C	C	C	G	G	A	......	U	C	C	G	G	G	C	A	C	C	A
Ala yeast(B)	pG	G	/G/	C	G	/U/	G	......	C	/U/	C	G	/U/	C	C	A	C	C	A
Asp yeast	pU	C	C	G	/U/	G	A	......	U	C	/G/	C	G	G	A	G	C	C	A
Trp E.coli	pA	G	G	G	G	C	G	......	C	G	C	C	C	C	U	G	C	C	A
Met E.coli	pG	G	C	U	A	C	G	......	C	G	U	A	G	C	C	A	C	C	A
Met F E.coli	pC	G	C	G	G	G	G	......	C	C	C	C	G	C	A	A	C	C	A

Figures 3–7. Summary of tRNA nucleotide sequences. The nucleotides have been arranged according to the various arms. Underlined bases indicate those base paired in the clover-leaf model. Yeast(B) = Baker's yeast, yeast(T) = Torula yeast. For the abbreviations of minor nucleotides the system using small letters for substitutions has been used (cf. *Handbook of Biochemistry*; Ed. SOBER, The Chemical Rubber Comp., Cleveland 1968, pp. H64–65), i.e. hU = Dihydrouridine, Ψ = Ribosyluracil, m^{1}G = 1-Methylguanosine, m^{2}G = N^2-Methylguanosine; m$^2_2$G = N^2-Dimethylguanosine; m^{7}G = 7-Methylguanosine; m^{1}I = 1-Methylinosine; m^{1}A = 1-Methyladenosine; m^{6}A = N^6-Methyladenosine; m^{5}U = Ribothymidine; m^{5}C = 5-Methylcytidine; m^{3}C = 3-Methylcytidine; Um = 2′-*O*-Methyluridine; Cm = 2′-*O*-Methylcytidine; Ψm = 2′-*O*-Methylpseudouridine; Gm = 2′-*O*-Methylguanosine; I = Inosine; s^{4}U = 4-Thiouridine; i^{6}A = N^6-Isopentenyladenosine; m^{2}si^{6}A = 2-Methylthio-N^6-isopentenyl-adenosine; ac^{4}C = N^4-Acetylcytidine; tcP = N-(purin-6-yl-carbamoyl)-threonine; Y = Fluorescent base in phenylalanine tRNA; X = Unknown derivatives of parent bases. Pseudouridine Ψ is indicated as an oblique-stroked 'U'.

only 6 base pairs. There are 10 exceptions to the classical G–C and A–U base pairs, namely 7 G–U base pairs, 1 U–U, 1 A–C and 1 A–G pair. No differences in the structure of isoaccepting tRNAs have been reported so far in the amino acid arm. The lack of heterogeneity in this region of the reported tRNAs from one source is actually rather surprising for several reasons. First of all, tRNAs from different sources specific for one amino acid like phenylalanine, tyrosine and valine tRNA from baker's yeast, Torula yeast or *E. coli*, and serine-tRNAs from yeast and rat liver vary in the stem region quite considerably. Furthermore, evidence of the heterogeneity in the stem region of isoaccepting tRNAs has come from the observation made in several laboratories[30–33] that by acylating tRNAs with a labelled amino acid followed by digestion with RNase T_1 fragments of different chain length containing the same amino acids can be obtained. In addition, our own experiments have indicated that great variation occurs in the stem region of the rat liver serine-tRNAs. Thus, pGU is the 5′ end in serine-tRNA I, pGC was found in species II and pGGU in species III. The recognition of a variability in the base-paired region of the amino acid arm within isoaccepting tRNAs may be important in pointing out possible pitfalls in sequence determinations. Furthermore, it also indicates that this region may not play an important role in enzyme recognition.

In the dihydroU arm (Figure 4), we can distinguish between tRNAs with 3 or 4 base pairs. Those with 3 base pairs have only G–C bonds, with the exception of valine-tRNA which has one GU bond. In all tRNAs both terminal base pairs are G–C base pairs, with the exception of yeast aspartic acid-tRNA, which has 2 G–U bonds. *E. coli* tyrosine tRNA is exceptional in that it is the only tRNA with a pyrimidine in position 9, which might form a fourth base pair with the A in position 31. The number of nucleotides in the DHU arm is variable; the nucleotides have therefore been arranged in Figure 7 in such a way that some regularities

