Inhibition of transfer-messenger RNA aminoacylation and trans-translation by aminoglycoside antibiotics.

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Abbreviations footnote: tmRNA: transfer messenger RNA; tRNA: transfer RNA; aaRS: aminoacyl-tRNA synthetase;
Summary

Transfer-messenger RNA directs the modification of proteins whose biosynthesis has stalled or has been interrupted. Here, we report that aminoglycosides can interfere with this quality control system in bacteria, termed trans-translation. Neomycin B is the strongest inhibitor of tmRNA aminoacylation with alanine ($K_i$ value of ~35 $\mu$M), an essential step during trans-translation. The binding sites of neomycin B do not overlap with the identity determinants for alanylation, but the aminoglycoside perturbs the conformation of the acceptor stem that contains the aminoacylation signals. Aminoglycosides reduce the conformational freedom of the tRNA-like domain of tmRNA. Additional contacts between aminoglycosides and tmRNA are within the tag reading frame, probably also disturbing reprogramming of the stalled ribosomes prior protein tagging. Aminoglycosides impair tmRNA aminoacylation in presence of all the tRNAs from Escherichia coli, small protein B and elongation Factor Tu, but when both proteins are present, the inhibition constant is one order of magnitude higher. SmpB and EF-Tu have RNA chaperone activities, ensuring that tmRNA adopts an optimal conformation during aminoacylation.
**Introduction**

In bacteria, transfer-messenger RNA, tmRNA, known alternatively as SsrA RNA or 10Sa RNA, rescues stalled ribosomes and contribute to the degradation of incompletely synthesized peptides (for reviews, see 1-3). In a process termed *trans*-translation, tmRNA acts first as a tRNA, being aminoacylated at its 3’ end with alanine by alanyl-tRNA synthetase (AlaRS 4,5) and adding an alanine to the stalled polypeptide chain. Resumption of translation ensues not on the mRNA on which the ribosomes were stalled but at an internal position in tmRNA. Translation termination occurs and permits ribosome recycling. *Trans*-translation plays at least two physiological roles in bacteria: removing ribosomes stalled upon mRNAs, and tagging the resulting truncated proteins for degradation.

Because tmRNA is unique to prokaryotes, and is required for viability of some bacteria, it has attracted the attention of those interested in novel targets for antibiotic therapy. tmRNA has to be aminoacylated before directing the addition of a peptide tag to the problematic protein. Moreover, in *Neisseria gonorrhoea*, and probably also in other bacteria, tmRNA aminoacylation is essential for resolving stalled translation complexes and preventing depletion of free ribosomes (6), whereas tagging for proteolysis is dispensable. tmRNA aminoacylation is therefore an attractive target for blocking *trans*-translation in pathogenic bacteria responsible of infectious diseases.

The interactions of aminoglycosides with RNAs represent a paradigm in the use of small molecules as effectors of RNA function. Aminoglycosides bind and modulate the function of a variety of therapeutically useful RNA targets (for a review, see 7), and show antimicrobial as well as antiviral activities. Aminoglycoside antibiotics disrupt the bacterial membrane and induce miscoding during prokaryotic protein synthesis by binding to the ribosomal A site (8). They also interfere with translational control (9), with HIV replication (10), inhibit the activity of several catalytic RNAs by displacing essential metal ions, such as self-splicing
group I introns (11, 12), hammerhead (13), human hepatitis delta virus (HDV, 14) and hairpin ribozymes (15) as well as the tRNA processing activity of RNase P RNA (16).

