A human tRNA^{Glu} gene of high transcriptional activity

J.P.Goddard*, M.Squire, M.Bienz and J.D.Smith

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

SUMMARY
    A mixture of low molecular weight RNAs, in which only tRNAs were radio-
labelled, was used as a hybridisation probe to select for tRNA-like sequences
within a bank of human genomic DNA in λ Charon 4A.
    A restriction enzyme digest of one of the selected λ Charon 4A recom-
binants contained two fragments (2.4 Kb & 1.8 Kb) which hybridised tRNA and
which, when subcloned into pAT153, were transcribed in Xenopus oocyte
nuclei.   Analysis of the subcloned 2.4 Kb fragment, which was of remarkably
high transcriptional activity, revealed the presence of a single gene for
tRNA^{Glu} in the middle of the fragment.   The sequence immediately preceding
the gene has the potential for forming a tRNA-like structure.

INTRODUCTION

     During the past few years, the application of gene cloning and DNA

sequencing has led to a great increase in our knowledge of the structure and

function of tRNA genes of higher eukaryotes (1, 2).   Studies so far on tRNA

gene organisation show that many types of gene arrangement occur.   There are

examples of clusters of different tRNA genes in Xenopus and Drosophila (3,

4).   In Xenopus the studied cluster occurs as a 300-fold tandem repeat,

whereas in most other organisms tRNA genes are widely scattered throughout the

genome.   Relatively few studies have been made of mammalian tRNA genes.   In

rat, different tRNA genes appear to occur in small interspersed clusters

(5).   One of these clusters is repeated about ten times on the haploid DNA

and shows sequence variation between the repeats including the presence of

incomplete genes (pseudogenes) (6).   In man, tRNA^{Met} genes are widely

scattered (7), but clusters of different tRNA genes have also been found

(8).   Clearly further studies are required to relate this apparent anarchy of

gene organisation to, for example, differences in the expression of tRNA genes

in different tissues or at different stages of development of an organism.

     Expression studies, on the other hand, have produced clearer, if

unexpected, results.   In Xenopus, Drosophila and C. elegans the promoter for

transcription of tRNA genes comprises noncontiguous regions within the coding

sequence (for review see (1)).   Modulation of the transcription is less well understood but the sequence preceding the gene has been implicated in some cases (9-11).   No comparable studies have been made on mammalian tRNA genes.

In this paper we report the isolation of several tDNA-containing clones from a human genomic library and the structure and expression _in vivo_ of a tRNA gene from one of these.

MATERIALS & METHODS

MATERIALS

The human genome library of 15-20 Kb fragments of foetal liver DNA cloned in λ Charon 4A (12) was a gift from T. Maniatis.   pBR322 clones of X. laevis tDNAs (13), used as control during library screening and clone character- ization were supplied by S.G. Clarkson.   tRNA nucleotidyl transferase was prepared from Baker's Yeast by the method of Sternbach et al (14).   DNA ligase was a gift from D. Bentley.   RNA ligase was from P.L. Biochemicals, snake venom phosphodiesterase from Worthington and restriction enzymes Sst I and Sst II from BRL (UK).   All other enzymes were from Biolabs.   All radio- chemicals were from Amersham International.

Radioactive tRNA for Hybridisation Probes

Crude tRNA was prepared from human placenta by the method of Roe (15). Three different probes were prepared from this.   (a) "Crude tRNA [^{32}P]pCp" (2 to 5 x 10^7 cpm μg^{-1}) was obtained by ligation of cytidine 3', 5' [5'-^{32}P] bisphosphate ($\geqslant$ 3,000 Ci/mmol) to the tRNA for 2-12 hours using the method of England et al (16);   (b) Crude tRNA was aminoacylated using a freshly desalted mixture of aminoacyl tRNA synthetases from human placenta by the method of Roe (15) except that twenty amino acids were used, ten of which (Phe, Pro, Tyr, Ser, Arg, Ala, Asp, Glu, Asn, Leu) were ^{14}C labelled at 0.1 Ci/mmol.   The mixed aminoacyl tRNAs (average specific activity 48 pmol each aminoacid/A$_{260}$ unit) were treated with periodate by the method of Traboni et al (17) and excess periodate removed by addition of rhamnose to 0.1 M.   The protected aminoacyl tRNAs were then deacylated by incubation with 0.5 M lysine pH 8.8 which also caused β-elimination of the periodate oxidised uncharged RNA.   This material, chargeable to the same extent as untreated tRNA, was radiolabelled as described in (a) above to yield "periodate-treated tRNA [^{32}P]pCP" (approx. 2 x 10^7 cpm/μg);   (c) Crude tRNA (1 μg) was treated with snake venom phosphodiesterase and repaired with yeast tRNA-nucleotidyl transferase, CTP and [α-^{32}P] ATP by the method of Silberklang et al (18), modified to reduce the reaction volume from 10 μl to 4 μl.   This gave

"crude tRNACpC[^{32}P]pA" of approx. 10^6 cpm/µg.

Isolation of λ clones

This and subsequent manipulations were performed in compliance with GMAG requirements.

