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# Macromolecular recognition through electrostatic repulsion

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**In the process of genetic translation, each aminoacyl-tRNA synthetase specifically aminoacylates its cognate tRNAs and rejects the 19 other species of tRNAs. A decrease in the specificity of this reaction can result in misincorporations of amino acids into proteins and be deleterious to the cell. In the case of tyrosyl-tRNA synthetase from *Bacillus stearothermophilus*, the change of residue Glu152 into Ala results in erroneous interactions with non-cognate tRNAs. To analyse how Glu152 contributes to the discrimination between tRNAs by tyrosyl-tRNA synthetase, 11 changes to this residue were created by mutagenesis. The misaminoacylations of $tRNA^{Phe}$ and $tRNA^{Val}$ with tyrosine *in vitro* (on a scale going from 1 to 30) and the toxicity of tyrosyl-tRNA synthetase *in vivo* (on a scale from 1 to $10^7$) increased in a correlated way when the nature of the side chain in position 152 varied from negatively charged to uncharged then to positively charged. The aminoacylation of $tRNA^{Tyr}$ was unaffected by the mutations. The results show that the role of Glu152 in the discrimination between tRNAs is purely negative, that it acts by electrostatic repulsion of non-cognate tRNAs and that this mechanism has been conserved throughout evolution.**

*Key words*: aminoacyl-tRNA synthetase/discrimination/specificity/transfer RNA/tyrosyl-tRNA synthetase

## Introduction

The specificity of recognition between biological macromolecules is considered to result mainly from the complementarity of shapes and the formation of non-covalent bonds. The specific aminoacylation of the cognate tRNAs by an aminoacyl-tRNA synthetase and the rejection of the 19 other species of tRNAs involve these general mechanisms of macromolecular recognition (Giegé *et al.*, 1993; McClain, 1993; Schimmel *et al.*, 1993). This enzymatic reaction is the crucial step in the translation of the genetic code since it puts the amino acids and anticodons into correspondence. A decrease in its specificity can result in misincorporations of amino acids into proteins and be deleterious to the cell.

Three related factors complicate the study of the recognition between tRNAs and synthetases. All the tRNAs have similar structures (Sprinzl *et al.*, 1989). The tRNAs and synthetases form a network of interactions: several tRNAs enter into competition for each synthetase and several synthetases compete for each tRNA (Yarus, 1972). The cellular concentrations of tRNAs and synthetases are equimolar (~1.6 μM for tyrosyl-tRNA synthetase and $tRNA^{Tyr}$ from *Escherichia coli*), and their balance contributes to the precision of aminoacylation (Calendar and Berg, 1966; Jakubowski and Goldman, 1984; Swanson *et al.*, 1988; Bedouelle *et al.*, 1990; Sherman *et al.*, 1992). These complications make the comparison of *in vitro* and *in vivo* studies necessary.

We use the tyrosyl-tRNA synthetase (TyrRS) from *Bacillus stearothermophilus* to study this problem of macromolecular recognition (Fersht, 1987; Brick *et al.*, 1989; Bedouelle, 1990; Bedouelle *et al.*, 1993). In a previous work, we have found that TyrRS(E152A), a mutant synthetase carrying the change of residue Glu152 into Ala, is toxic for the producing cells in conditions where TyrRS(wt), the wild-type synthetase, is not toxic. A genetic analysis of this cellular toxicity and experiments of tRNA aminoacylation *in vitro* have shown that mutation E152A results in erroneous interactions between TyrRS and non-cognate tRNAs (Vidal-Cros and Bedouelle, 1992). In the present work, we created a set of 11 changes to Glu152 by site-directed mutagenesis to characterize the mechanism by which this residue contributes to the discrimination between tRNAs by TyrRS. Some changes altered the discriminating properties of TyrRS much more strongly than E152A. We improved the sensitivity of our toxicity assay and established the existence of a correlation between the toxicity of the mutant TyrRSs and their ability to misaminoacylate non-cognate tRNAs *in vitro*. This correlation indicates that cellular toxicity can be used to measure the accuracy of essential macromolecular interactions *in vivo*. The results show that Glu152 acts by electrostatic repulsion of non-cognate tRNAs and thus reveal a new mechanism of macromolecular recognition.