Recent structural (17) and functional (18) evidences indicate that aminoglycosides can bind and inhibit the aminoacylation of two canonical tRNAs, *Escherichia coli* (*E. coli*) tRNA\(^{\text{Phe}}\) and yeast tRNA\(^{\text{Asp}}\). The crystal structure of yeast tRNA\(^{\text{Phe}}\) in complex with neomycin B reveals that the aminoglycoside binds in the deep groove below the D-loop. Inhibition of aminoacylation of tRNAs is proposed to be either because the aminoglycoside interferes with the interaction between the aminocyl-tRNA synthetase (aaRS) and its cognate tRNA through its binding to major recognition elements (the phenylalanine system; 17) or via a conformational change of the RNA (the acid aspartic system; 18). For the alanine system, aminoacylation is determined by a single G\(^3\)·U\(^70\) pair (19) and also by minor identity elements including the discriminator base A\(^73\) and a G\(^2\)·C\(^71\) pair. This limited set of nucleotides (circled nucleotides in Fig. 1A-B) is conserved in all the 293 tmRNA genes that have been sequenced (20). tmRNA is a partial structural mimic of canonical tRNAs thanks to the presence of an acceptor stem, a T-stem loop and a D analog (21). tmRNA is, however, ~five fold bigger compared to canonical tRNAs, contains several pseudoknots, an internal open reading frame, probably lacks most of the key tertiary interactions present in tRNAs and does not contain an anticodon stem-loop. Here, we report that several aminoglycosides interact with tmRNA with affinity and specificity, preventing its aminoacylation with alanine. The tRNA-like domain of tmRNA is altered upon aminoglycoside binding. Chemical footprints in solution have explored the structural basis of the interaction between tmRNA and several aminoglycosides. *Trans*-translation is therefore a novel target for aminoglycosides.
Experimental Procedures

Enzymes and RNAs

Alkaline phosphatase and T4 polynucleotide kinase are from New England Biolabs (Beverly, MA, USA). T4 RNA ligase was from Gibco BRL Life Technologies (Cergy-Pontoise, France). RNases S1, V1, U2, and T1 were from Amersham-Pharmacia-Biotech (Orsay, France). [$\gamma$-$^{32}$P]-ATP (3000 mCi/mmol) and [$\alpha$-$^{32}$P]-pCp (3000 mCi/mmol) were from Perkin Elmer (Boston, USA). E. coli tmRNA (22) and tmRNA-TLD (Gaudin et al., unpublished results) were over-expressed in E. coli cells and purified as described. Appropriate bands were electroeluted and pure RNAs were recovered by ethanol precipitation. Total tRNAs from E. coli are from Sigma-Aldrich (Saint-Quentin, France). Purified E. coli tRNA$^{\text{AlaUGC}}$ was a gift from Dr R. Gillet (MRC, Cambridge, UK).

Aminoacylation experiments

Recombinant alanyl-tRNA synthetase was purified on Ni$^{2+}$-NTA-agarose (QIAGEN), and purity was confirmed on a 10% SDS-PAGE. Five independent protein purifications were required. Final protein concentration ranged from 0.5 to 3.5 $\mu$M. Assays were performed at 20 or 37°C in a medium containing 50 mM Tris-HCl (pH 7.5), 10 mM KCl, 20 mM $\beta$-mercapto ethanol, 10 mM MgCl$_2$, 2 mM ATP ([MgCl$_2$]/[ATP] = 5), 0.05 mg/ml of BSA and 42-59 $\mu$M L-[14C] alanine (170 mCi/mmol) and 50 to 330 nM of purified E. coli AlaRS. When varying the pH from 5.5 to 9.5, 50 mM [N-Morpholino]Ethane Sulfonic acid was used for pH 5.5, and 50 mM Tris-HCl was used for pHs from 6.5 to 9.5. Usually, 1$\mu$M of tmRNA or tmRNA-TLD was denatured 3 min. at 75°C followed by 10 min. at room temperature. Then, 1$\mu$M of purified E. coli (His)$_6$-tagged SmpB, and/or (His)$_6$-tagged EF-Tu.GDP, and/or EF-Tu.GTP were added and incubated 15 minutes at room temperature. All proteins were at least 98% pure as judged by SDS-PAGE analysis. EF-Tu.GDP was activated to EF-Tu.GTP immediately before use by incubation at 37°C for 15 minutes in 50 mM Tris-HCl (pH 7.6), 7
mM MgCl₂, 60 mM NH₄Cl complemented with 100 µM GTP, 6 mM phosphoenolpyruvate (PEP) and 10 µg/ml of pyruvate kinase. After the incubation, the mixture was kept on ice before use. Various concentrations of aminoglycosides were added, followed by the aminoacylation buffer, the labeled alanine and the AlaRS. Aliquots were spotted onto 3MM Whatman papers at different times and trichloroacetic acid precipitated. Kinetic parameters ($K_M$, $V_{max}$) in the presence and absence of aminoglycosides were performed under steady-state conditions of enzyme (50-330 nM AlaRS) and substrate concentrations, tmRNA (0.5-3.4 µM) or tmRNA-TLD (0.5-6 µM), and determined from Lineweaver-Burk plots. These experimental conditions were also applied for tmRNA aminoacylation in the presence of all tRNAs from *E. coli*.

