The EMBO Journal vol.3 no.5 pp.1091 – 1096, 1984

# The molecular basis for the differential translation of TMV RNA in tobacco protoplasts and wheat germ extracts

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*Communicated by H.J. Gross*

**Translation of tobacco mosaic virus (TMV) RNA in tobacco protoplasts yields the 17.5-K coat protein, a 126-K protein and a 183-K protein which is generated by an efficient readthrough over the UAG termination codon at the end of the 126-K cistron. In wheat germ extracts, however, only the 5′-proximal 126-K cistron is translated whereas the 183-K readthrough protein is not synthesized. Purification and sequence analysis of the endogenous tyrosine tRNAs revealed that the uninfected tobacco plant contains two $tRNAs^{Tyr}$, both with GΨA anticodons which stimulate the UAG readthrough *in vitro* and presumably *in vivo*. In contrast, ~85% of the $tRNA^{Tyr}$ from wheat germ contains a QΨA anticodon and ~15% has a GΨA anticodon. Otherwise the sequences of $tRNAs^{Tyr}$ from wheat germ and tobacco are identical. UAG readthrough and hence synthesis of the 183-K protein is only stimulated by $tRNA^{Tyr}_{G\Psi A}$ and not at all by $tRNA^{Tyr}_{Q\Psi A}$. The $tRNAs^{Tyr}$ from wheat leaves were also sequenced. This revealed that adult wheat contains $tRNA^{Tyr}_{G\Psi A}$ only. This is very much in contrast to the situation in animals, where Q-containing tRNAs are characteristic for adult tissues whereas Q deficiency is typical for the neoplastic and embryonic state.**

*Key words:* readthrough/tobacco mosaic virus (TMV)/wheat germ $tRNAs^{Tyr}$/translation/UAG suppression

## Introduction

Pioneering studies of replication, transcription and protein synthesis have preferentially been performed in defined virus-infected cellular systems. Plant protoplasts, in particular, have become a powerful tool for elucidating some of the basic features of plant viral gene expression. Thus, tobacco protoplasts inoculated with tobacco mosaic virus (TMV) *in vitro* have been used most successfully for studying the TMV-dependent protein synthesis. The major viral proteins observed in this system are a 17.5-K protein, the coat protein, and two high mol. wt. proteins of 126 and 183 K, supposedly related to a viral replicase or replicase subunit. The 183-K protein is the product of a UAG readthrough at the end of the 5′-proximal cistron coding for the 126-K protein (Pelham, 1978; Goelet *et al.*, 1982). This is now one of the best known cases of natural suppression in a completely homologous system, since we have recently isolated and sequenced the two major cytoplasmic tyrosine tRNAs of the tobacco plant which promote this readthrough *in vitro* and presumably *in vivo*. These $tRNAs^{Tyr}$ both have a GΨA anticodon and differ in a base pair and in a methyl group only (Beier *et al.*, 1984).

Translation of TMV RNA has also been studied in wheat germ extracts (Roberts and Paterson, 1973; Davies and Kaesberg, 1974; Bruening *et al.*, 1976; Beachy and Zaitlin, 1977). Here, the major product is the 126-K protein, whereas the 183-K readthrough protein is not synthesized at all, or in traces only. Here we show that the structural and functional comparison of the tyrosine tRNAs from wheat germ with the corresponding $tRNAs^{Tyr}$ from tobacco reveals the molecular basis for the presence of 183-K readthrough synthesis in tobacco protoplasts and for its absence in wheat germ extracts.

## Results

Two *in vitro* translation systems have been routinely used for studying the translation of TMV RNA, i.e., the reticulocyte lysate and the wheat germ extract. In the latter, the synthesis of the 126-K and 183-K TMV-specific proteins is reduced as compared with the efficient translation of TMV RNA in the nuclease-treated reticulocyte lysate and has often been regarded as a general failure of the wheat germ extract to generate high mol. wt. polypeptides. We could increase the synthesis of the 126-K protein by 30% by supplementing the wheat germ extract with 10 μg/ml of calf liver tRNA (Figure 1, lane b), however, no stimulation of the readthrough product synthesis (e.g., 183-K protein) was observed. Only the addition