## Results

### *Construction and production of mutant tyrosyl-tRNA synthetases (TyrRS)*

We changed residue Glu152 of TyrRS by site-directed mutagenesis of the encoding gene, *tyrS*, placed under the control of promoter *Ptac*. We used two different vectors of *tyrS*, phage M13-BY(Ptac) and plasmid pEMBL9-BY(Ptac). *Ptac* is repressed by repressor LacI and can be induced with lactose or IPTG. The changes were constructed in conditions where *Ptac* was repressed and therefore where TyrRS was not produced. The induced productions of the mutant TyrRSs from the derivatives of M13-BY(Ptac) either were not toxic or were only slightly toxic for strain TG2 (*lacI$^q$*, *recA*), which made possible their purification for *in vitro* aminoacylation experiments. In contrast, the production of some of the mutant TyrRSs from the derivatives of pEMBL9-BY(Ptac) were toxic

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for strain HB2202 (a $lacZ^+Y^+$ derivative of TG2). We quantified this toxicity and used it as an assay for the discrimination between tRNAs by TyrRS *in vivo*.

### Aminoacylation of $tRNA^{Tyr}$

We measured the rate of aminoacylation of $tRNA^{Tyr}$ by pure preparations of the wild-type and mutant TyrRSs *in vitro*. The concentration of $tRNA^{Tyr}$ in these experiments (1.1 μM) was close to its $K_m$ for the wild-type TyrRS (1.38 μM; Vidal-Cros and Bedouelle, 1992; Avis and Fersht, 1993). This rate was equal to 1.8 $s^{-1}$ at 25°C for TyrRS(wt) and comprised between 1.3 and 3.1 $s^{-1}$ for the 10 mutant TyrRSs. The results of the present work extend those of a previous work, in which we found that mutations E152A, E152D and E152Q only weakly affect the kinetic parameters $K_m$ and $k_{cat}/K_m$ for the aminoacylation of $tRNA^{Tyr}$ by TyrRS (Vidal-Cros and Bedouelle, 1992). Mutation E152A deletes the side chain in position 152 and the other mutations change it for very varied chemical groups. Thus, this set of results showed that the side chain in position 152 was not involved in the aminoacylation of $tRNA^{Tyr}$ by TyrRS *in vitro*.

The *Escherichia coli* strain HB2109 [$lacI^+$, *recA*, *tyrS(Ts)*] carries a thermosensitive mutation in its own *tyrS* gene, which makes it unable to grow at 42°C. An active *tyrS* gene from *B.stearothermophilus*, but not an inactive one, can replace the mutant *tyrS(Ts)* gene of HB2109 for growth at 42°C (Bedouelle and Winter, 1986). We found that plasmid pEMBL9-BY(Ptac) and its mutant derivatives, but not the parental vector pEMBL9$^+$, enabled HB2109 to grow at 42°C. We performed these experiments of genetic complementation in the absence of IPTG to avoid an overproduction of the mutant TyrRSs. The results showed that the mutant TyrRSs were at least partially active for the charging of $tRNA^{Tyr}$ *in vivo*.

### Misacylations of $tRNA^{Phe}$ and $tRNA^{Val}$ in vitro

We tested the ability of the mutant TyrRSs to tyrosylate $tRNA^{Phe}$ and $tRNA^{Val}$ *in vitro* (Figure 1). The misaminoacylation (mischarging) of a tRNA is incomplete in this type of experiment and its level results from a kinetic competition between the enzymatic aminoacylation and spontaneous deacylations (Giegé *et al.*, 1993). Most changes of residue Glu152 increased the mischargings of $tRNA^{Phe}$ and $tRNA^{Val}$ with tyrosine (Figure 2).

The mischarging experiments of Figures 1 and 2 were performed at 25°C. To analyse how temperature affected mischarging *in vitro*, we ran reactions in parallel at 25 and 37°C with $tRNA^{Phe}$ as the substrate and TyrRS(wt), TyrRS(E152R), TyrRS(E152K) and TyrRS(E152W) as the enzymes. The levels of mischarging were higher at 37°C than at 25°C by the same factor (1.7 ± 0.2) for all the TyrRSs tested.