Grids of 400 mixed plaques were prepared from nearly confluent plates of the Maniatis library grown on E. coli K803. Filters (Millipore HA or Schleicher and Schuell BA85) were prepared from these gridded plates and hybridised with crude tRNA [^{32}P]pCp or periodate-treated tRNA [^{32}P]pCp for 16-24 hr in 50% formamide, 5 x SSC at 42°C. Filters were washed (x 3) at room temperature in hybridisation buffer and (X2) in 2 x SSC, dried and auto-radiographed. Plaques which hybridised either form of tRNA were purified and rescreened. Clones showing hybridisation to periodate-treated tRNA [^{32}P]pCp were selected.

Production, separation, hybridization subcloning and sequencing of restricton fragments

These were performed using standard methods essentially the same as those compiled by Maniatis et al (19). DNA fragment size markers were λ DNA digested with Hind III and M13mp2RF DNA digested with Hae III. The conditions of RNA-DNA hybridisation were the same as for plaque filters. The vectors used were pAT153 (20, 21), M13mp8(RF) and M13mp9(RF) (22). Nucleotide sequences of fragments subcloned in M13 were determined by the method of Sanger et al (23).

Oocyte Injection and RNA analysis

Plasmid DNA (5-10 ng/oocyte) was co-injected with [α-^{32}P]GTP·(410 Ci/mmol; 0.5 µCi/oocyte) into oocyte nuclei as described by Kressman et al (24) and the oocytes were incubated overnight. Total RNA was then extracted (25) and separated on 10% polyacrylamide gels containing 4 M urea (26).

RESULTS AND DISCUSSION

Isolation and physical mapping of a tDNA fragment

A mixture of radiolabelled tRNAs was chosen to probe the Maniatis human genomic library in order to increase the likelihood of selecting a wider variety of tRNA genes than might be expected using either a purified single species tRNA or eukaryotic tDNA as a probe. Furthermore it was hoped that the use of mixed tRNAs would favour selection of those genes the products of which predominate in crude tRNA and which, therefore, are present in high copy number or of high transcriptional activity. In the initial screening of 4,000 mixed plaques (estimated to contain up to ten or more different recom-
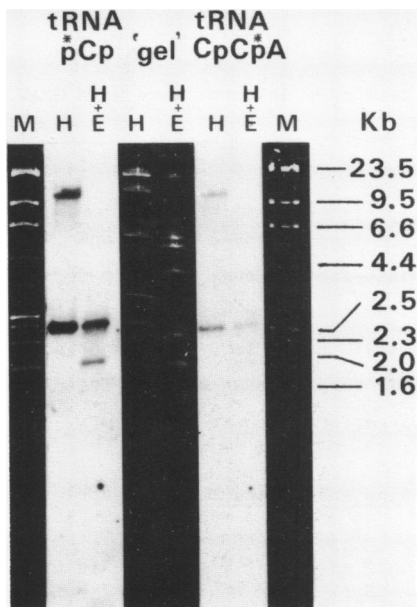
binants per plaque), 268 putative positives were selected of which approx. 50 showed strong hybridization, approx. 50 hybridised weakly and the rest showed intermediate hybridisation to crude tRNA [^{32}P]pCp. Screening with periodate-treated tRNA [^{32}P]pCp, plaque purification and rescreening, permitted selection of eight recombinants. Only one of these originated from a mixed plaque which strongly hybridised to crude tRNA [^{32}P]pCp, but three were from weakly hybridising plaques. The clone reported here, λ ht137, originally showed weak to intermediate hybridisation.

In view of the high incidence of 'false' positives, which we tentatively ascribe to radiolabelled small RNAs (e.g. 5S RNA, degraded rRNA etc.) present in crude tRNA [^{32}P]pCp but not radiolabelled in periodate-treated tRNA [^{32}P]pCp, the selected clones were further screened using another indepen-dent and unambiguous probe. The post-transcriptional addition and repair of the 3' terminus by tRNA nucleotidyltransferase permitted selective labelling of RNAs with a tRNA-like structure to less than 10^6 cpm μ g^{-1}, irrespective of the time (zero to 20 min) of the prior treatment of the tRNA with venom phosphodiesterase. This precluded the probe's use in plaque hybridisation but was just sufficient for hybridisation of Southern blots of the restriction fragments of the recombinant DNAs.