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Fig. 4 DHU-ARM

	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
$Tyr_{yeast(B)}$	U	A	^{2}mG	C	C	A	-	-	A	G	hU	hU	-	Gm	G	hU	hU	hU	A	A	G	G	C	m_2^2G
$Tyr_{yeast(T)}$	U	^{1}mG	^{2}mG	C	C	A	-	-	A	G	hU	hU	-	Gm	G	hU	hU	hU	A	A	G	G	C	m_2^2G
$Val_{yeast(B)}$	U	^{1}mG	G	U	C	Ø	-	-	A	G	hU	C	-	G	G	hU	hU	-	A	U	G	G	C	A
$Val_{yeast(T)}$	U	^{1}mG	G	U	C	Ø	-	-	A	G	hU	hU	-	G	G	hU	C	-	A	U	G	G	C	A
$Ile_{yeast(T)}$	U	G	G	C	C	C	-	-	A	G	hU	hU	-	G	G	hU	hU	-	A	A	G	G	C	m_2^2G
$Ser_{yeast(B)}$	U	G	G	C	$A^{c}C$	G	-	-	A	G	hU	-	-	Gm	G	hU	hU	-	A	A	G	G	C	m_2^2G
Ser_{rat}	U	G	G	C	$A^{c}C$	G	-	-	A	G	hU	-	-	Gm	G	hU	hU	-	A	A	G	G	C	m_2^2G
$Tyr_{E.coli}$	sU	sU	C	C	C	G	-	-	A	G	C	-	-	Gm	G	C	C	A	A	A	G	G	G	A
$Leu_{E.coli}$	U	G	G	C	G	G	-	-	A	A	hU	hU	-	G	G	hU	A	-	G	A	C	G	C	G
$Val_{E.coli}$	sU	A	G	C	U	C	-	-	A	G	C	hU	-	G	G	G	-	-	A	G	A	G	C	A
$Phe_{yeast(B)}$	U	A	^{2}mG	C	U	C	-	-	A	G	hU	hU	-	G	G	G	-	-	A	G	A	G	C	m_2^2G
Phe_{wheat}	U	A	^{2}mG	C	U	C	-	-	A	G	hU	hU	-	G	G	G	-	-	A	G	A	G	C	m_2^2G
$Phe_{E.coli}$	U	A	G	C	U	C	-	-	A	G	hU	C	-	G	G	hU	-	-	A	G	A	G	C	A
$Ala_{yeast(B)}$	U	^{1}mG	G	C	G	C	G	U	A	G	hU	C	-	G	G	hU	-	-	A	G	C	G	C	m_2^2G
Asp_{yeast}	U	A	G	U	U	Ø	-	-	A	A	hU	-	-	G	G	hU	C	-	A	G	A	A	U	G
$Trp_{E.coli}$	sU	A	G	U	U	C	-	-	A	A	hU	hU	-	G	G	hU	-	-	A	G	A	G	C	A
$Met_{E.coli}$	sU	A	G	C	U	C	-	-	A	G	hU	hU	-	Gm	G	hU	hU	-	A	G	A	G	C	A
$Met\ F_{E.coli}$	sU	G	G	A	G	C	-	-	A	G	..U.. C	C	U	G	G	hU	-	-	A	G	C	U	C	G

become more apparent. Regularities which are always found are the following:

1. Nucleotide 8 is always U or t^4U.
2. 16 is always A.
3. 17 is always a purine.
4. 18, 19 are always pyrimidines.
5. 21, 22 is always GG or GmG.
6. 31 is always a purine.

Regularities with only one exception are the following:

1. 9 is a purine.
2. 10 is G.
3. 14, 15 are missing.
4. 20 is missing.
5. 26 is A.

Additional fairly common features in this region appear striking. Thus, for instance, with one exception, the first and last base pairs are G–C bonds, the one to the center of the clover leaf having G on the 5′ side and the one towards the loop having G on the 3′ side. And with the exception of yeast valine-tRNA the last non-base-paired nucleotide at the 3′ side is always A.

The next region, i.e. the anticodon arm (Figure 5), shows no variation in length. All tRNAs show 5 base pairs, where in many instances Ψ or Ψm substitutes for U. The 7 non base-paired nucleotides show the following regularities: 37 = pyrimidine, 38 = U, 42 = purine, in most cases modified A. The bases on the 3′ side of the anticodon are especially remarkable in that a number of very unusual bases have been found in this position, e.g. isopentenyl-adenine and 2-thiomethyl-6-isopentenyl-adenine. These bases are extremely lipophilic. In countercurrent distribution, partition chromatography and reverse phase chromatography the different tRNAs are eluted according to their degree of lipophilicity. All the tRNAs containing these bases are among the most lipophilic species[34]. This might indicate that these bases confer this character on the whole tRNA. It is interesting to compare the degree of lipophilicity as revealed by the elution pattern from a partition column with their coding properties. Figure 8 shows the elution profile of *E. coli* tRNAs from a partition column in relation to the coding properties of the tRNAs. There appears to be a very remarkable connection between the two properties. Among the most lipophilic species, which are eluted first, those tRNAs are found that recognize a codon starting with U, whereas those where the first letter