The only radioactive molecule in the mischarging reactions was [$^{14}$C]Tyr. Tyrosine is not activated or transferred to tRNAs by synthetases other than TyrRS. Therefore, mischarging was not due to impurities in our preparations of purified TyrRSs. We performed mischarging experiments of $tRNA^{Phe}$ and $tRNA^{Val}$ with an impure preparation of TyrRS(wt) to test this point directly. We stopped its purification after the chromatography on a DEAE column and before the MonoQ column (Materials and methods). We found that this impure preparation of TyrRS(wt)

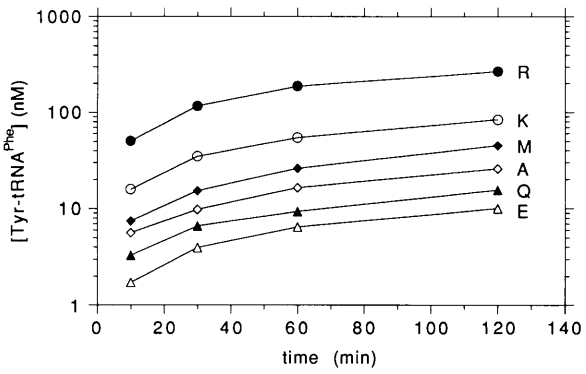


**Fig. 1.** Kinetics of $tRNA^{Phe}$ tyrosylation at 25°C. The concentration of tyrosyl-$tRNA^{Phe}$ in the reaction (on a logarithmic scale) is given as a function of time. The nature of the side chain in position 152 of TyrRS is given on the right of the curves. The curves for Asp and Glu were close, as were those for Gly, His and Met, and those for Ser, Trp and Lys. $tRNA^{Phe}$ was at 1.5 μM and the purified mutant TyrRSs at 1.1 μM in the reactions. Each data point (at 10, 30, 60 and 120 min) is the average of the measurements in three independent experiments. $tRNA^{Val}$ was mischarged with similar kinetics.

mischarged $tRNA^{Phe}$ and $tRNA^{Val}$ no more than a pure preparation (0.56% versus 0.65% of the $tRNA^{Phe}$ molecules and 0.23% versus 0.33% of the $tRNA^{Val}$ molecules).

### Toxicities of the mutant TyrRSs

We streaked strain HB2202[pEMBL9-BY(Ptac)] and its mutant derivatives onto plates of McConkey indicator medium, containing lactose, to observe their phenotypes in conditions where promoter *Ptac* is induced. We recorded two traits of the colonies, as functions of the growth temperature and of the mutation in codon 152 of the *tyrS* gene: their colour and their size (Table I). The phenotypes varied more widely at 37°C than at 30°C. Some mutations were very toxic at both temperatures; some others decreased the fermentation of lactose without inhibiting growth at 30°C.

We performed bacterial platings to quantify the toxicities of the mutant TyrRSs. Strain HB2202[pEMBL9-BY(Ptac)] and its mutant derivatives were first grown in conditions where TyrRS was not produced, i.e. on plates of LB medium, without IPTG and at 30°C. Equal portions of a bacterial suspension were then spread on plates of growth medium, supplemented or not with IPTG, and the plates were incubated at 30° or 37°C. Figure 3 gives the ratios of the efficiencies of plating (e.o.p.) for the derivatives of HB2202 in these four conditions. Figure 3A and B correspond to platings on LB medium and on minimal medium, respectively. The toxicities were higher at 37° than at 30°C, and on minimal medium than on LB medium. The sensitivity of the toxicity assay on minimal medium at 37°C was remarkable since the scale of the measures extended from 1 to $10^7$. The use of minimal medium enabled us to compare mutations that we could not differentiate on LB medium.