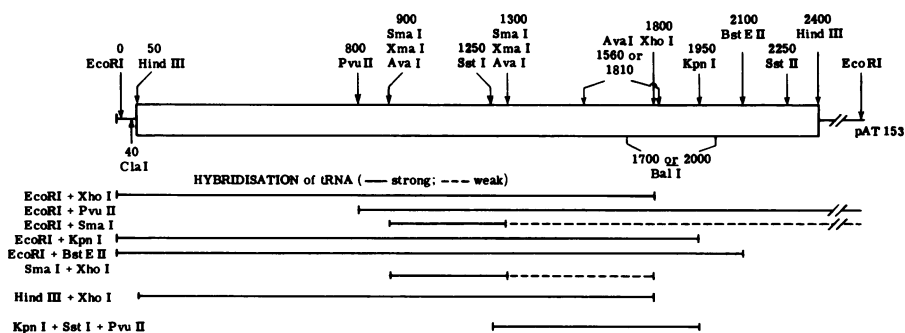The result for the recombinant λ ht137 is shown in Figure 1. Digestion

```
                          900         1300                  2100    2400
                          Sma I       Sma I         1800    Bst E II Hind III
           0    50        800  Xma I  1250 Xma I   AvaI Xho I        2250
         EcoRI Hind III   PvuII Ava I Sst I Ava I  1560 or  1950     Sst II      EcoRI
                                                   1810     Kpn I
                                                                              pAT 153
           40                                              1700 or 2000
           ClaI                                            Bal I
                 HYBRIDISATION of tRNA ( —— strong; --- weak )
 EcoRI + Xho I
 EcoRI + Pvu II
 EcoRI + Sma I
 EcoRI + Kpn I
 EcoRI + Bst E II
 Sma I + Xho I

 Hind III + Xho I

 Kpn I + Sst I + Pvu II
```

Figure 2
     Restriction map of the 2.4 Kb Hind III fragment (box) showing hybridiz-
ation of tRNA to restriction fragments.

with Hind III released, _inter_ _alia_, two fragments of 10 Kb and approx. 2.4 Kb
which hybridised to both periodate-treated [^{32}P]pCp and tRNA CpC[^{32}P]pA.
Further digestion with EcoRI appeared not to cut the 2.4 Kb Hind III fragment
but the larger fragment was cut to release a 1.8 Kb fragment which hybridised
to both probes.   The 2.4 Kb and 1.8 Kb fragments were isolated and subcloned
into Hind III-cut pAT153 or (Hind III + EcoRI)-cut pAT153 to yield PLB4 and
pTC163 respectively.

     In order to establish the location of the tRNA gene(s) within the 2.4 Kb
fragment, pLB4 was linearised by digestion with EcoRI which cuts the DNA
uniquely at a position 49 bases from the unique Hind III site into which the
2.4 Kb fragment was inserted (20, 21).   The linearised plasmid was then
digested with one of several restriction endonucleases which recognise se-
quences of 6 nucleotides absent from pAT153 or present in a few precisely
known positions.   The size of the fragments measured directly, and in most
cases unambiguously, the position of restriction sites, if any, to the right
of the unique EcoRI site.   These are shown in Figure 2.   Ambiguity with
respect to the position of one of the Sma I sites was removed by digestion of
the circular plasmid recombinant with Sma I.   This released a fragment of
400 bp consistant with Sma I sites at approx. 900 bp and 1300 bp from the
EcoRI site.   The fragments which hybridised crude tRNA [^{32}P]pCp, shown in
Figure 2, indicated tRNA-like sequences were confined to the central portion
of the 2.4 Kb fragment.

Transcriptional studies
     The RNA produced by _Xenopus_ oocytes following microinjection of the

(b)

A in M13mp8

	900	1300	1800	2400	
Hind III	XmaI	XmaI	XhoI	Hind III	
B in pAT153					B in pAT153

tRNA^{Glu}
XmaI

C in M13mp9

D in M13mp8

E in M13mp9

F in M13mp8

G in M13mp9

(a)

pLB4

pTC163

pNB21 pt210 C

M

(c)