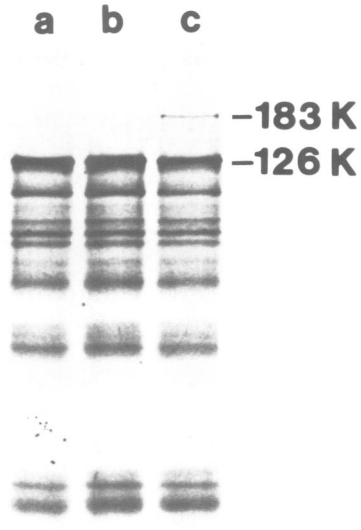


**Fig. 1.** *In vitro* translation of TMV RNA in wheat germ extracts. TMV RNA was translated in wheat germ extracts as described in Materials and methods in the absence of any added tRNA **(a)** or in the presence of 10 μg/ml of calf liver tRNA **(b)** and 50 μg/ml of suppressor $tRNA^{Tyr}_{G\Psi A}$ from tobacco (c), respectively. The suppressor tRNA used in this experiment had been partially purified by BD-cellulose and Sepharose 4B chromatography as described (Beier *et al.*, 1984). Translation products were separated on a 10% polyacrylamide gel and identified by autoradiography.

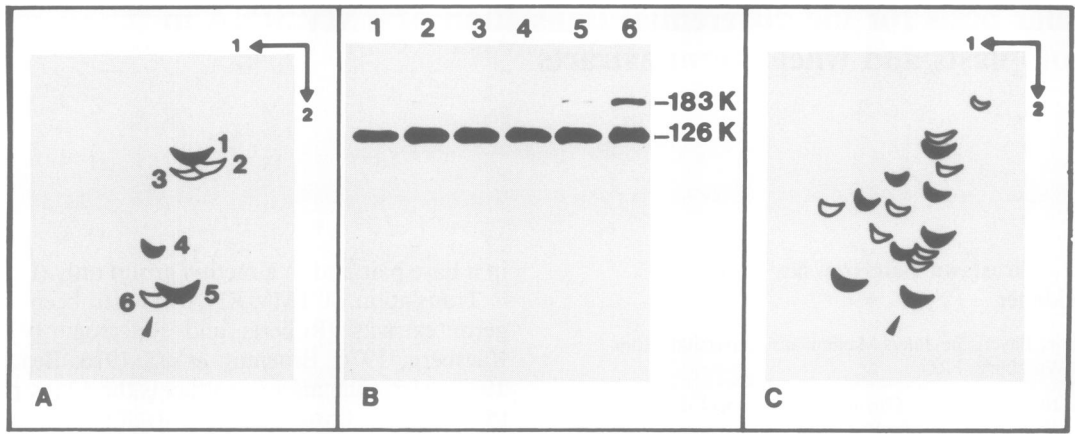


**Fig. 2.** Identification of suppressor tRNAs from wheat germ and wheat leaves. About 10 μg of wheat germ tRNAs$^{Tyr}$ partially purified by successive chromatography on BD cellulose and Sepharose 4B columns (**A**) and 40 μg of tRNAs$^{Tyr}$ (fraction 1) from wheat leaves partially purified by BD-cellulose chromatography only (**C**) were first loaded on a 10% polyacrylamide gel, pH 3.5 (from right to left), followed by a second dimension (12.5% polyacrylamide, pH 8.3, from top to bottom). Gels were stained with toluidine blue and destained as described in Materials and methods. Under these conditions, ~0.5 μg of tRNA per spot can be detected. The stained spots are presented schematically according to their staining intensity (open and shaded spots). All tRNAs visible in **panels A** and **C** were excised from the gel and the eluted material was directly used for aminoacylation with [$^3$H]tyrosine and for *in vitro* translation experiments with TMV RNA in parallel assays. An example for the latter is given in **panel B** which shows a fluorogram of a 10% SDS polyacrylamide gel of viral proteins synthesized in a reticulocyte lysate in the presence of tRNA species 1 to 6 from **A**. The arrow in **panels A** and **C** indicates the tRNA species which showed both, readthrough activity and [$^3$H]tyrosine acceptance. A second tyrosine tRNA species (No. 5, **panel A**) which did not significantly stimulate readthrough (No.5, **panel B**) was detected in the tRNA fraction isolated from wheat germ only.

of purified major cytoplasmic tRNA$^{Tyr}_{G\Psi A}$ from uninfected tobacco resulted in the synthesis of the 183-K protein (Figure 1, lane c). This latter tRNA has recently been isolated and characterized in our laboratory (Beier *et al.*, 1984) as a natural UAG suppressor tRNA. Thus, *in vitro* translation of TMV RNA in the wheat germ extract is hampered by a lack of appropriate tRNAs rather than an incapability to generate high mol. wt. polypeptides. These observations prompted us to study the pattern of tyrosine tRNAs present in wheat germ extracts.