**Ultraviolet absorbance melting curves**

The progressive melting of tmRNA and tmRNA-TLD was monitored by following their UV absorbency at 258 nm as a function of temperature on a UVikon™ (Bio-tek instruments) equipped with a temperature regulator and with a six-cell holder. Temperature was increased gradually at 0.5°C/min from 15°C to 93°C. Measurements were done in 20 mM potassium phosphate pH 5.8, 0.5 mM EDTA and 5 mM MgCl₂ for tmRNA-TLD and in 20 mM potassium phosphate pH 5.8, 50 mM NaCl and 5 mM MgCl₂ for tmRNA, in the absence and presence of 500 µM neomycin B, 10 mM tobramycin and 2 mM paromomycin.

**Chemical footprints**

Labeling at the 5’-ends of tmRNA and tmRNA-TLD were performed with [$\gamma$-³²P]ATP and phage T4 polynucleotide kinase on RNA dephosphorylated previously with alkaline phosphatase. Labeling at their 3’-ends was carried out by ligation of [$\gamma$-³²P]pCp using T4 RNA ligase. After labeling, tmRNA was gel purified (5% PAGE), eluted, and ethanol precipitated. Labeled tmRNA is heated 2 min at 80°C and slowly cooled down for 20 minutes at room temperature. Reaction mixtures (15 µl) contained 200 000cpm of [$³²P$]
tmRNA or tmRNA-TLD and increasing amounts of aminoglycosides (50 to 500 µM of neomycin B, 0 to 1000 µM of paromomycin and 0 to 6 mM of tobramycin) in 50 mM Hepes pH 7.5, 25 mM KCl and 2 mM MgCl₂. After an incubation of 10 min at room temperature, ENU, lead-acetate or iron-EDTA were added: 6.25 µl of a solution of ENU saturated in 100% EtOH supplemented with 1 µg of total tRNA, 0.3 mM of lead acetate supplemented with 2.5 µg of total tRNA and 1 mg/ml of Fe(NH₄)₂(SO₄)₂, 5 mM EDTA, 12.5 mM DTT and 0.25% H₂O₂ supplemented with 2µg of total tRNA for Fe-EDTA mapping (a quick centrifuge spin mix all four chemicals deposited at four locations inside the eppendorf tube). Incubation times were 4 hours at 37°C for ENU, 7 minutes at 37°C for lead acetate and 10 minutes at 0°C for Fe-EDTA footprints. RNAs are ethanol precipitated, the pellets are washed twice with 80% EtOH, dried and counted. Then, the RNA fragments are submitted to 8 or 12% PAGE. The results were analysed on a PhosphorImager (Molecular Dynamics, Sunnyvale, CA, USA). During lead mapping of tmRNA in the presence of either tobramycin or paromomycin, we noticed that gradually increasing the concentration of the aminoglycosides increases the ratio of the uncleaved versus the cleaved RNA. Modifying the lead concentration, the incubation times, or the amount of total RNA added during the reaction could solve the issue. Since the overall conformation of tmRNA changes in the presence of both aminoglycosides, the conformation of tmRNA in the presence of the aminoglycosides might be less sensitive to the action of the structural probe.