The lack of toxicity that we observed for some of the mutant TyrRSs, could come from a lower level of production. We therefore measured the concentration of TyrRS in soluble extracts of HB2202[pEMBL9-BY(Ptac)] and its mutant derivatives by active site titration. The extracts were prepared from bacteria that had been induced with IPTG and grown in LB medium at 30°C, because

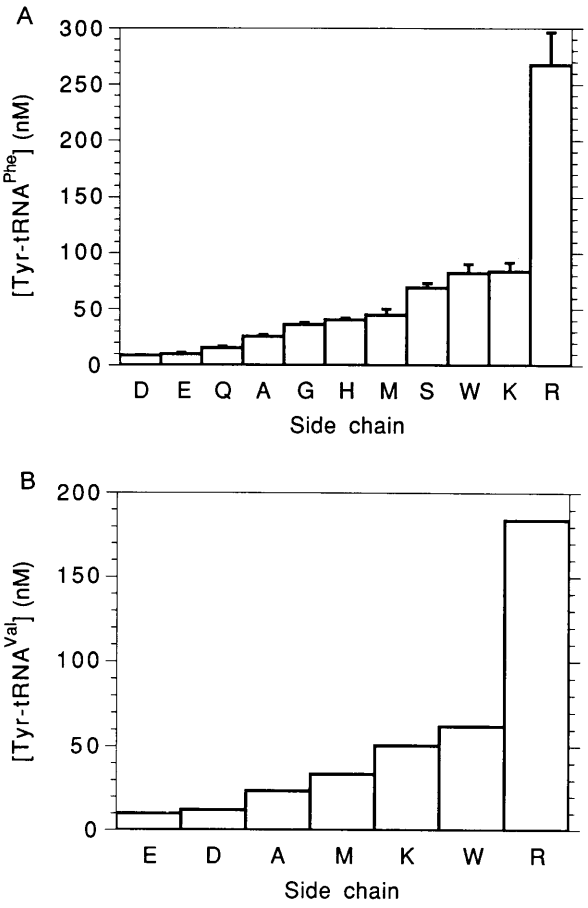


**Fig. 2.** Mischargings of $tRNA^{Phe}$ and $tRNA^{Val}$ by mutant TyrRSs at 25°C. The graphs give the nature of the side chain in position 152 of TyrRS along the *x*-axis, and the concentrations of (**A**) tyrosyl-$tRNA^{Phe}$ and (**B**) tyrosyl-$tRNA^{Val}$ after 120 min of reaction along the *y*-axis. The aminoacylation reactions were conducted as described in Figure 1. The average value and the standard error from at least three independent experiments are given for $tRNA^{Phe}$. $tRNA^{Phe}$ was at 1.5 μM, $tRNA^{Val}$ at 3.0 μM and the mutant TyrRSs at 1.1 μM in the reactions.

some of the mutant TyrRSs were too toxic to allow growth of the producing cells at 37°C. The concentration of active TyrRS varied little with the nature of the side chain in position 152. It was equal to 2% of the soluble proteins for TyrRS(wt) and comprised between 0.93 and 2.3% for the 10 mutant TyrRSs. There was no correlation between the levels of production and the toxicities of the TyrRSs.

## Discussion

### *Glu152 and the specificity of TyrRS in vitro*

Most changes of residue Glu152 increased the mischargings of $tRNA^{Phe}$ and $tRNA^{Val}$ with tyrosine *in vitro* (Figures 1 and 2). TyrRS(E152R) highly mischarged $tRNA^{Phe}$ (>17.5% of the molecules at the plateau of the reaction) and $tRNA^{Val}$ (>6.1%). Thus, the nature of the side chain in position 152 was important for the rejection of these two tRNAs. In contrast, the changes of Glu152 only weakly affected the rate of aminoacylation of $tRNA^{Tyr}$ by TyrRS. Thus, Glu152 was not significantly involved in the interactions between TyrRS and $tRNA^{Tyr}$ that resulted in its aminoacylation. We conclude that Glu152 plays a role in the rejection of non-cognate tRNAs but no significant role in the aminoacylation of the cognate $tRNA^{Tyr}$ by TyrRS *in vitro*: Glu152 is a purely negative determinant of the specificity for $tRNA^{Tyr}$.

**Table I.** Phenotypes of HB2202[pEMBL9-BY(Ptac)] and its mutant derivatives on McConkey medium

| Codon | 30°C | | 37°C | |
|---|---|---|---|---|
| | Growth | Lac | Growth | Lac |
| A | ++ | – | – | – |
| D | +++ | +++ | +++ | +++ |
| E (wt) | +++ | +++ | +++ | +++ |
| G | +++ | + | ± | + |
| H | +++ | ++ | +++ | ++ |
| K | ± | – | – | – |
| M | +++ | ++ | +++ | ++ |
| Q | +++ | ++ | +++ | ++ |
| R | – | – | – | – |
| S | +++ | ± | – | – |
| W | +++ | + | + | + |

Strain HB2202[pEMBL9-BY(Ptac)] and its derivatives were streaked on McConkey indicator medium, containing lactose, and the plates were incubated either at 30°C or at 37°C. Two phenotypic traits were recorded: growth (–, no colony; +++, large colonies) and fermentation of lactose (–, white colonies, no fermentation; +++, dark red colonies, fermentation). The first column gives the nature of the codon in position 152 of the plasmidic *tyrS* gene.