*Purification of tRNAs$^{Tyr}$ from wheat germ and detection of UAG suppressor activity*

Wheat germ tRNAs$^{Tyr}$ were isolated from unfractionated total tRNA by BD-cellulose and Sepharose 4B chromatography using essentially the same procedure as for the isolation of tobacco tRNAs$^{Tyr}$ (Beier *et al.*, 1984). One major tyrosine tRNA peak eluted from the BD-cellulose column, as shown by aminoacylation assays with [$^3$H]tyrosine. This material stimulated only to a low extent readthrough over the UAG codon in TMV RNA, i.e., the synthesis of the 183-K protein in the reticulocyte lysate (not shown). The ratio of 183 K: (126 K + 183 K) was ~0.1 in fractions without suppressor activity and increased to 0.17 in fractions enriched in tRNA$^{Tyr}$. Fractions from the BD-cellulose column with tyrosine-accepting activity were pooled and used for further fractionation on a Sepharose 4B column. Again one major tRNA peak containing tyrosine acceptor and suppressor activity eluted from the column.

The final purification of wheat germ tyrosine tRNAs consisted of a two-dimensional gel electrophoretic step resulting in the separation of two tRNA species which could be charged with tyrosine (Figure 2A, spots No. 5 and No. 6). Only the minor tRNA$^{Tyr}$ showed significant suppressor activity (Figure 2B, No. 6) as was revealed by the determination of the percentage of 183-K protein synthesized *in vitro* in relation to the synthesis of both, 126-K and 183-K proteins. Thus, the non-supplemented lysate showed a 183 K: (126 K + 183 K) ratio of 0.11, whereas the tRNA fractions 1, 2, 3, 4, 5 and 6 produced a ratio of 0.09, 0.12, 0.12, 0.1, 0.14 and 0.26, respectively (Figure 2B). The major tRNA$^{Tyr}$ from wheat germ which is present to ~85% in the tyrosine-accepting material pooled after Sepharose 4B chromatography did not stimulate readthrough (Figure 2B, No. 5). The observed increase of the 183 K: (126 K + 183 K) ratio from 0.11 to 0.14 is most likely due to cross-contamination with the suppressor tRNA$^{Tyr}$ (species No. 6).

We eluted the tRNA species from spot No. 5 (Figure 2A) and did a controlled acid hydrolysis of the 3′-[$^{32}$P]pCp-labeled material to obtain a preliminary indication of the nature of the major tRNA$^{Tyr}$ from wheat germ. At the position where one would expect the anticodon we observed a big gap in the ladder (Figure 3, No. 34 and 35) characteristic for a queuosine (Q) nucleotide (Lankat, 1981). These interesting findings prompted us to elucidate the complete sequence of both, the major and minor tRNAs$^{Tyr}$ from wheat germ.

*Nucleotide sequences of the major and minor tRNAs$^{Tyr}$ from wheat germ*

The nucleotide sequences of the two tRNAs$^{Tyr}$ were determined by the two-dimensional mobility shift method (Jay *et al.*, 1974; Gillum *et al.*, 1975; Tyc *et al.*, 1983) and by the fragment analysis of partially digested RNA according to Stanley and Vassilenko (1978). The 5′-nucleotide of each eluted fragment was analysed by cellulose t.l.c. as shown in Figure 4. Modified nucleotides were identified according to their relative position in the two solvents described by Nishimura (1979). In some cases they were further examined by re-chromatography with authentic standards. The nucleotide at the 3′ side of the anticodon was identified as pm$^1$G (Figure 4) by comparison with authentic [$^{32}$P]pm$^1$G. This was obtained from pure yeast tRNA$^{Trp}$ by parallel sequence and nucleotide analyses according to Stanley and Vassilenko (1978).

The complete nucleotide sequence of the major tRNA$^{Tyr}$ from wheat germ is shown in Figure 5. Ribothymidine in the TΨC-loop of the tRNA$^{Tyr}$ is replaced by unmodified U. The modified U* next to m$^7$G is most likely 3-(3-amino-3-

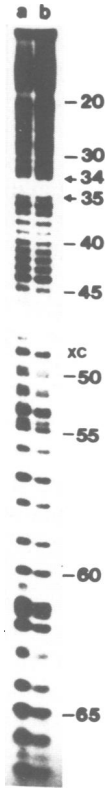


**Fig. 3.** Autoradiogram of fragments produced by limited acid hydrolysis from major 3′ $^{32}$P-labeled wheat germ $tRNA^{Tyr}_{(Q\Psi A)}$. The material eluted from spot No. 5 (Figure 2, panel A) was 3′ [$^{32}$P]pCp-labeled with T4 RNA ligase and subjected to controlled acid hydrolysis with 0.2 N (**a**) and 0.1 N $H_2SO_4$ (**b**), respectively, for 3 min at 95°C. Analysis was on a 20% polyacrylamide gel containing 8 M urea, pH 8.3. The numbers refer to the nucleotides in the tRNA (Figure 4) and indicate the 5′-terminal nucleotide of the corresponding 3′ end-labeled fragment. The arrows indicate the gap in the ladder produced by the Q nucleotide. XC = xylene cyanol.