Results

Inhibition of tmRNA aminocacylation by aminoglycoside antibiotics. Aminoacylation experiments with purified E. coli alanyl-tRNA synthetase were performed on two RNAs purified in vivo: tmRNA from E. coli (363 nucleotides, Fig. 1A) and tmRNA-TLD (61 nucleotides, Fig. 1B), a shorter RNA recapitulating the tRNA-like domain of tmRNA. tmRNA-TLD is capable of being aminoacylated with alanine in vitro (Gaudin et al.,
unpublished results). The structures of the six aminoglycosides tested are shown in Fig. 1C. Aminoglycosides are subdivided into three major families (23), and representatives of each family were tested: paromomycin and neomycin B belong to the neomycin family; amikacin, tobramycin and kanamycin A are members of the kanamycin family, and gentamicin represents the gentamicin family. All six aminoglycosides inhibit aminoacylation of tmRNA with alanine, in a concentration dependent manner (Fig. 2). In presence of each aminoglycoside, the decrease of aminoacylation is not a consequence of tmRNA or tmRNA-TLD degradation (not shown). Neomycin B is the most efficient inhibitor, with apparent inhibition constants \( K_i \) of \( \sim 35 \) µM for tmRNA-TLD and \( \sim 70 \) µM for tmRNA (Fig. 2 and Table I). Neomycin B inhibits the aminoacylation of tmRNA-TLD with alanine at a \( \sim 30 \)-fold lower concentration than that required to inhibit tRNA\(^\text{Ala}\) aminoacylation (Table I). Paromomycin, gentamicin and amikacin are also potent inhibitors, whereas kanamycin A and tobramycin are modest ones, with a substantial difference in activity between the two RNAs (kanamycin A and tobramycin have no measurable \( K_i \) on tmRNA-TLD alanylation). Compared to tmRNA, aminoacylation of tmRNA-TLD is half inhibited at approximately two-fold higher concentrations of paromomycin, gentamicin and amikacin (the opposite effect is observed for neomycin B). Aminoacylation with alanine of a tRNA\(^\text{Ala}\) transcript can also be altered by these aminoglycosides, but the inhibition constants are 3 to 15 fold higher than that of tmRNA. Amikacin, kanamycin A and tobramycin have related structures (Fig. 1C), although different inhibition constants (Table I), especially between amikacin and the two others. Between amikacin and tobramycin, the only differences are at positions R1 and R2 (Fig. 1C). Therefore, the chemical groups substituted at positions R1 and/or R2 of amikacin are responsible for its lower \( K_i \). Replacing a hydroxyl group (paromomycin) by an amino group (neomycin B) decreases the \( K_i \) for tmRNA three fold (Table I), indicating that a
minor modification on the chemical structure of an aminoglycoside can increase significantly its inhibitory activity on tmRNA aminoacylation. All the subsequent functional and structural investigations were conducted using three representatives, neomycin B and paromomycin as potent inhibitors and tobramycin as a modest inhibitor of tmRNA aminoacylation.

The number of charged amino groups is important for inhibition of RNA function by aminoglycosides. Neomycin B has 6 protonatable aminogroups, their corresponding pKₐ values are indicated on Figure 1C. In most cases, inhibition of RNA function by aminoglycosides depends on the number of charged aminogroups (24). tmRNA-TLD inhibition by neomycin B (at its Kᵢ of 50µM) was monitored at five different pHs (5.5, 6.5, 7.5, 8.5 and 9.5), centered on pH 7.5 that was used in our assays (not shown). At pH 7.5, neomycin B inhibits 50% of aminoacylation; when pH increases up to 9.5, neomycin B has no inhibitory effect on aminoacylation (the aminogroups are mostly deprotonated); at pH 8.5, 70% of aminoacylation is detected; when pH decreases to 6.5 and 5.5, inhibition of aminoacylation by neomycin B is more efficient, leaving only 35% and 20% residual aminoacylation, respectively. Therefore, inhibition of tmRNA aminoacylation by neomycin B depends on the number of charged aminogroups, and suggests that complex formation between tmRNA and neomycin B is mainly driven by electrostatic interactions.