A B C D E F G

5.8S —

5S —
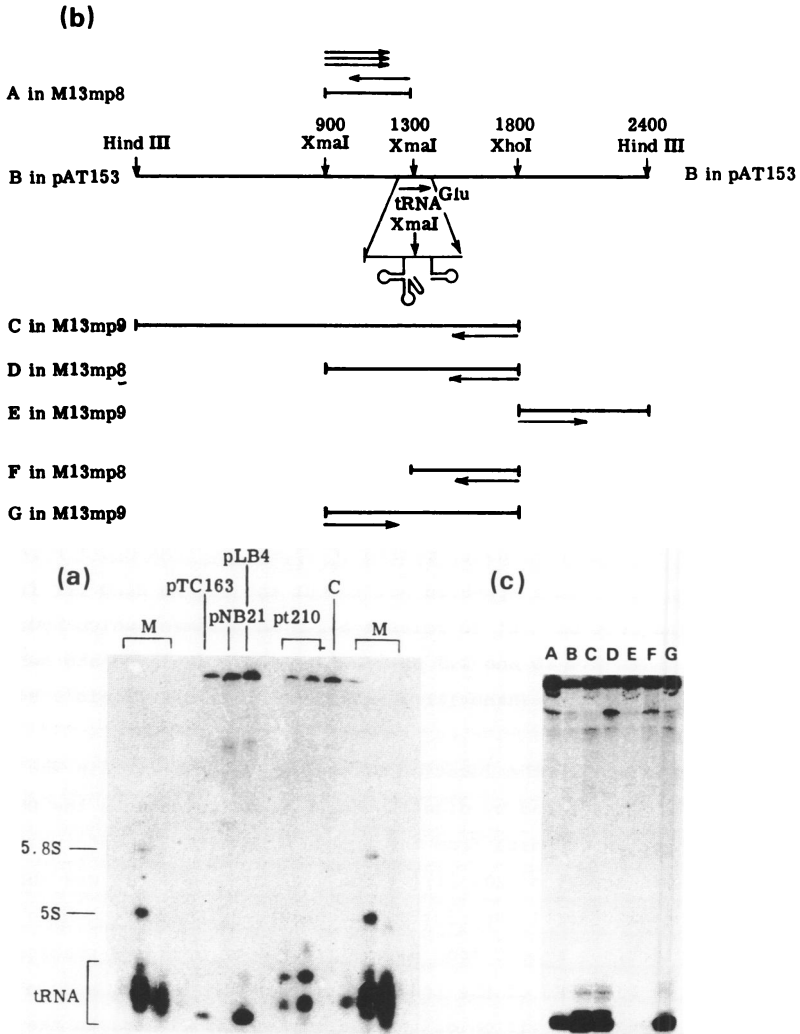
tRNA

Figure 3

(a) RNAs synthesised in Xenopus oocytes following nuclear injection with
recombinant DNAs and [α-^{32}P]-GTP. M = Marker tRNA [^{32}P]pCp (left,
crude tRNA; right; "periodate-treated tRNA"); pTC163 = 1.8 Kb Hind
III-EcoRI fragment from λ ht137 in pAT153; pNB21 = A pBR322 subclone of
human DNA being characterized; pLB4 = The 2.4 Kb Hind III fragment
containing the tRNA^{Glu} gene; pt210 =3.18 Kb X. laevis tDNA cluster in
pBR322 (only in the right hand lane were conditions identical to all
other experiments). C = Control (only [α -^{32}P]GTP])

(b) Fragments of the 2.4 Kb subcloned into M13 for sequence analysis and
transcriptional studies.   The direction and approximate extent of
sequence data is shown by the arrows.   An additional subclone equivalent
to F but in M13mp9 was used to determine the sequence 1300 to 1600.   The

conclusion from these studies is shown diagrammatically below the parent
fragment B.
(c) Autoradiograph of gel containing RNAs produced by subclones A-G (Fig. 3b)
following their nuclear co-injection, with [α -^{32}P]GTP, into <u>Xenopus</u>
oocytes.

subcloned fragment into their nuclei was resolved by polyacrylamide gel

electrophoresis.   In this system endogenous transcription of the oocyte tRNA

genes is very low and almost all the tRNA transcripts are derived from the

large number of injected tRNA genes.   Brief (2 hr) autoradioagraphy of the

gel (Fig. 3a) indicated that probably only one tRNA species, of relatively

high electrophoretic mobility, was transcribed following microinjection of

either pLB4 or pTC163.   The discontinuous gel system used (26) resolved most

of the species transcribed from the pBR322 clone of the <u>Xenopus</u> <u>laevis</u> tDNA

cluster, pt210 (3, 13).   A longer exposure (16 hr - data not shown) revealed

minor tRNA 4S bands and endogenous transcripts.   The former were larger than

the major species and may be ascribed either to tRNAs produced by less active

tRNA genes in the recombinant or to precursors to the major tRNA species.

The most striking feature of Figure 3a is the amount of tRNA produced by

oocytes microinjected with pLB4 compared to that produced on microinjection of

the same amounts of either pTC163 or the control recombinant pt210.   This

control contains within its 3.18 Kb insert a cluster of 8 tRNA genes of which

7 are transcriptionally active in the homologous <u>Xenopus</u> oocyte nuclei or

germinal vesicle extracts (3).   The density of the autoradiograph bands (Fig.

3a) indicate that the major tRNA bands produced by pLB4 is approximately 10

times that from pTC163, 3 times that of the strongest band from pt210 and is

10-20% greater than the sum of all of the tRNA bands produced by pt210.

The 2.4 Kb fragment contained the restriction sites Xma I, Hind III and

Xho I (compatible with Sal I).   This permitted subcloning of suitable

portions of the fragment into M13mp8 or M13mp9 for both sequence analysis and

further transcriptional studies (Fig. 3b).   Analysis of the RNA produced in

oocytes following nuclear injection of RFDNA from several of these M13 recom-

binants is shown in Figure 3c.   No RNA was produced from the Xho I-Hind III

fragment in M13mp9 (E in Fig. 3b and c) confirming the hybridization studies

which suggested no tRNA-like sequence in the region 1800-2400.   Subclones of

the two fragments from the central region, Xma I (900)-Xma I (1300) (A in Fig.