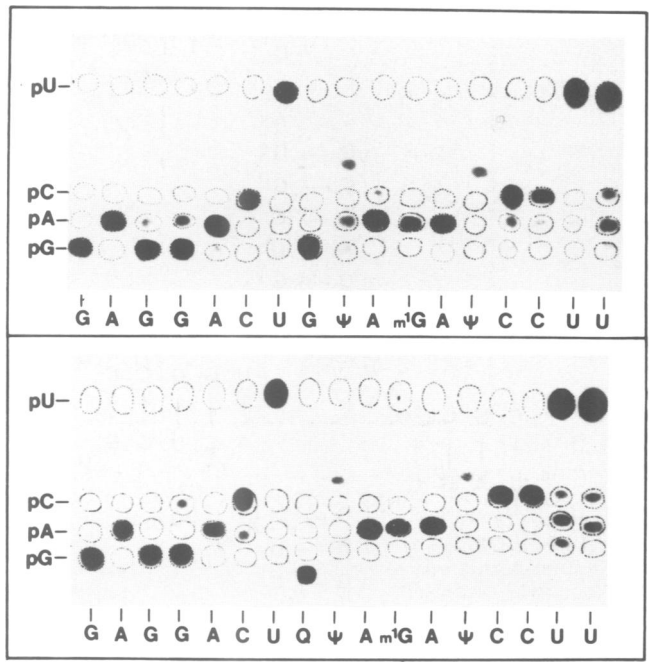


**Fig. 4.** Analysis of 5′-terminal nucleotides of 5′ $^{32}$P-labeled fragments from major and minor wheat germ $tRNAs^{Tyr}$ by cellulose t.l.c. Purified wheat germ $tRNAs^{Tyr}$ were subjected to limited hydrolysis with water, the fragments were 5′ $^{32}$P-labeled and then separated on a 12.5% polyacrylamide gel as described in Materials and methods. Each fragment was subjected to nuclease P1 digestion and the 5′-nucleotide identified by t.l.c. in two different solvents (Nishimura, 1979). As an example, the nucleotide sequence analysis of the anticodon stem and loop of minor $tRNA^{Tyr}_{(G\Psi A)}$ (upper panel) and of major $tRNA^{Tyr}_{(Q\Psi A)}$ (lower panel) is shown using isopropanol/conc. HCl/water as a solvent. The dotted circles indicate the location of unlabeled pN markers as visualized under u.v. light.

carboxypropyl) uridine, acp$^3$U, for reasons described earlier (Beier *et al.*, 1984). The major and minor $tRNAs^{Tyr}$ differ only in one nucleotide at the first position of the anticodon. The major $tRNA^{Tyr}$ contains queuosine (Q) instead of guanosine (Figure 4 and Figure 5) as had been expected from our preliminary observations shown in Figure 3. The Q nucleoside has very likely no sugar (mannose or galactose) linked to the cyclopentene diol moiety, as indicated by its chromatographic behaviour in two different solvents (Okada and Nishimura, 1977). This Q derivative, Q*, has only been found in tRNAs from animal tissue (Kasai *et al.*, 1976). Although $tRNA^{Tyr}_{G\Psi A}$ can recognize the UAG codon on TMV RNA, $tRNA^{Tyr}_{Q\Psi A}$ cannot act as a suppressor tRNA (Figure 2, panels A and B), indicating that queuosine prevents the recognition between the anticodon and the UAG termination codon. The $tRNAs^{Tyr}$ from wheat germ are essentially identical in sequence with those from tobacco (Beier *et al.*, 1984). Two major $tRNAs^{Tyr}_{G\Psi A}$ were purified from tobacco plants, both acting as UAG suppressor tRNAs. These two $tRNAs^{Tyr}$ differ in an A:U pair at the base of the TΨC-stem ($tRNA_1^{Tyr}$) and a G:C pair in the same position ($tRNA_2^{Tyr}$), respectively. $tRNA_1^{Tyr}$ was only isolated with an unmodified $G_{10}$, whereas $tRNA_2^{Tyr}$ contained a m$^2$G in this position. The two $tRNAs^{Tyr}$ from wheat germ contained mainly an A:U pair (>80%) at the base of the TΨC-stem and, to a low extent, a G:C pair, but only $m_2G$ in position 10.