The mischargings of $tRNA^{Phe}$ and $tRNA^{Val}$ increased when the nature of the side chain in position 152 of TyrRS varied from negatively charged to uncharged then to positively charged (Figure 2). This increase shows that the discrimination between tRNAs by TyrRS *in vitro* involves an electrostatic repulsion between the side chain of Glu152 and the tRNAs. Mutation E152R gave much higher levels of $tRNA^{Phe}$ and $tRNA^{Val}$ mischargings than E152K. This result suggests that the arginine and lysine side chains in position 152 of TyrRS interacted differently with the tRNAs. The side chain of arginine contains two $NH_2$ groups and one NH group. It can form a network of hydrogen bonds with an RNA through these groups and contact two adjacent phosphates. Lysine contains a single $NH_3$ group and cannot form such a network (Calnan *et al.*, 1991).

### *Glu152 and the specificity of TyrRS in vivo*

Plasmid pEMBL9-BY(Ptac) and its mutant derivatives were innocuous for their bacterial host when the promoter *Ptac* of the *tyrS* gene was repressed. The wild-type plasmid remained innocuous when *Ptac* was induced with IPTG, whereas most of its derivatives, mutated in codon 152 of *tyrS*, became toxic (Figure 3). The dependence of toxicity on the induction of *Ptac* showed that it was due to the production of the mutant TyrRSs. In a previous work, we have shown that the toxicity of mutation E152A is due to erroneous interactions between the mutant synthetase, TyrRS(E152A), and tRNAs. For this, we used two mutations of TyrRS, K410N and K411N, which strongly diminish the binding of $tRNA^{Tyr}$ without affecting the formation of tyrosyl-adenylate (Bedouelle and Winter, 1986). We found that K410N (or K411N) abolishes the toxicity of E152A when the two mutations are combined in the same molecule of TyrRS (Vidal-Cros and Bedouelle, 1992). In the present work, we did not repeat this experiment for the other mutations of Glu152, but we assumed