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Fig. 5 ANTICODON ARM

$Tyr_{yeast(B)}$	m_2^2G	C	A	A	G	A	C	U	G	Ψ	A	i^6A	A	Ψ	C	U	U	G	A
$Tyr_{yeast(T)}$	m_2^2G	Ψ	C	A	G	A	C	U	G	Ψ	A	i^6A	A	Ψ	C	U	G	A	A
$Val_{yeast(B)}$	A	Ψ	C	U	G	C	Ψ	U	I	A	C	A	C	G	C	A	G	A	A
$Val_{yeast(T)}$	A	Ψ	C	U	G	C	Ψ	U	I	A	C	A	C	G	C	A	G	A	A
$Ile_{yeast(T)}$	m_2^2G	Ψ	G	G	U	G	C	U	I	A	U	tcP	A	C	G	C	C	A	A
$Ser_{yeast(B)}$	m_2^2G	A	A	A	G	A	Ψ	U	I	G	A	i^6A	A	Ψ	C	U	U	U	Um
Ser_{rat}	m_2^2G	A	Ψ	G	G	A	m^3C	U	I	G	A	i^6A	A	Ψm	C	C	A	U	Um
$Tyr_{E.coli}$	A	G	C	A	G	A	C	U	XG	U	A	s^iA	A	Ψ	C	U	G	C	C
$Leu_{E.coli}$	G	C	U	A	/G/	C	U	U	C	A	G	G*	Ψ	G	/Ψ/	U	A	G	U
$Val_{E.coli}$	A	C	C	U	C	C	C	U	X	A	C	m^6A	A	G	G	A	G	G	G
$Phe_{yeast(B)}$	m_2^2G	C	C	A	G	A	Cm	U	Gm	A	A	Y	A	Ψ	m^5C	U	G	G	A
Phe_{wheat}	m_2^2G	Ψ	C	A	G	A	Cm	U	Gm	A	A	Y	A	Ψ	C	U	G	A	A
$Phe_{E.coli}$	A	G	G	G	G	A	Ψ	U	G	A	A	s^iA	A	Ψ	C	C	C	C	G
$Ala_{yeast(B)}$	m_2^2G	C	U	C	C	C	U	U	I	G	C	mI	Ψ	G	G	G	A	G	A
Asp_{yeast}	G	G	G	C	/G/	C	Ψ	U	G	U	C	m^1G	C	G	/U/	G	C	C	A
$Trp_{E.coli}$	A	C	C	G	G	U	Cm	U	C	C	A	A*	A	A	C	C	G	G	G
$Met_{E.coli}$	A	C	A	U	C	A	C	U	XC	A	U	XA	A	Ψ	G	A	U	G	G
$Met\ F_{E.coli}$	G	U	C	G	G	G	Cm	U	C	A	U	A	A	C	C	C	G	A	A
		31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49

of the codon is A, C or especially G are found among the more hydrophilic species. ISHIKURA[35] has found that several tRNAs from Torula yeast with coding properties for triplets starting with A have all purinyl-6-carbamoyl-threonin, which is acidic at neutral pH, next to the anticodon. In serine-tRNA III from rat liver and methionine-tRNA from *E. coli*, which code for triplets starting with A, the base next to the anticodon was also found to be a similar acid adenine derivative. This seems therefore to be characteristic of tRNAs coding for triplets starting with A.

The nucleotides in the anticodon itself pose an especially interesting problem. Although the code words for the amino acids are known, very little work has been reported on the coding properties of the actual tRNA whose sequences have been determined. To account for the distribution of the amino acids in the genetic code the wobble hypothesis proposed by CRICK[4] postulates that only the bases corresponding to the first 2 nucleotides of the messenger codon form classical G–C or A–U base pairs. In the third position of the codon-anticodon interaction CRICK has proposed that the bases on the tRNA could form the following base pairs with the messenger: A = U, C = G, U = A or G, G = C or U, I = U, C or A. This is certainly correct in all cases where coding properties with tRNAs of known sequences have been determined. But it may not be the entire story, because in addition to G, C and I, 2 unknown nucleotides XG and XC have been reported in this position of the anticodon, i.e. nucleotide 39 of Figure 5. XG in *E. coli* tyrosine has remarkable similarities to a nucleotide found in the minor dinucleotides from a pancreatic RNase digest of rat liver tRNA[36]. It seems to be a derivative of G, since unmodified G has also been found in this position. Another unusual nucleotide was found in this position in a subspecies of serine-tRNA II[21]. In 4 different thin-layer chromatographic solvent systems this nucleotide runs exactly like Ψ. It has also the same spectrum at acid and neutral pH, but it does not show a bathochromic shift at alkaline pH. It is probably a pyrimidine, since it is cleaved by pancreatic RNase and not by RNase T_1. The tRNA containing this nucleotide in the anticodon shows unusual coding properties, since

[35] H. ISHIKURA, Y. YAMADA, K. MURAO, M. SANEYOSHI and S. NISHIMURA, Biochem. Biophys. Res. Commun. *37*, 990 (1969).
[36] H. ROGG and M. STAEHELIN, Biochim. biophys. Acta *195*, 16 (1969).