### *Purification of tRNAs$^{Tyr}$ from wheat leaves and detection of suppressor activity*

To decide whether the abundant presence of $tRNA^{Tyr}_{Q\Psi A}$ in wheat germ reflects a basic difference between dicotyledonae (tobacco) and monocotyledonae (wheat) or between the adult plant (tobacco leaves) and embryonic tissue (wheat germ) we also studied the tyrosine tRNA composition of wheat leaves. Total tRNA was isolated from the leaves of 2-week-old wheat plants and further purified by fractionation on a BD-cellulose column by the same conditions used for the purification of wheat germ $tRNAs^{Tyr}$. Two peaks of major tyrosine tRNAs eluted closely together from the BD-cellulose column at high salt concentrations and were separately further purified by 2-dimensional gel electrophoresis. Since the two pooled $tRNA^{Tyr}$-enriched fractions (fractions 1 and 2) comprise all fractions containing tyrosine-accepting activity eluting from the BD-cellulose column, they should contain both $tRNA^{Tyr}$ species, if present at all, which then would be detected in our two dimensional gel electrophoresis system which separates $tRNA^{Tyr}_{Q\Psi A}$ and $tRNA^{Tyr}_{G\Psi A}$ as shown in Figure 2A. Figure 2C shows a typical pattern of the $tRNA^{Tyr}$ (fraction 1). The two-dimensional pattern of fraction 2 was exactly the same.

All tRNAs visible in panel C were excised from the gel and the eluted material was used for aminoacylation with [$^3$H]-tyrosine and for *in vitro* translation experiments with TMV RNA. Only one $tRNA^{Tyr}$ species, being a UAG suppressor tRNA, could be identified in wheat leaves (the position of this tRNA species is marked by an arrow in Figure 2C), quite in contrast to wheat germ, where two $tRNAs^{Tyr}$ were detected

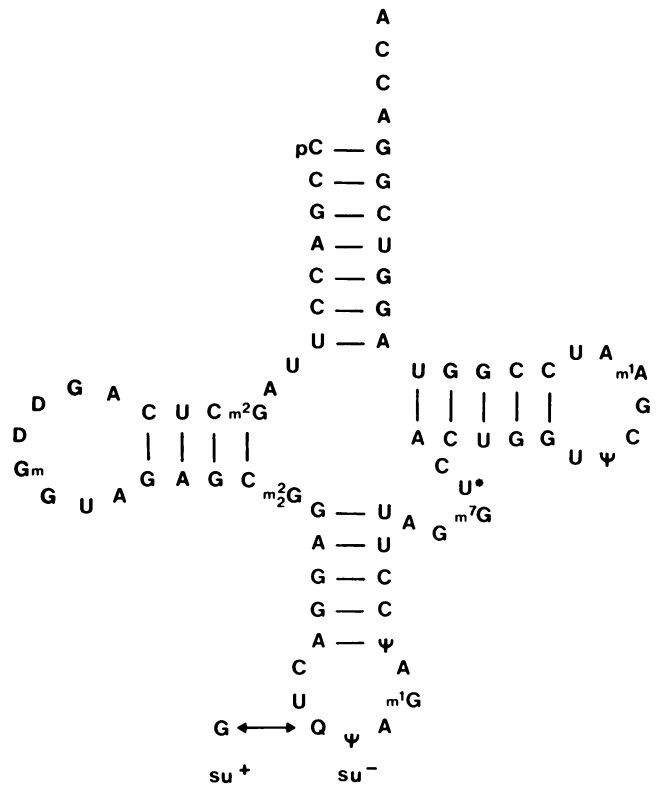


**Fig. 5.** Nucleotide sequence of major and minor wheat germ $tRNAs^{Tyr}$. The clover leaf model shows the nucleotide sequence of the major $tRNA^{Tyr}_{(Q\Psi A)}$. The minor $tRNA^{Tyr}_{(G\Psi A)}$ contains a G instead of a Q nucleoside in the first position of the anticodon and acts as a suppressor for the UAG termination codon in TMV RNA. There are indications that U* in position 47 is 3-(3-amino-3-carboxypropyl)uridine, $acp^3U$. The nucleotide sequence shown here is identical with that of tobacco $tRNA^{Tyr}$ (Beier *et al.*, 1984).

(Figure 2A). To make sure that we were really dealing with one species only and to verify the structure we sequenced this $tRNA^{Tyr}$ (fraction 1) from wheat leaves using the eluted tRNA material as shown in Figure 2C, and in parallel we also determined the sequence of $tRNA^{Tyr}$ from fraction 2. Both $tRNAs^{Tyr}$ from wheat leaves have a GΨA anticodon and are otherwise identical in sequence with the $tRNAs^{Tyr}$ from wheat germ. The $tRNA^{Tyr}$ (fraction 2) from wheat leaves contains a higher percentage of a G:C pair at the base of the TΨC-stem than that from fraction 1. No $tRNA^{Tyr}$ with a QΨA anticodon could be detected in either of the two $tRNA^{Tyr}$ fractions. It is yet open whether these findings also apply to $tRNA^{His}$, $tRNA^{Asp}$ and $tRNA^{Asn}$, which also have G or Q in the first position of the anticodon.