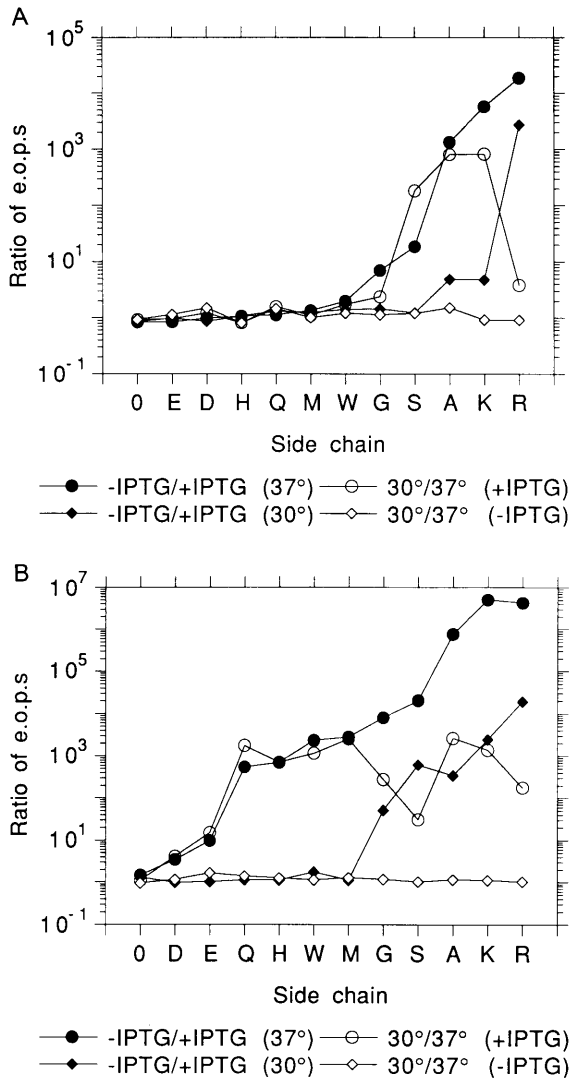


**Fig. 3.** Toxicities of mutant TyrRSs. Strain HB2202[pEMBL9-BY(Ptac)], its mutant derivatives and the control strain HB2202(pEMBL9$^+$) were plated either on LB medium (**A**) or on glucose minimal medium (**B**). They were grown under four conditions: at 30°C or at 37°C, without IPTG or with IPTG (Materials and methods). The figures give the nature of the side chain in position 152 of TyrRS along the *x*-axis, and the ratios of the four efficiencies of plating (e.o.p.) that were measured for each strain, along the *y*-axis. '0', control strain; –IPTG/+IPTG, ratio of the e.o.p.s obtained after growth without IPTG and with IPTG; 30°/37°, ratio of the e.o.p.s obtained after growth at 30°C and 37°C. All the strains plated with similar efficiencies in the absence of IPTG. Each data point corresponds to the average value of at least three independent experiments. The standard errors were below 60% of the values. The lines between the points are drawn only for clarity.

that its result could be extended to them. The experiments of aminoacylation *in vitro* and genetic complementation *in vivo* showed that residue Glu152 was not involved in the interaction between TyrRS and tRNA$^{Tyr}$. Thus, the toxicity of the mutant TyrRSs was not due to defective interactions with the cognate tRNA$^{Tyr}$. We then conclude that the toxicity of the mutations in position 152 was due to erroneous interactions between the mutant TyrRSs and non-cognate tRNAs.

Toxicity increased when the nature of the side chain in position 152 of TyrRS varied from negatively charged to uncharged bulky, then small, then positively charged (Figure 3B). This ranking of the toxicities shows that Glu152 contributes to the rejection of non-cognate tRNAs by TyrRS *in vivo,* mainly through an electrostatic repulsion and additionally through steric repulsions.

### *Mutant phenotypes and imprecision of translation*

Our *in vivo* experiments showed that some mutant side chains in position 152 caused erroneous interactions between TyrRS and non-cognate tRNAs. Several observations suggest that the production of the corresponding mutant TyrRSs resulted, directly or indirectly, in misincorporations of amino acids into proteins. Generally speaking, one expects that the incorporation of an amino acid in place of other amino acids into proteins will destabilize or inactivate some of them, in particular essential proteins. Therefore, this misincorporation will be toxic to the cell. Because the proteins are less stable at high temperature, the resulting toxicity will increase with temperature. Because the biosynthesis of the cellular components necessitates a larger number of enzymes when it is done from a unique carbon source rather than from a cellular extract, the toxicity will be higher when growth occurs on synthetic medium rather than on rich medium. We found that several mutant TyrRSs were toxic for the producing strain, HB2202, and this toxicity increased with the growth temperature (Figure 3). The mutant TyrRSs were more toxic for HB2202 when this strain grew on glucose minimal medium (Figure 3B) rather than on LB medium (Figure 3A), but the ranking of the mutations according to their toxicity was the same with both media. We also found that several mutant TyrRSs decreased the ability of HB2202 to ferment lactose without inhibiting its growth on McConkey indicator medium at 30°C. This decrease was strong for the changes of Glu152 into Gly, Ser and Trp, and noticeable for those into Gln, His or Met (Table I).