Fig. 6

										EXTRA-ARM							
$Tyr_{yeast(B)}$	A	-	-	-	-	G	A	hU	-	-	-	-	-	-	m^5C
$Tyr_{yeast(T)}$	A	-	-	-	-	C	A	hU	-	-	-	-	-	-	m^5C
$Val_{yeast(B)}$	A	-	-	-	-	C	-	hU	-	-	-	-	-	-	m^5C
$Val_{yeast(T)}$	A	-	-	-	-	C	-	-	-	-	-	-	-	-	m^5C
$Ile_{yeast(T)}$	A	-	-	-	-	G	A	hU	-	-	-	-	-	-	m^5C
$Ser_{yeast(B)}$	Um	G	G	G	C	U	C	U	-	G	C	C	C	G	m^5C
Ser_{rat}	Um	G	G	G	G	U	m^2C	U	-	C	C	C	C	G	m^5C
$Tyr_{E.coli}$	C	-	G	U	C	A	U	C	-	G	A	C	U	U	C
$Leu_{E.coli}$	U	G	U	C	C	U	U	A	C	G	G	A	C	G	U
$Val_{E.coli}$	G	-	-	-	-	G	m^7G	U	-	-	-	-	-	-	C
$Phe_{yeast(B)}$	A	-	-	-	-	G	m^7G	U	-	-	-	-	-	-	C
Phe_{wheat}	A	-	-	-	-	G	m^7G	hU	-	-	-	-	-	-	C
$Phe_{E.coli}$	G	-	-	-	-	U	m^7G	X	-	-	-	-	-	-	C
$Ala_{yeast(B)}$	A	-	-	-	-	G	-	U	-	-	-	-	-	-	C
Asp_{yeast}	A	-	-	-	-	G	-	A	-	-	-	-	-	-	U
$Trp_{E.coli}$	G	-	-	-	-	U	m^7G	U	-	-	-	-	-	-	U
$Met_{E.coli}$	G	-	-	-	-	G	m^7G	X	-	-	-	-	-	-	C
$Met\ F_{E.coli}$	A	-	-	-	-	G	A	U	-	-	-	-	-	-	C
							m^7G								
	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63

it codes very well for PolyUC, it also codes for UCU and UCA but not for UCC or UCG. Furthermore, 2 sulfur containing pyrimidines occur in this position of the anticodon, namely 2-thio-5-uridine acetic acid methylester in glutamic acid tRNA from yeast[37] and 2-thio-5-methylaminomethyl-uridine in glutamic acid tRNA from *E. coli*[38]. Both tRNAs recognize GAA but not GAG. It seems therefore that in addition to the wobble possibilities suggested by CRICK there are also other possibilities, such as the one found in rat liver serine-tRNA, i.e. 'Ψ' = U or A, and that in glutamic acid tRNAs, i.e. s^2U = A.

The next region, i.e. that of the extra arm, again shows considerable variation in length (Figure 6). The tRNAs with the short arm contain 3–5 nucleotides, those with the long arm 13–15. The last non-base-paired nucleotide is always a pyrimidine. In 3 instances the nucleotides in the center of this region were found to differ within various subspecies.

Lastly, in the TΨC arm we always find 5 base pairs. In 4 instances non-classical base pairs (3 GU and 1 AC pair) have been reported. With the exception of valine-tRNA from baker's yeast, these base pairs are directly linked to the base-paired region in the amino acid arm. The last base pair is always formed of a G at the 5′ and a C at the 3′ side. The non-base-paired region consists of 7 nucleotides with the following regularities: 1 = T, 2 = Ψ, 3 = C, 4 = Pu, 5 = A or m^1A, 7 = Py.

What do these sequences tell us about the general structure of the molecule? The nature of the base pairs in the various stem regions is indicated in Table II. Of all base pairs, over $^2/_3$ are G–C bonds and $^1/_4$ are A–U pairs. But it is interesting that these are not randomly distributed. Whereas in the amino acid arm the same distribution is found as in the whole tRNA, in the TΨC arm and DHU over $^3/_4$ of all bonds are G–C bonds and only $^1/_6$ A–U bonds. But this ratio is markedly different in the anticodon arm where 40% of all bonds are A–U bonds. This may be a reflection on the needs for stability of the various regions in the tertiary structure of the molecule. As far as the general structure of the bases not base-paired in the clover-leaf model is concerned, the regularities and possible base pairings

[37] M. YOSHIDA, K. TAKEISHI and T. UKITA, Biochem. Biophys. Res. Commun. *39*, 852 (1970).
[38] Z. OHASHI, M. SANEYOSHI, F. HARADA, H. HARA and S. NISHIMURA, Biochem. biophys. Res. Commun. *40*, 866 (1970).