## Discussion

Two high mol. wt. proteins of 126 K and 183 K are synthesized in addition to the 17.5-K coat protein in TMV-infected tobacco protoplasts (Paterson and Knight, 1975; Siegel *et al.*, 1978; Beier *et al.*, 1984). TMV RNA translation has also been studied in two *in vitro* systems, namely in the reticulocyte lysate (Pelham, 1978; Scalla *et al.*, 1978; Beier *et al.*, 1980) and in wheat germ extracts (Roberts and Paterson, 1973; Davies and Kaesberg, 1974; Bruening *et al.*, 1976; Beachy and Zaitlin, 1977). Apart from the fact that the viral coat protein is not synthesized at all *in vitro*, TMV translation in protoplasts and reticulocyte lysates is similar with regard to the ratio of the 126-K and 183-K proteins. In contrast, only the viral 126-K protein is synthesized in wheat germ extracts, whereas the 183-K protein is not produced at all or in small amounts only. This is somewhat surprising, since an *in vitro* system derived from plant tissue should have more properties in common with the protoplast system than one would expect for the unrelated reticulocyte lysate. This observation led us to the question of whether the inability of the wheat germ extract to synthesize the 183-K readthrough protein is due to a general failure to produce high mol. wt. translation products, or whether specifically the suppression of the UAG codon at the 3′ end of the first cistron of TMV is impaired due to a deficiency in natural suppressor tRNA.

We have recently identified the two major $tRNAs^{Tyr}_{G\Psi A}$ from tobacco plants as the UAG suppressor tRNAs which promote the TMV RNA-dependent 183-K protein synthesis *in vitro* and most likely *in vivo* (Beier *et al.*, 1984). Here we show that addition of purified suppressor $tRNA^{Tyr}_{G\Psi A}$ from tobacco to a protein synthesizing wheat germ extract results in the clear appearance of the 183-K readthrough product (Figure 1, lane c).

Purification and sequence analysis of the endogenous tyrosine tRNAs revealed that wheat germ contains two $tRNAs^{Tyr}$ differing in the first letter of the anticodon (Figure 4), quite in contrast to the situation in tobacco: a major $tRNA^{Tyr}$ with a QΨA anticodon and a minor $tRNA^{Tyr}$ with a GΨA anticodon. The $tRNA^{Tyr}_{Q\Psi A}$ is totally unable to stimulate the readthrough over the UAG codon of TMV RNA (Figure 1C), whereas the minor $tRNA^{Tyr}_{G\Psi A}$ was characterized as a UAG suppressor tRNA. These findings, together with the results shown in Figure 1, strongly support our assumption mentioned above that the wheat germ system primed with TMV RNA is able to synthesize the 183-K polypeptide but does not do so because it lacks sufficient amounts of suppressor $tRNA^{Tyr}_{G\Psi A}$. The suppressor activity of the small amount of $tRNA^{Tyr}_{G\Psi A}$ (~15% *versus* ~85% $tRNA^{Tyr}_{Q\Psi A}$) may be even further reduced by an inhibitory (competitive) influence of the major $tRNA^{Tyr}_{Q\Psi A}$ on any of the steps involved in protein biosynthesis. Consequently, the significant amount of readthrough protein synthesis in reticulocyte lysates without added suppressor tRNA (Beier *et al.*, 1984) may therefore indicate that a $tRNA^{Tyr}_{G\Psi A}$ is present in reticulocytes.

At present we can only accept as a fact, but not explain, that the GΨA anticodon interacts with the UAG termination codon and with the codons for tyrosine, whereas QΨA anticodon recognizes the tyrosine codons only. Assuming that the interaction between the GΨA anticodon and the UAG codon occurs via classical base pairing, only one A:U and one A:Ψ pair would be formed here. We have discussed several features, without any reasonable explanation, of this type of interaction earlier (Beier *et al.*, 1984). We would like to offer two additional ideas concerning the GΨA-UAG interaction. (i) The Ψ in the second position of the anticodon, a unique feature of eukaryotic $tRNAs^{Tyr}$, may interact with A more firmly than generally expected. Ward and Reich (1968) have shown that alternating A-Ψ polynucleotides have a dramatically higher *T*m (58°C) as compared with corresponding A-U polymers (32°C). This idea of a strong A:Ψ interaction may also explain the recent observation, that the suppressor activity of a yeast $tRNA^{Tyr}$ is lost when the Ψ in the anticodon is changed to U (Johnson and Abelson, 1983). (ii) We have not observed any suppression of the UAA codon at the end of the 183-K protein cistron of TMV (not shown), albeit this interaction would also involve the same A:U and