### *Correlation between cellular toxicity and in vitro misacylation*

The levels of tRNA$^{Phe}$ mischarging by the mutant TyrRSs *in vitro* and the toxicities of these mutant TyrRSs for the producing cells *in vivo* were correlated when the side chain varied in position 152 (Figure 4). This correlation strongly suggests that toxicity was due to the mischarging of non-cognate tRNAs (tRNA$^{Phe}$, tRNA$^{Val}$ or other tRNAs) with tyrosine. The correlation is imperfect and suggests that the mischarging of tRNA$^{Phe}$ (or tRNA$^{Val}$) was not the main cause of the toxicity for some of the mutations. Thus, TyrRS(E152A) and TyrRS(E152K) may recognize other non-cognate tRNAs better than tRNA$^{Phe}$ *in vivo*, whereas TyrRS(E152H) and TyrRS(E152W) may interact preferentially with tRNA$^{Phe}$. The effect of temperature on the *in vitro* mischarging of tRNA$^{Phe}$ was independent of the side chain in position 152 of TyrRS, whereas its effect on the *in vivo* toxicity varied with the nature of this side chain (Figure 3). These differences in the effect of temperature, between the mutant TyrRSs and between the *in vivo* and *in vitro* experiments, also suggest that the erroneous interactions that resulted in toxicity slightly varied from one mutant TyrRS to an other.

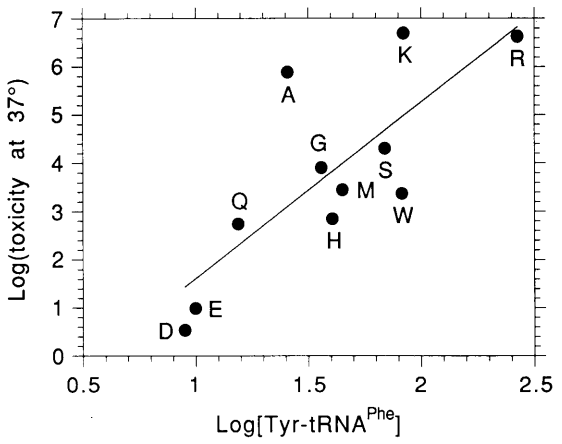


**Fig. 4.** Correlation between *in vitro* tyrosylation of $tRNA^{Phe}$ at 25°C and *in vivo* toxicity of mutant TyrRSs at 37°C. The concentration of $Tyr\text{-}tRNA^{Phe}$ (nM) after 120 min of reaction is given along the *x*-axis (see Figure 2). The ratio of the e.o.p.s on glucose minimal medium, after growth of the host strain without IPTG and with IPTG, is given along the *y*-axis (see Figure 3B). We used the logarithm of the values in order to spread them. The correlation coefficient of the logarithms was equal to 0.79. The side chain in position 152 is indicated near the corresponding point of the distribution.

### *Comparison with a structural model of the complex between TyrRS and $tRNA^{Tyr}$*

Glu152 is close to the phosphate groups of nucleotides 73 and 74 in a structural model of the complex between TyrRS and $tRNA^{Tyr}$ that we have previously constructed (Labouze and Bedouelle, 1989). Our results of mutagenesis are compatible with this specific prediction of the model, since they show that Glu152 contributes to the discrimination between tRNAs by TyrRS through electrostatic and steric repulsions. According to the model, Glu152 cannot discriminate between tRNAs by recognition of their local sequence or structure, as found for acidic residues of other aminoacyl-tRNA synthetases (Perona et al., 1989; Schmitt *et al.*, 1993; Weygand-Durasevic *et al.*, 1993). Indeed, phosphates 73 and 74 are common to all the tRNAs and 10 tRNA species of *E.coli* (among which $tRNA^{Tyr}$, $tRNA^{Phe}$ and $tRNA^{Val}$) have the sequence ...CACCA3′ at their 3′-terminal end, between positions 72 and 76 (Sprinzl *et al.*, 1989). Moreover, the acceptor end ...CCA3′ is mobile or adopts very different conformations in the known structures of tRNAs, free (Jack *et al.*, 1976; Sussman *et al.*, 1978; Woo *et al.*, 1980; Westhof *et al.*, 1985) or complexed with their aminoacyl-tRNA synthetases (Rould *et al.*, 1989; Biou *et al.*, 1994; Cavarelli *et al.*, 1994). Probably, the orientation of the acceptor end of a tRNA in the active site of a synthetase is dictated by the whole set of the elementary interactions between the two macromolecules. The acceptor end of non-cognate tRNAs could thus be misaligned relatively to the active site. We have previously shown that the discriminator base A73 of $tRNA^{Tyr}$ is fully recognized by TyrRS only in the transition state for the aminoacylation reaction (Labouze and Bedouelle, 1989; Bedouelle *et al.*, 1993). Residue Glu152 could prevent the mispositioned acceptor end of non-cognate tRNAs from forming the interactions with TyrRS that are necessary to reach and stabilize the transition state of the aminoacylation reaction.