Fig. 7 TΨC-ARM

	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81
$Tyr_{yeast(B)}$	G	G	G	C	G	T	Ψ	C	G	^{1}mA	C	U	C	G	C	C	C	-
$Tyr_{yeast(T)}$	G	G	G	C	G	T	Ψ	C	G	^{1}mA	A	U	C	G	C	C	C	-
$Val_{yeast(B)}$	C	C	C	A	G	T	Ψ	C	G	^{1}mA	U	C	C	U	G	G	G	G
$Val_{yeast(T)}$	C	C	C	A	G	T	Ψ	C	G	^{1}mA	U	C	C	U	G	G	G	-
$Ile_{yeast(T)}$	A	G	C	A	G	T	Ψ	C	G	^{1}mA	U	C	C	U	G	C	U	-
$Ser_{yeast(B)}$	G	C	A	G	G	T	Ψ	C	A	A	A	U	C	C	U	G	C	-
Ser_{rat}	G	C	A	G	G	T	Ψ	C	··G··	^{1}mA	··G··	U	C	C	U	G	C	-
$Tyr_{E.coli}$	G	A	A	G	G	T	Ψ	C	G	A	A	U	C	C	U	U	C	-
$Leu_{E.coli}$	G	G	G	G	G	T	Ψ	C	A	A	G	U	C	C	C	C	C	-
$Val_{E.coli}$	G	/G/	C	G	G	T	Ψ	C	G	A	U	C	C	C	G	/U/	C	-
$Phe_{yeast(B)}$	m^5C	U	G	U	G	T	Ψ	C	G	^{1}mA	U	C	C	A	C	A	G	-
Phe_{wheat}	G	C	G	U	G	T	Ψ	C	G	^{1}mA	U	C	C	A	C	G	C	-
$Phe_{E.coli}$	C	U	/U/	G	G	T	Ψ	C	G	A	U	U	C	C	/G/	A	G	-
$Ala_{yeast(B)}$	U	C	C	G	G	T	Ψ	C	G	A	U	U	C	C	G	G	A	-
Asp_{yeast}	m^5C	G	G	G	G	T	Ψ	C	A	A	U	U	C	C	C	C	G	-
$Trp_{E.coli}$	G	/G/	G	A	G	T	Ψ	C	G	A	G	U	C	U	C	/U/	C	-
$Met_{E.coli}$	A	C	/A/	G	G	T	Ψ	C	G	A	A	U	C	C	/C/	G	U	-
$Met\ F_{E.coli}$	G	/U//	C/	G	G	T	Ψ	C	A	A	A	U	C	C	G	/G/	C	-

depicted in Figure 9 have been pointed out by LEVITT[29]. The fact that all tRNAs which contain G in position 17 have C in position 63, whereas those which contain A in position 17 have U in position 63, renders this general type of base pairing very likely.

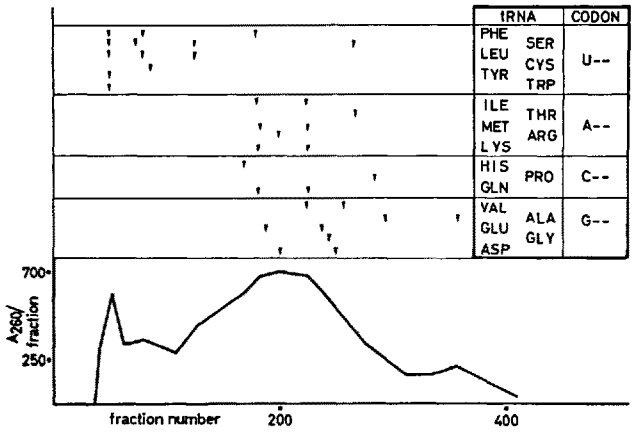

Fig. 8. Order of elution of *E. coli* tRNAs on partition chromatography Data from MÜNCH and BERG[34] as well as from our own experiments were compiled to construct this composite diagram.

Table II. Distribution of base pairs in various arms of the clover-leaf

	Number of base pairs					
	G–C	A–U	G–U	A–C	G–A	U–U
Amino acid arm	83	32	7	1	1	1
TΨC-arm	70	15	4	1		
Anticodon arm	52	36	2			
DHU-arm	49	9	5			

It was hoped that among other things a detailed knowledge of many tRNA sequences might throw some light on the mechanism of enzyme recognition. Although it is probably of importance that the only region which is absolutely identical in all rat liver and yeast serine-tRNAs is the dihydroU arm, this does not mean that these nucleotides are directly involved in enzyme recognition, because the fact that they also have exactly the same number of nucleotides might also indicate that the general shape of the molecule is more important than the actual nucleotide sequence. The work of CERUTTI[39] has indicated furthermore that dihydrouridine residues in this region can be destroyed without impairment of aminoacylation, although the pyrophosphorylase may not be able to recognize the molecule anymore. On the other hand the interesting observation has been made that *E. coli* valine-tRNA synthetase also recognizes phenylalanine tRNA from yeast and wheat embryo but not from *E. coli*[40]. The primary sequences of these 4 tRNAs are represented in the first 4 tRNAs of class III in Figures 3–7. They all contain the same number of nucleotides. Those that are recognized by the enzyme differ from phenylalanine tRNA (*E. coli*) in the spacing of hU and G in positions 19 and 23 of the DHU-arm, and in having G and C residues in positions 54 and 75.

[39] P. CERUTTI and N. MILLER, J. molec. Biol. *26*, 55 (1967).
[40] B. S. DUDOCK, C. DIPERI and M. S. MICHAEL, J. biol. Chem. *245*, 2465 (1970).
[41] J. N. ABELSON, M. L. GEFTER, L. BARNETT, A. LANDY, R. L. RUSSELL and J. D. SMITH, J. molec. Biol. *47*, 15 (1970).
[42] J. D. SMITH, L. BARNETT, S. BRENNER and R. L. RUSSELL, J. molec. Biol., in press.