ATP is required during the first step of all the aminoacylation reactions, to form the activated aminoacyl-adenylate (in our case an alanyl-adenylate). Therefore, aminoglycosides could bind specifically to the catalytic site of E. coli AlaRS as structural analogs of ATP. To test this hypothesis, increasing concentrations of ATP, from 1 to 64 mM, were added in the aminoacylation reaction of tmRNA-TLD with and without 500 µM paromomycin, and in the aminoacylation reaction of tmRNA with or without 2 mM of tobramycin ([MgCl₂] is kept constant at 20mM). Increasing the concentration of ATP does not relieve the inhibitory effect.
caused by the two aminoglycosides on the aminoacylation of both RNAs (not shown). Thus, neither paromomycin nor tobramycin function as structural analogues of ATP in the aminoacylation reaction of tmRNA-TLD and tmRNA with alanine, respectively.

The Michaelis-Menten parameters of tmRNA-TLD and tmRNA aminoacylation with alanine, in the absence and presence of neomycin B, paromomycin or tobramycin, have been determined (Table II). In the absence of aminoglycosides, the $K_M$ for tmRNA-TLD and tmRNA are essentially the same (5 to 6 $\mu$M) but the $V_{max}$ for tmRNA aminoacylation is one order of magnitude lower than that of tmRNA-TLD (Table II). Compared with tmRNA, the smaller size of tmRNA-TLD may allow a faster turnover of the substrate during the aminoacylation reaction. For both RNAs, increasing gradually the concentration of either neomycin B (15 to 60 $\mu$M for tmRNA-TLD), paromomycin (from 50 to 500 $\mu$M for tmRNA and from 100 to 1000 $\mu$M for tmRNA-TLD) or tobramycin (from 1 to 3 mM for tmRNA and from 3 to 10 mM for tmRNA-TLD) increases the $K_M$ up to thirty-fold (Table II), but the $V_{max}$ also increases (not shown). Alanylation is not completely inhibited at saturating antibiotic concentration: there is significant residual aminoacylation level at antibiotic saturation with tmRNA-TLD and in the presence of amikacin and kanamycin A with tmRNA (Fig. 2). Thus, the inhibition of tmRNA aminoacylation by aminoglycosides is neither competitive nor non-competitive; therefore the $K_i$ values could not be measured.

**Inhibition of tmRNA aminoacylation in the presence of all tRNAs from E. coli.** We tested whether or not aminoglycosides can inhibit tmRNA aminoacylation in the presence of all the 46 tRNAs from *E. coli* (Table III). For this experiment, we selected paromomycin, at a concentration corresponding to its apparent inhibition constant for tmRNA alanylation (Table I). Native tRNA$^{Ala}$ isoacceptors represent ~6% of the total tRNA population in *E. coli* (25). In all the tRNAs from *E. coli*, up to 60% of the tRNA$^{Ala}$ isoacceptors are able to charge alanine (100% of charging is not observed probably because our calculated number of
tRNAAla present in the mixture is overestimated; alternatively, a fraction of tRNAAla might be degraded. In the presence of all tRNAs from *E. coli*, there is no inhibition on the aminoacylation of the tRNA alanine isoacceptors (Table III). This result is consistent with the fact that purified tRNAAla UGC transcript has a ~4 fold higher $K_i$ for paromomycin, compared with tmRNA (Table I). When tmRNA is mixed with all tRNAs from *E. coli*, paromomycin decreases the aminoacylation level, even in the presence of a ten-fold excess of total tRNAs (Table III). Since paromomycin, at that concentration, has no effect on the alanylation of the tRNAs, the decrease in tmRNA aminoacylation with alanine is responsible for the overall decrease in the charging levels of the RNA mixture containing both tRNAs and tmRNA. Thus, paromomycin can inhibit tmRNA alanylation in the presence of all tRNAs from *E. coli*.