Residue Glu152 is widely exposed to the solvent in the crystal structure of the TyrRS dimer: its accessible surface area is equal to 93 $Å^2$ when the radius of the sphere probe is equal to 1.4 Å (Connolly, 1983). There is no other charged group of TyrRS within 10 Å of the negatively charged atoms, Oε1 and Oε2, of Glu152. These data allow us to eliminate an alternative interpretation of our results, according to which Glu152 would act indirectly in the discrimination between tRNAs, by structuring a recognition element of TyrRS without making direct contacts with the tRNAs.

### *Conclusions*

Residue Glu152 of TyrRS from *B.stearothermophilus* is conserved or replaced by Asp in the nine prokaryotic or mitochondrial TyrRSs whose sequences are known (for references, see Vidal-Cros and Bedouelle, 1992; Kämper *et al.*, 1992; Salazar *et al.*, 1994). Therefore, our work shows that electrostatic repulsion can be a mechanism of molecular recognition and that such a mechanism can be maintained during evolution. We used cellular viability, a global phenomenon, to study the discrimination between tRNAs by an aminoacyl-tRNA synthetase *in vivo*, at the atomic level. This approach could be extended to other macromolecular recognitions, the precision of which is vital.

## Materials and methods

### *Media, strains and bacterial techniques*

The media and buffers (Bedouelle *et al.*, 1990), strains RZ1032 (Kunkel *et al.*, 1987), TG2 and HB2202 (Bedouelle *et al.*, 1990), plasmids $pEMBL9^+$ and pEMBL9-BY(Ptac), and phage M13-BY(Ptac) (Vidal-Cros and Bedouelle, 1992) have been described. HB2109 is the immediate $F^-$ ancestor of HB2111 (Bedouelle and Winter, 1986). We selected or tested for the presence of the $F'lacI^q$ episomes in TG2, HB2202 and their derivatives by growing these strains on plates of glucose minimal medium. The strains that contained a derivative of $pEMBL9^+$ were grown in the presence of 100 μg/ml ampicillin; they were grown at 30°C in the absence of IPTG unless otherwise indicated. The plating experiments were performed from resuspended colonies as described (Bedouelle *et al.*, 1990). Briefly, the HB2202 derivatives were grown on plates of LB medium at 30°C for 48 h. An isolated colony was resuspended in buffer, dilutions of the suspension were spread on plates of medium, and the bacteria were grown in four conditions. The colonies were counted after 42 h of growth and the ratios of the colony counts were calculated.

### *Mutagenesis*

We chose codons that are frequent in the abundant proteins of *E.coli* to replace the codon of Glu152 in the *tyrS* gene of *B.stearothermophilus*: Ala, GCG; Asp, GAC; Arg, CGT; Gly, GGC; Gln, CAG; Glu, GAG; His, CAC; Lys, AAG; Met, ATG; Ser, TCT; Trp, TGG (Grantham *et al.*, 1981). The changes to codon 152 were introduced into phage M13-BY(Ptac) by a published method of mutagenesis (Kunkel *et al.*, 1987). The template DNA was produced in strain RZ1032 (*dut, ung*) and the mutated DNA was transfected into strain TG2 (*$lacI^q$, recA*). We checked the full sequence of the mutant *tyrS* genes and of their promoters. The mutant derivatives of pEMBL9-BY(Ptac) were constructed from the mutant derivatives of M13-BY(Ptac) as described for the wild-type (Vidal-Cros and Bedouelle, 1992).

### *Preparations of TyrRS and enzymatic assays*

The preparation of soluble cellular extracts, the purification of the wild-type and mutant TyrRSs, the measurement of the concentration of TyrRS in the soluble extracts or in the purified preparations of TyrRS by active site titration and the experiments of aminoacylation or misaminoacylation of tRNAs with tyrosine were performed as described (Vidal-Cros and Bedouelle, 1992). Pure *E.coli* $tRNA^{Tyr}$ (1232 pmol/$A_{260}$ unit), $tRNA^{Phe}$ (957 pmol/$A_{260}$ unit) and $tRNA^{Val}$ (1896 pmol/$A_{260}$ unit) were purchased from Boehringer-Mannheim.

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