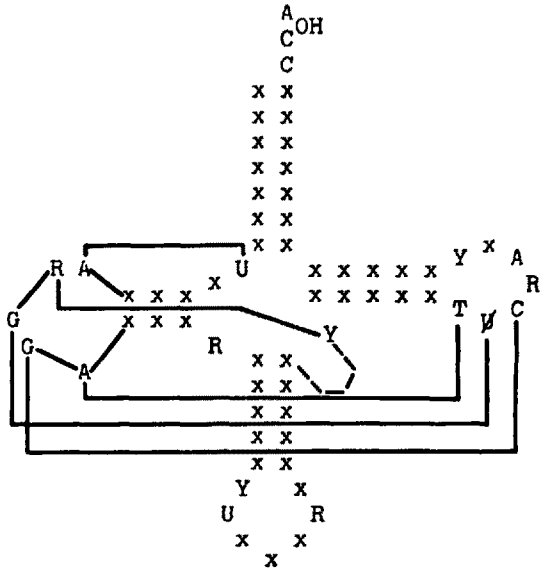

Fig. 9. Common features of all tRNAs and possible base pairings, according to LEVITT[29]. Y indicates a pyrimidine, R a purine.

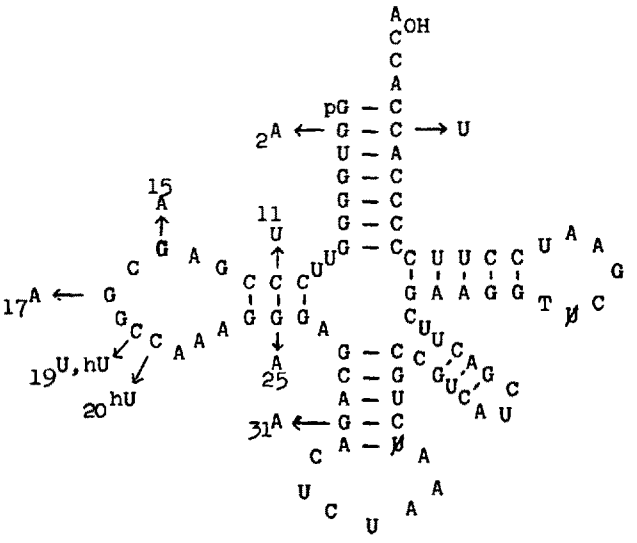

Fig. 10. Sites of mutations in su^{+}_{III} Tyrosine tRNA (from ABELSON et al.[41] and SMITH et al.[42]).

Probably the most important work which might throw some light on the importance of individual nucleotides in the primary structure is that carried out in mutants containing modified su_{III}^{+} tyrosine-tRNA[41]. All the mutant tRNAs shown in Figure 10 were defective in one or more functions, but in some where the mutated nucleotide is involved in base pairs, full function could be regained by a double mutation, which gave rise to a new correct base pair. So far such a complementarity has only been reported in those regions already known to be base-paired from the clover-leaf model. This, in itself is important in view of the fact that quite a number of non-correct base pairs have been reported in double-stranded regions of the clover-leaf structure. When by this technique double mutants regaining full activity can be obtained in nucleotides other than those involved in base-paired regions of the clover-leaf molecule, the analysis of primary structures might be of help to elucidate the tertiary structure of the tRNA molecule.

One of the most important information primary sequence has given us is the location of minor bases. A dozen or so minor nucleotides have been found in various tRNAs which occur always at the same sites (Figure 11). Four minor nucleotides (Gm, m^5C, m^3C and m^1G) have been found at 2 sites, whereas dihydrouridine and pseudouridine have been found to occur at 6 and 9 different sites, respectively. Figures 12 and 13

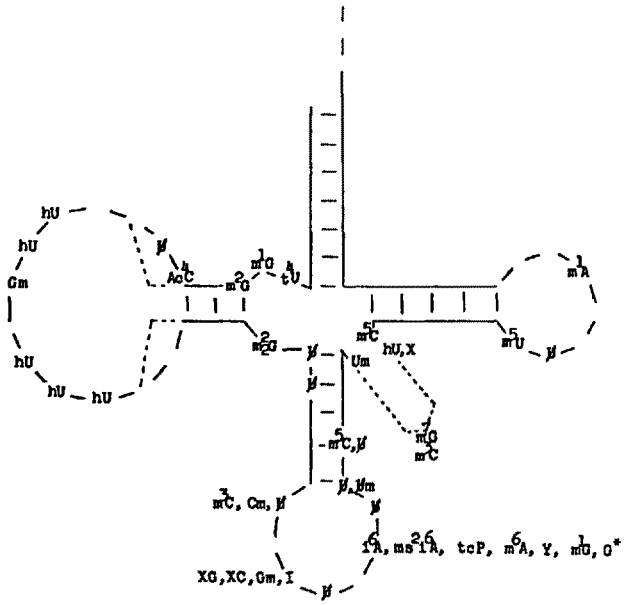

Fig. 11. Location of minor nucleotides in known sequences.