3b, c) and Xma I (1300)-Xho I (1800) (F in Fig. 3b, c) also produced no RNA

although the larger Xma I (900)-Xho (1800) fragment in either M13mp8 or M13mp9

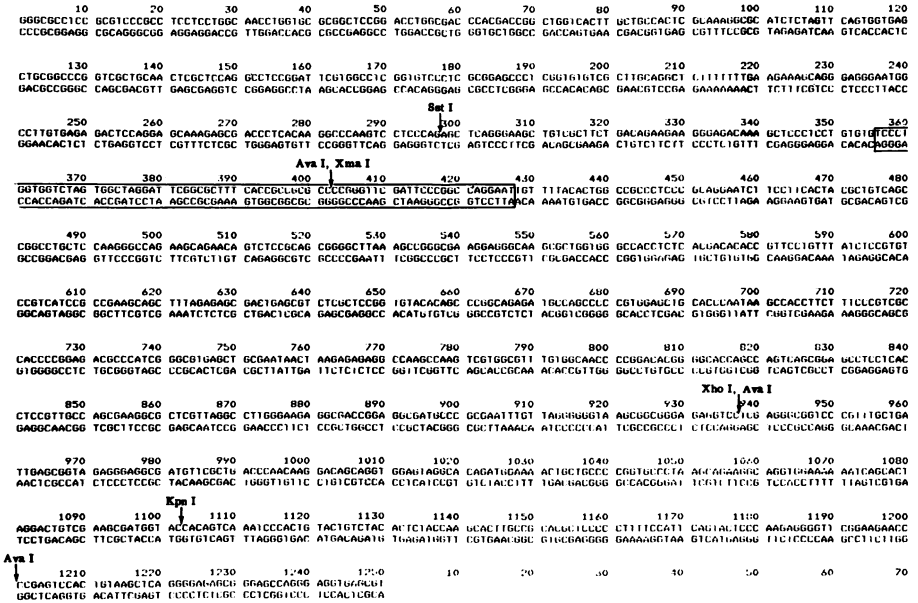(D and G in Fig. 3b, c) produced tRNA of the same size and quantity as the

```
        10        20        30        40        50        60        70        80        90       100       110       120
GGGCGCC1CC GCGTCCCGCC TCCTCCTGGC AACCTGG1GC GCGGCTCCGG ACCTGGCGAC CCACGACCGG CTGG1CACTT BCTGCCCAC1C GLAAAAGGCC ATCTCTAGTT CAGTGGTGAG
CCCGCGGAGG CGCAGGGCGG AGGAGGACCG TTGGACCACG CGCCGAGGCC TGGACCGCTG GGTGCTGGCC GACCAGTGAA CGACGGTGAG CGTTTCCGCG TAGAGATCAA GTCACCACTC

       130       140       150       160       170       180       190       200       210       220       230       240
CTGCGGCCCG GTCGCTGCAA CTCGCTCCAG GCCTCCGGAT TCGTGGCC1C GG1GTCCCTC GCGGAGCCC1 CGG1G1GTCG C1TGCAGGC1 C11111TTGA AGAAAGCAGG GAGGGAATGG
GACGCCGGGC CAGCGACGTT GAGCGAGGTC CGGAGGCCTA AGCACLGGAG CCACAGGGAG CGCCTCGGGA GCCACACAGC GAACGTCCGA GAAAAAAACT TCT11CGTCC CTCCCTTACC

                                                                    Sst I
       250       260       270       280       290       300       310       320       330       340       350       360
CCTTGTGAGA GACTCCAGGA GCAAAGAGCG ACCCTCACAA GGCCCAAGTC CTCCCAGAGC TCAGGGAAGC TGTCGCT1CT GACAGAAGAA GGGAGACAAA GCTCCC1CCT GTGTGTCCCT
GGAACACTC1 CTGAGGTCCT CGTTTCTCGC TGGGAGTGTT CCGGGTTCAG GAGGGTCTCG AGTCCC1TCG ACAGCGAAGG AC1GTC1TCT1 CCCTC1GTTT CGAGGGAGGA CACACAGGGA

                           Ava I, Xma I
       370       380       390       400       410       420       430       440       450       460       470       480
GGTGGTCTAG TGGCTAGGAT TCGGCGCTTT CACCGCLGCG CCCCGGGT1C GATTCCCGGC CAGGAAT1GT TT1ACACTGG CCGCCCTCGC GLAGGAATC1 TCC11CACTA CGCTGTCAGC
CCACCAGATC ACCGATCC1A AGCCGCGAAA GTGGCGGCGC GGGGCCCAAG C1AAGGCCG GTCCTTAACA AAATGTGACC GGCGGGAGGG CGTCCT1AGA AGGAAGTGAT GCGACAGTCG

       490       500       510       520       530       540       550       560       570       580       590       600
CGGCCTGCTC CAAGGGCCAG AAGCAGAACA GTCTCGCAG CGGGGCTTAA AGCCGGGCGA AGGAGGGCAA GCGCTGG1GG GCCACCTCTC ACGACACACC GTTCC1GTTT A1C1CCGTGT
GCCGGACGAG GTTCCCGGTC TTCGTC11GT CAGAGGCGTC GCCCCGAATT 1CGGCCCGCT TCCTCCCGT1 CGLGACCACC CGG1GAGGAG 1GCTG1G1GG CAAGGACAAA 1AGAGGCACA