A:Ψ pairs only. This implies that the G of the GΨA anticodon plays a selective role in this recognition process, possibly being able to interact in the *syn* conformation (Topal and Fresco, 1976) with the G of UAG and not with the A of UAA. In fact, the possibility of a *syn* conformation in the first position of the anticodon has already been discussed earlier (Jank *et al.*, 1977) to explain the recognition of all four valine codons by one $tRNA^{Val}$. Following this line of reasoning, the Q nucleoside in the *syn* conformation would not be able to interact with the G of the UAG codon due to its space-filling cyclopentene diol side chain.

Modified nucleosides in tRNA are synthesized by post-transcriptional modification (Söll, 1971; Nishimura, 1979). The highly modified Q nucleoside, however, is a unique exception in that it is synthesized as the free Q base (queuine) which is then inserted into tRNA by a transglycosylase to replace guanine (Okada *et al.*, 1979). Bacterial tRNAs normally are completely modified with respect to Q (Katze and Mosteller, 1976; Vold, 1978) but in eukaryotes tRNAs may exhibit a variable Q content depending on the developmental state and/or growth conditions (White *et al.*, 1973; Katze, 1975). It has been shown that undermodified tRNAs containing guanine instead of queuine specifically appear in a variety of tumour cells (Okada *et al.*, 1978). The presence of these hypomodified tRNAs in tumour tissues seems to be due to a lack of Q base or its precursors, and not due to a deficiency of the transglycosylase (Shindo-Okada *et al.*, 1981). Katze and Beck (1980) showed that continuous infusion of Q base into tumour-bearing mice reverses the Q-deficiency in Ehrlich ascites cell tRNAs and coincidently causes an inhibition of tumour growth.

Sheep reticulocytes and fetal liver contain significant amounts of tRNAs which contain G in place of Q in the first position of the anticodon, whereas adult liver does not (Landin *et al.*, 1979). Thus, Q-deficient tRNAs are probably specific for cells in a less differentiated stage. Adult *Drosophila melanogaster* contain two major $tRNAs^{Tyr}$ which are supposed to differ in the presence of Q and G, respectively (Bienz and Kubli, 1981) and significant changes in the Q-content in tRNAs isolated from different ontogenetic stages have been observed (White *et al.*, 1973). Our finding of the exclusive presence of GΨA anticodons in $tRNAs^{Tyr}$ from mature tobacco and wheat leaves on the one hand, and the abundant presence of a $tRNA^{Tyr}$ with a QΨA anticodon in embryonic tissue (wheat germ) on the other hand, is rather surprising because this is just the reverse of the situation in animals.

It remains obscure what the suppressor activity of $tRNAs^{Tyr}$ means for the plant, since it is questionable how frequently the UAG termination codon is used. It should be stressed, however, that the Q-deficiency in adult plants as reported here, should have some yet unknown biological significance. Plants in a differentiated stage might generally need $tRNAs^{Tyr}$ which are more flexible with respect to their codon recognition. In this context it may be important to mention that Q has a greater affinity for U than for C (Harada and Nishimura, 1972), whereas $tRNA^{Tyr}_{G\Psi A}$ would be able to serve equally well both tyrosine codons, UAU and UAC.

## Materials and methods

T4 polynucleotide kinase and T4 RNA ligase were obtained from Boehringer and P-L Biochemicals, respectively. The reticulocyte lysate was a generous gift from Dr. O.Martini, Würzburg, and pure yeast $tRNA^{Trp}$ was kindly provided by Dr. G.Keith, Strasbourg. The vulgare strain of TMV was from the collection of Dr. K.W.Mundry, Stuttgart. RNA was isolated from purified virus by phenol extraction.

*Preparation of unfractionated tRNA from wheat leaves and wheat germ*

Wheat leaves were harvested 2 weeks after germination (height ~25 cm) and stored frozen at −20°C. Unfractionated tRNA was prepared from this material by phenol extraction in a buffer containing 0.1 M Tris-HCl, pH 7.5, 0.1 M NaCl, 0.01 M $MgCl_2$, 2 mM EDTA, 5 mM mercaptoethanol, and 0.1% SDS. High mol. wt. RNA was precipitated with 3 M NaOAc, pH 6.0. Total tRNA from wheat germ was isolated essentially by the same procedure and was further purified by a DEAE-cellulose fractionation step.