Fig. 12 Sites of hU and Ψ in Various tRNAs

	13	18	19	23	24	25	43	44	69	32	33	37	40	45	56
$Tyr_{yeast(B)}$	A	hU	hU	hU	hU	hU	A	Ψ	Ψ	C	A	C	Ψ	C	hU
$Tyr_{yeast(T)}$	A	hU	hU	hU	hU	hU	A	Ψ	Ψ	Ψ	C	C	Ψ	C	hU
$Val_{yeast(B)}$	Ψ	hU	C	hU	hU	-	C	G	Ψ	Ψ	C	Ψ	A	C	hU
$Val_{yeast(T)}$	Ψ	hU	hU	hU	C	-	C	G	Ψ	Ψ	C	Ψ	A	C	-
$Ile_{yeast(T)}$	C	hU	hU	hU	hU	-	A	C	Ψ	Ψ	G	C	A	G	hU
$Ser_{yeast(B)}$	G	hU	-	hU	hU	-	A	Ψ	Ψ	A	A	Ψ	G	C	U
Ser_{rat}	G	hU	-	hU	hU	-	A	Ψm	Ψ	A	Ψ	m^3C	G	C	U
$Tyr_{E.coli}$	G	C	-	C	C	A	A	Ψ	Ψ	G	C	C	U	C	C ..A..
$Leu_{E.coli}$	G	hU	hU	hU	A	-	Ψ	G	Ψ	C	U	U	A	Ψ	A
$Val_{E.coli}$	C	C	hU	G	-	-	A	G	Ψ	C	C	C	A	G	U
$Phe_{yeast(B)}$	C	hU	hU	G	-	-	A	Ψ	Ψ	C	C	Cm	A	m^5C	U
Phe_{wheat}	C	hU	hU	G	-	-	A	Ψ	Ψ	Ψ	C	Cm	A	C	hU
$Phe_{E.coli}$	C	hU	C	hU	-	-	A	Ψ	Ψ	G	G	Ψ	A	C	X
$Ala_{yeast(B)}$	C	hU	C	hU	-	-	Ψ	G	Ψ	C	U	U	G	G	U ..hU..
Asp_{yeast}	Ψ	hU	-	hU	C	-	C	G	Ψ	G	G	Ψ	U	U	A
$Trp_{E.coli}$	C	hU	hU	hU	-	-	A	A	Ψ	C	C	Cm	C	C	U
$Met_{E.coli}$	C	hU ..U..	hU	hU	hU	-	A	Ψ	Ψ	C	A	C	A	G	X
$Met\ F_{E.coli}$	C	C	hU	hU	-	-	A	C	Ψ	U	C	Cm	A	C	U

give a survey of the sites in all tRNAs where modification of U to hU or Ψ and where some of the methylations have occurred. It can be seen that in all 5 sites in the DHU-arm, where hU has been reported to occur, all U residues have been modified. The same is true of the Ψ sites in the DHU-arm, at the 3′ side of the anticodon and in the TΨC-arm. On the other hand, the U residues 32, 33 and 45 in the base paired region of the anticodon arm as well as the free residues 40 and 43 and the hU site in the extra arm are only partially modified. The same applies to all sites of methylation with the exception of T, which is present in all tRNAs (Figure 13).

Since the content of minor nucleosides varies in different species, species differences in the extent of modifications to minor nucleosides have also to be considered. But although none of the *E. coli* tRNAs contains m^1G, m^2G, $m^2{}_2G$, m^1A or m^5C, in no case have the parent bases in all yeast tRNAs been methylated. The only exception is possibly position 55 where all G residues in Figure 13 were found to be modified to m^7G. Interestingly all 7 reported m^7G residues are found in class III tRNAs whereas m^5C is only found in class I and II tRNAs, where the C residues of all tRNAs from eukaryotic cells have been modified to m^5C. This leads to the question: what do these modifying enzymes recognize and why are only some tRNAs modified? One aspect of this problem has been investigated in relation to 1-adenine-methylase[43]. In pancreatic RNase digests of yeast tRNA 1-methyl-adenine was found in 6 different sequences, i.e. 3 tri- and 3 tetranucleotides. In vitro methylation of yeast tRNA by purified 1-adenine-methylase from rat liver yields the same 6 sequences (Table III). Three of these are found in the 8 instances, where m^1A has been located in complete tRNA sequences. Methylation of highly purified serine-tRNA known to have the sequence CAAAU in the TΨC-arm has yielded the pancreatic RNase digest fragment Am^1AAU, indicating enzymatic methylation of nucleotide 73, i.e. of the only nucleotide where m^1A occurs in the established tRNA sequences[44]. This indicates that the tRNA-methylases recognize very specific sites in the molecule. On the one hand this facilitates the construction of new tRNA sequences containing minor bases, on the other hand it poses

[43] B. C. BAGULEY and M. STAEHELIN, Biochemistry *8*, 257 (1969).
[44] B. C. BAGULEY, W. WEHRLI and M. STAEHELIN, Biochemistry *9*, 1645 (1970).