       610       620       630       640       650       660       670       680       690       700       710       720
CCGTCATCCG CCGAAGCAGC TTTAGAGAGC GACTGAGCGT CTCGCTCCGG 1GTACACAGC CCGGCAGAGA 1GCCAGCCLC CGTGGGAGC1G CACLCAATAA GCCACCTTCT T1CLCGTCGC
GGCAGTAGGC GGCTTCGTCG AAATCTCTCG CTGACTCGCA GAGCGAGGCC ACAT1GTCG GGCCGTCTC1 ACGG1CGGGG GCACCTCGAC G1GGG11ATT CGGTCGAAGA AAGGGCAGCG

       730       740       750       760       770       780       790       800       810       820       830       840
CACCCCGGAG ACGCCCATCG GGCGTGAGCT GGGAATAACT AAGAGAGAGG CCAAGCCAAG TCGTGGCG11 TG1GGCAACC CCGGACALGG GGCACCAGCC AGTCAGCGGA GCCTCCTCAC
G1GGGGCCTC TGCGGGTAGC CCGCACTCGA CGCTTA1TGA 11CTC1CTCC GG1TCGGTTC AGCACCGCAA ACACCG1TGG GGCCTGTGCC CCGTGGTCGG 1CAGTCGCCT CGGAGGAGTG

                                                                                          Xho I, Ava I
       850       860       870       880       890       900       910       920       930       940       950       960
CTCCGTTGCC AGCGAAGGCG CTCGTTAGGC CT1GGGAAGA GGCGACCGGA GGCGATGCCC GCGAAT1TGT TAGGGGGG1A AGCGGCGGGA GAHGTCC1CG AGGGCGGTCC CG11TGCTGA
GAGGCAACGG TCGCT1CCGC GAGCAATCCG GAACCC11C1 CCGCTGGCC1 1CCGCTACGGG CGC1TAAACA ATCCCCTA1 TCGGCGCCC1 C1CCAAGAGC 1CCGCCAGG GLAAACGAC1

       970       980       990      1000      1010      1020      1030      1040      1050      1060      1070      1080
TTGAGCGGTA GAGGGAGGCG ATGT1CGCTG ACCCAACAAG GACAGCAGG1 GGAG1AGGCA CAGATGGAAA AC1GCTGCCC CGGTGCCC1A AGCGGGAGGC AGGTGGAA1A AA1CAGGAC1
AACTCGCCAT CTCCCTCCGC TACAAGCGAC 1GGGT1G11C C1G1CGTCCA CC1CA1CCG1 G1C1ACC11T 1GA1GACGGG GCCACCGGA1 1CG11TCCG TCCACCT111 11AGTCGTGA

                  Kpn I
      1090      1100      1110      1120      1130      1140      1150      1160      1170      1180      1190      1200
AGGACTGTCG AAGCGATGGT ACCACAGTCA AA1CCCACTG TACTGTCTAC AC1C1ACCAA GCACT1GCCG CACGC1CCCC CT1T1CCAT1 CAG1AC1CCC AAGAGGGGT1 CGGAAGAACC
TCCTGACAGC TTCGCTACCA TGGTGTCAGT TTAGGGTGAC A1GACAGATG TGAGA1GGT1 CGTGAACGGC G1GCGAGGGG GAAAAGGTAA GTCA1GAGGG 11C1CCCCAA GCC11C11GG

Ava I
      1210      1220      1230      1240      1250              10        20        30        40        50        60        70
TCGAGTCCAC 1GTAAGCTCA GGGGA1AGCG GGAGCCAGGG AGG1GAGCG1
GGCTCAGGTG ACATTCGAGT CCCCTC1LGC CCTCGG1CCL 1CCAC1CGCA
```