*Fractionation and isolation of $tRNAs^{Tyr}$ from total tRNA of wheat leaves and wheat germ*

Unfractionated tRNA was loaded onto a BD-cellulose column (1.2 x 35 cm) and fractionated by elution with a linear gradient of 0.35 M NaCl (0.01 M $MgCl_2$, 0.02 M NaOAc, pH 4.5) to 1 M NaCl in the same buffer. The wheat germ tRNA fractions enriched in $tRNA^{Tyr}$ were further fractionated by Sepharose 4B chromatography on a column of 0.7 x 28 cm (Holmes *et al.*, 1975). Elution was performed by a reverse gradient of 2 M to 1 M $(NH_4)_2SO_4$ in 0.01 M $MgCl_2$, 0.01 M NaOAc, pH 4.5.

Two-dimensional polyacrylamide gel electrophoresis was used as the final step in purification of $tRNAs^{Tyr}$ (Lockard *et al.*, 1978; Walker and RajBhandary, 1978). Partially purified $tRNAs^{Tyr}$ (10−40 μg) were applied to a 10% polyacrylamide/7 M urea gel at pH 3.5 (thickness, 0.3 mm). Strips of the appropriate section of the gel were cut out and polymerized into a 12.5% polyacrylamide/7 M urea gel (pH 8.3). Individual spots containing tRNA were visualized by staining with 0.4% toluidine blue '0' in 50% methanol, 1% acetic acid for 15 min and destaining in 35% methanol, 1% acetic acid for 20 min. Elution of the RNA was performed in a buffer containing $NH_4OAc$, EDTA, $MgCl_2$, and SDS (Gross *et al.*, 1982).

*Sequencing of tRNA by post-labeling techniques*

Nucleotide sequences were determined by the fragment analysis procedure of Stanley and Vassilenko (1978) with some modifications (Gupta and Randerath, 1977). Limited hydrolysis of ~1 μg pure $tRNA^{Tyr}$ eluted from the gel after two-dimensional electrophoresis was performed in 1 μl $H_2O$ in a sealed glass capillary by incubation in a boiling water bath for 30 s. After 5′ $^{32}P$-labeling the fragments were separated on a 12.5% polyacrylamide/8 M urea gel (thickness, 0.3 mm; height, 88 cm). After autoradiography (~1 h) individual bands were eluted (Gross *et al.*, 1982) in the presence of 5 μg carrier tRNA. Complete digestion with 100 ng nuclease P1 in 10 μl 50 mM $NH_4OAc$ (pH 5.3) was performed for 2 h at 50°C. The 5′-nucleotide of each eluted fragment was identified by cellulose t.l.c. using the two-solvent system of Nishimura (1979) in parallel runs: (a) isobutyric acid/conc. ammonia/$H_2O$ = 580:38:385 (vol); (b) isopropanol/conc. HCl/$H_2O$ = 700:150:150 (vol).

The 5′- and 3′-terminal sequences were established by the two-dimensional mobility shift method (Jay *et al.*, 1974; Gillum *et al.*, 1975; Tyc *et al.*, 1983).

*Translation in vitro*

Translation in the nuclease-treated reticulocyte lysate was performed as described (Pelham and Jackson, 1976; Beier *et al.*, 1980) with the exception that the lysate was not supplemented with calf liver tRNA. TMV RNA was added at 100 μg/ml. The reaction mixture was incubated for 60 min at 30°C.

S23 cell-free extract was prepared from wheat embryos as described by Zagorski (1978). The standard 50 μl incorporation mixture contained 10 μl of 23 000 *g* extract, 100 μCi/ml $^{35}S$-labeled methionine (1420 Ci/mmol), 40 μM each of 19 amino acids, 375 mM GTP, 2.5 mM ATP, 60 μg/ml creatine kinase, 10 mM creatine phosphate, 80 μM spermine, 3 mM magnesium acetate, 125 mM potassium acetate and 20 mM Hepes-KOH, pH 7.6. TMV RNA was added at final concentrations of 100 μg/ml. The reaction mixture was incubated for 90 min at 30°C.

*Analysis of translation products*

Proteins were analysed by gel electrophoresis in 10% polyacrylamide gel slabs containing SDS (Laemmli, 1970). Gels were fixed overnight and either autoradiographed or fluorographed as described by Laskey and Mills (1975) and exposed to RX Fuji X-Ray films at −70°C. To determine the 183 K: (126 K + 183 K) ratio individual bands were excised from the dried gel of a fluorogram and the radioactivity was counted directly without adding scintillation fluid.

## Acknowledgements

We are grateful to Professor H.J.Gross for his stimulating interest and valuable suggestions during the course of this work. We thank Mrs H. Petrásek for help in preparing and Dr. R. Radloff for critically reading the

manuscript. This work was supported by grants from the Deutsche Forschungsgemeinschaft to H.B. and H.J. Gross.

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*Received on 23 January 1984*