Fig. 13 Some Sites of Methylation in tRNAs

A	$\overset{2}{m}G$	Gm	m^2_2G	A	m^5C	T	$\overset{1}{m}A$
$\overset{1}{m}G$	$\overset{2}{m}G$	Gm	m^2_2G	A	m^5C	T	$\overset{1}{m}A$
$\overset{1}{m}G$	G	G	A	-	m^5C	T	$\overset{1}{m}A$
$\overset{1}{m}G$	G	G	A	-	m^5C	T	$\overset{1}{m}A$
G	G	G	m^2_2G	A	m^5C	T	$\overset{1}{m}A$
G	G	Gm	m^2_2G	C	m^5C	T	A
G	G	Gm	m^2_2G	..U.. m^7C	m^5C	T	$\overset{1}{m}A$
sU	C	Gm	A	U	C	T	A
G	G	G	G	..C.. U	U	T	A
A	G	G	A	$\overset{7}{m}G$	-	T	A
A	$\overset{2}{m}G$	G	m^2_2G	$\overset{7}{m}G$	C	T	$\overset{1}{m}A$
A	$\overset{2}{m}G$	G	m^2_2G	$\overset{7}{m}G$	C	T	$\overset{1}{m}A$
A	G	G	A	$\overset{7}{m}G$	C	T	A
$\overset{1}{m}G$	G	G	m^2_2G	-	C	T	A
A	G	G	G	-	U	T	A
A	G	G	A	$\overset{7}{m}G$	U	T	A
A	G	Gm	A	$\overset{7}{m}G$	C	T	A
G	G	G	G	A ..$\overset{7}{m}G$...	C	T	A
9	10	21	31	55	63	69	73

Table III. 1-Methyladenine-containing sequences (from 41)

Base residues per tRNA chain	Methylation in vitro		Endogenous 1-Methyladenine	
	E. coli tRNA 0.9	Yeast tRNA 0.2	Yeast tRNA 0.55	Liver tRNA 0.90
Sequences				
Py-A-1MA-U	–	4%	0.05	0.05
Py-G-1MA-U	–	35%	0.20	0.20
Py-G-1MA-C	–	8%	0.05	0.10
Py-A-1MA-A-U	10%	20%	0.10	0.30
Py-G-1MA-A-U	90%	23%	0.10	0.20
Py-G-1MA-A-C	–	10%	0.05	0.05

the problem why some tRNAs are methylated and others are not. Of the known yeast tRNAs, for instance, tyrosine-, valine-, isoleucine- and phenylalanine-tRNA contain m^1A, whereas the three others don't, although they can be methylated by rat liver methylase. Thus as yet no clear picture as to the reason for the different extent of methylation emerges. From our knowledge of the specificity of tRNA methylases and from the known sequences it appears only that the question to be raised should not be anymore: why have some nucleotides been modified, but rather: why has the modification not occurred in all tRNAs, since there are apparently enzymes capable of modifying the parent residues in these positions?

Zusammenfassung. Aus einem Vergleich der beschriebenen Sequenzen verschiedener Transferribonukleinsäuren lassen sich Schlüsse über die allgemeine Struktur dieser Moleküle ziehen. Sämtliche Sequenzen lassen Basenpaarungen zu, die sich zweidimensional in einer Kleeblattform darstellen lassen. Die bisher bekannten Transferribonukleinsäuren lassen sich in 3 Klassen aufteilen, je nachdem, ob sie 3–4 Basenpaare im DHU- und einen kurzen oder langen Extra-Arm besitzen. Die Verteilung der seltenen Nukleotide weist eine auffallende Regelmässigkeit auf. MethylierteBasen kommen meistens nur an einer bestimmten Stelle des Moleküls vor, nur vier wurden an zwei Stellen beschrieben. Dihydrouridin und Pseudouridin wurden an 6 bzw. 9 Stellen gefunden. Dies dürfte darauf hinweisen, dass die Enzyme, die Nukleotide in der Transferribonukleinsäure modifizieren, nicht spezifische Transferribonukleinsäuren erkennen, sondern spezifische Stellen in den verschiedensten Molekülen. Anhand der serinspezifischen Transferribonukleinsäuren aus Rattenleber wird gezeigt, dass multiple Species vorliegen, die zum Teil das gleiche Codon erkennen können, sich aber in der Primärsequenz unterscheiden.

SPECIALIA

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Modifications of Lichen Substances and Morphology Induced by Mechanical Shock in *Cladonia pacifica*

Microchemical and crystalline tests of thalli of shocked *Cladonia pacifica* revealed additional substances not known to occur in this lichen and which did not occur in the controls. The only morphological alteration after shock was the color of the thallus. Control thalli were white, whereas shocked thalli were tan to khaki in color.

Lichens are considered to be hardy plants because of their wide geographic distribution and their adaptation to xeric habitats, being very resistent to extremes of temperature and drying. They are, however, highly responsive to environmental factors[1–6]. The present study examines the gross morphology of lichen thalli and lichen substances after exposing the lichens to mechanical shock, where shock is defined as a fast-rising pressure pulse of several seconds duration. The present work stems from an interest in the possibilities for using plants as experimental bio-indicators of underground shock[7].

Since lichens are such slow growing plants, only obvious changes in their appearance and obvious microchemical changes were observed in the present study in which unshocked plants were used as controls. Because the lichen acids are mostly phenolic derivatives, 2 post-shock periods were selected to allow for accumulation of any newly formed acids or degradation products of the existing acids.

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