Figure 4

The nucleotide sequence from the XmaI site at approximately 900 on the restriction map of Figure 2. The tRNA^{Glu} gene is boxed. The restriction sites found experimentally (Figure 2) are shown.

parent clone pLB4 (B in Fig. 3b, c) or the Hind III-Xho fragment (C in Fig. 3b, c). The minor 4S bands (Fig. 3c; lanes C, D and G) we ascribe to precursors of the tRNA which have been fully processed in the pLB4 parent clone transcript (Fig. 3c; lane B). The high molecular weight products, present in all lanes of Fig. 3c and strong in lanes A and D are presumably endogenous transcripts. This suggested that the 2.4 Kb fragment contained only one tRNA gene which was split by Xma I (900) to yield two transcriptionally inactive fragments.

Sequence Studies

Eleven of the M13 recombinants were sequenced as indicated in Figure 3b. The orientation of the Xma I fragment within the 2.4 Kb sequence was determined from the position of the Sst I site (at approx. 1250 bp from EcoRI - Fig. 2) within the Xma I fragment sequence. All other fragments were of known orientation permitting the alignment of sequence from approx. 900 bp to approx. 2150 bp from the EcoRI site of Figure 2. This is shown in Figure 4.

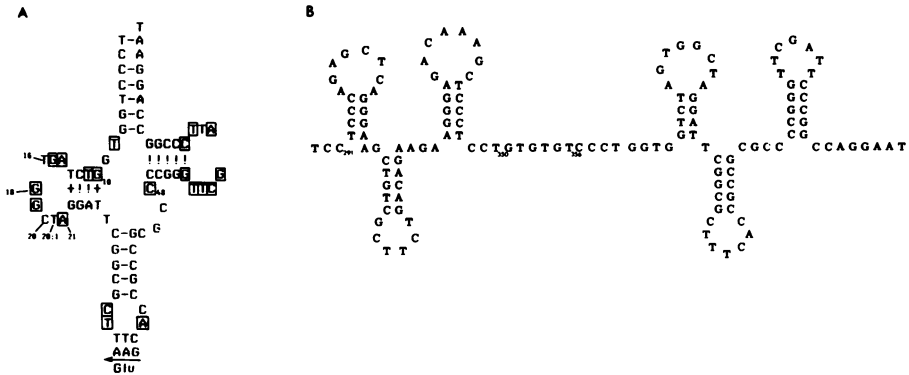The position of the only tRNA gene in the sequence (356 to 427, corres-

Figure 5

(A) Sequence of the tRNA^{Glu} gene (non-coding strand) arranged in a
cloverleaf secondary structure with the yeast tRNA^{Phe} numbering.
Conserved and semi-conserved nucleotides are boxed.

(B) A possible secondary structure in the 5' flanking sequence (residues
292-347) with features resembling that of a tRNA.   The tRNA^{Glu} gene,
drawn in the same manner, is also shown (residues 356-end).

ponding to approx. 1250 to approx. 1320 on the map in Fig. 2) confirmed the
inference from transcriptional studies that this inadvertantly disrupted human
tRNA gene, like tRNA genes of other eukaryotes, contains an intragenic split
promoter, both parts of which are required for transcription.   Xma I cuts
within the gene after residue 402, corresponding to the junction of the
variable loop and the TψC stem of the tRNA.

The structure of the tRNA^{Glu} gene is shown in Figure 5A where the non-
coding (tRNA-like) strand is folded as a cloverleaf with standard numbering of
residues according to those found in yeast tRNA^{Phe} (27).   The most unusual
structural feature is the presence of an C_2-A_{71} pair in the acceptor stem,
the first example of its occurrence in a eukaryotic tRNA (28).   Another un-
expected feature is the presence of T(U) at position 73, instead of a purine
previously found in every tRNA^{Glu} sequence (27).   The general structural
properties of tRNAs, including all the conserved or semi-conserved nucleotides
are present.   Some features characteristic of tRNA^{Glu}, e.g. the absence of
residues 17 and 47 and an extra residue (20:1) in the D loop, are found in
this gene.   The sequence reported here differs from the rat tRNA$^{Glu}_{GAA}$
(29) tDNA$^{Glu}_{GAG}$ of rat (6) and tDNA$^{Glu}_{GAA}$ of Drosophila (30) by 19, 7
and 17 residues respectively.

It is premature to attempt to generalise upon the organisation of tRNA
genes within the human genome.   Earlier studies (7) on tRNA^{Met} genes show

that about twelve genes are scattered in the genome, whereas more recently a cluster of three different tRNA genes, each separated by approx. 0.5 Kb, have been found in a human λ recombinant which contains several tRNA genes (8). The single tRNA^{Glu} gene reported here is separated from other tRNA genes by at least 1 Kb, being centrally located within a 2.4 Kb fragment. However there is evidence for a least one more tRNA gene within the clone, λ htl37 from which the 2.4 Kb fragment was derived (see Fig. 1).

We have inspected the sequence surrounding the tRNA^{Glu} gene (Fig. 4). No other tRNA genes were found using the program of Staden (31). No open reading frames of significant length were detected. However several repetitive sequences and inverted repeats of eight or more nucleotides were found. The 5' flanking sequence preceding the tRNA^{Glu} gene shares the feature, noted for other human tRNAs (8), of a block of purines followed by a block of pyrimidines although their lengths in the present sequence are considerably larger than those noted previously. In addition we note the presence of a 7 bp alternating purine-pyrimidine tract capable of forming left handed DNA which has been suggested may be an inhibitory sequence in a variant tRNA^{Met} gene of Xenopus laevis (32).

The interesting feature of this region however is that residues 292-347 have the potential to form a secondary structure resembling that of part of a tRNA. This is shown in Figure 5B. It would be misleading to call this sequence a tRNA pseudogene. In spite of the presence of TTC in the region corresponding to the anticodon loop it shows low homology with the adjacent tRNA^{Glu} gene, nor does it contain a convincingly large proportion of the conserved or semi-conserved nucleotides noted for tRNA - the most generous interpretation permits only about half of these residues to be present. The strongest resemblence of this sequence to tRNA lies in its secondary structure and the relative spacing of the arms to each other and to these in the tRNA gene. Although this is not perfect (with 6 and 8 residues in the anticodon and TψC loops instead of the normal 7 residue), we have been unable to generate a structure resembling this from other reported 5' flanking sequences. When two eukaryotic tRNA genes are in tandem they are usually separated by a greater distance than the structures shown in Figure 5B and, if of the same polarity, are separated by a T-rich "transcription termination" sequence (33). Although the RNA polymerase promoter is contained within the transcribed gene, there is evidence that the transcriptional control region for tRNA genes (9-11), are in the 5' flanking sequence immediately preceding the gene (for review see 32). For example Drosophila tRNA^{Lys} gene is re-

pressed by an oligo T-containing undecanucleotide 13 nucleotides upstream from the tRNA (9, 11), whereas the normal 5' flanking sequence of <u>Bombyx</u> <u>mori</u> tRNA^{Ala} is required for transcription by a homologous cell-free extract (10). It has been proposed that the intragenic promoter of tRNA genes is recognised as a tertiary structure that partially resembles tRNA itself (32). The present gene is remarkably active in transcription. We are therefore tempted to speculate that this peculiar structural feature of the 5' flanking may serve to enhance transcription of the gene.

*On leave of absence from the Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ, UK

REFERENCES
1. Kubli, E., Experientia 37, 1-9 (1981).
2. Söll, D., Abelson, J.N. and Schimmel, P.R. (1980) Transfer RNA: Biological Aspects, Cold Spring Harbor Laboratory.
3. Yen, P.H. and Davidson, N. (1980) Cell 22, 137-148.
4. Müller, F. and Clarkson, S.G. (1980) Cell 19, 345-353.
5. Lasser-Weiss, M., Bawnik, N., Rosen, A., Sarid, S. and Daniel, V. (1981) Nucl. Acids. Res. 9, 5965-5978.
6. Shibuya, K., Noguchi, S., Nishimura, S. and Sekiya, T. (1982) Nucl. Acids. Res. 10, 4441-4448.
7. Santos, T. and Zasloff, M. (1981) Cell 23, 699-709.
8. Roy, K.L., Cooke, H. and Buckland, R. (1982) Nucl. Acids. Res. 10, 7313-7322.
9. DeFranco, D., Schmidt, O. and Söll, D. (1980) Proc. Natl. Acad. Sci. USA 77, 3365-3368.
10. Sprague, K.U., Larson, D. and Morton, D. (1980) Cell 22, 171-178.
11. DeFranco, D., Sharp, S. and Söll, D. (1981) J. Biol. Chem. 256, 12424-12429.
12. Lawn, R.M., Fritsch, E.F., Parker, R.C., Blake, G. and Maniatis, T. (1978) Cell 15, 1157-1174.
13. Clarkson, S.G., Kurer, V. and Smith, H.O. (1978) Cell 14, 713-724.
14. Sternbach, H., von der Haar, F., Schlimme, E., Gaertner, E. and Cramer, F. (1971) Eur. J. Biochem. 22, 166-172.
15. Roe, B.A. (1975) Nucl. Acids. Res. 2, 21-42.
16. England, T.E., Bruce, A.G. and Uhlenbeck, O.C. (1980) in Methods in Enzymology Vol. 65 pp 65 -74, Academic Press, New York.

17. Traboni, C., Cortese R. and Salvatore F. (1980), Nucl. Acids. Res. 8, 5223-5232.
18. Silberklang, M., Gillum, A.M. and RajBhandary, U.L. (1979) in Methods in Enzymology Vol. LIX pp 58 -109, Academic Press, New York.
19. Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory.
20. Twigg, A.J. and Sherratt, D. (1980) Nature 283, 216-218.
21. Sutcliffe, J.G. (1978) Cold Spring Harbor symp. Quant. Biol. 43, 77-90.
22. Messing, J. and Vieira, J. (1982) Gene 19, 269-276.
23. Sanger, F., Coulson, A.R., Barrell, B.G., Smith, A.J.H. and Roe, B.H. (1980) J. Mol. Biol. 143, 161-178.
24. Kressmann, A., Clarkson, S.G., Pirrotta, V. and Birnstiel, M.L. (1978) Proc. Natl. Acad. Sci. USA 75, 1176-1180.
25. Bienz, M. and Gurdon, J.B. (1982) Cell 29, 811-819.
26. Fradin, A., Gruhl, H. and Feldmann, H. (1975) FEBS Letters 50, 185-189.
27. Sprinzl, M. and Gauss, D.H. (1983) Nucl. Acids Res. 11, r1-r55.
28. Grosjean. H. Cedergren, R.J. and McKay, W. (1982) Biochemie 64, 387-397.
29. Chan, J.C., Yang, J.A., Dunn, M.J., Agris, P.F. and Wong, T-W. (1982) Nucl. Acids Res. 10, 4605-4608.
30. Indik, Z.K. and Tartof, K.D. (1982) Nucl. Acids Res. 10, 4159-4172.
31. Staden, R. (1980) Nucl. Acids. Res. 8, 817-825.
32. Hall, B.D., Clarkson, S.G. and Tocchini-Valentini, G. (1982) Cell 29, 3-5.
33. Korn, L.J. and Brown, D.D. (1978) Cell 15, 1145-1156.