*Magnesium ions cannot reverse the inhibitory effect caused by the aminoglycosides.* Positively charged ammonium groups of aminoglycosides match negatively charged metal-ion binding pockets in RNA three-dimensional structures, displacing divalent metal ions (26). Recent structural (17) and functional (18) data indicate that the rule also applies for specific interactions between canonical tRNAs and aminoglycosides. If true for tmRNA, inhibition of tmRNA aminoacylation by aminoglycosides might be overcome by increasing the magnesium concentration. The [Mg$^{2+}$/[ATP] ratio was kept constant at 5, as in the previous aminoacylation assays, since it affects the specificity and efficiency of the aminoacylation reaction (27). The magnesium ion concentration was gradually increased, and the aminoacylation of tmRNA-TLD and tmRNA, with and without paromomycin and tobramycin, respectively, was measured (Fig. 3, top). In the presence of paromomycin, aminoacylation of tmRNA-TLD slightly increases up to 20 mM [Mg$^{2+}$] whereas in the presence of tobramycin, aminoacylation of tmRNA show almost no differences up to 10 mM [Mg$^{2+}$]. At higher magnesium concentrations, the aminoacylation plateau decreases for both RNAs with and without aminoglycosides, probably because elevated magnesium
concentrations start degrading the RNAs (not shown). Therefore, for both RNA-aminoglycoside interactions, increasing the magnesium concentration during the aminoacylation reaction cannot rescue the inhibitory effect caused by the aminoglycosides on aminoacylation. Using lead acetate as a probe, we performed a footprinting experiment between 500µM neomycin B and labeled tmRNA-TLD at various magnesium concentrations (1mM, 2mM, 5mM and 10 mM, not shown). The result is that the footprints do not depend on magnesium concentration, and therefore strengthen our claim that magnesium does not rescue aminoglycoside inhibition.

Inhibition of tmRNA aminoacylation by neomycin B in the presence of specific ligands. Small protein B (SmpB) and elongation factor Tu.GTP (EF-Tu.GTP) both enhance alanine-accepting activity of tmRNA, and SmpB protects the 3' end of Ala-tmRNA against enzymatic degradation (28-30). 1µM of tmRNA with post transcriptional modifications charges alanine to 26% on average, but there is a half increase in charging when either 1µM of purified SmpB or 1µM of purified EF-Tu.GTP are present (Fig. 4, table inset). A two-fold increase is observed when both proteins are present at 1µM. This indicates that in our aminoacylation assays, both SmpB and EF-Tu.GTP bind tmRNA and enhance its aminoacylation with alanine. With EF-Tu.GDP, the charging level of tmRNA is not significantly affected, but when in the presence of SmpB, aminoacylation plateau also increases two-fold (Fig. 4, inset). We verified by native gel shift assays that SmpB, EF-Tu.GTP and EF-Tu.GDP bind deacylated tmRNA (not shown). In presence of equimolar ratio of SmpB, neomycin B can still inhibit aminoacylation, but the $K_i$ increases 1.5-fold, compared with tmRNA alone. In presence of EF-Tu.GTP, the $K_i$ increases five-fold; in presence of both proteins, the $K_i$ increases ten-fold, compared to tmRNA alone (Fig. 4). In the presence of 1µM EF-Tu.GDP, the $K_i$ increases 2.5-fold; in the presence of 1µM SmpB and 1µM EF-Tu.GDP the $K_i$ also increases ten-fold, compared to tmRNA alone (Fig. 4,
These data indicate that in presence of SmpB and EF-Tu, tmRNA aminoacylation is significantly protected against the inhibitory effect of aminoglycosides.

_Termal UV melting profiles tmRNA-TLD in the presence of aminoglycosides._ Ultraviolet (UV) absorbance melting curves were performed to assay the effects of aminoglycosides on tmRNA and tmRNA-TLD structures. In the presence of 5 mM MgCl₂, the melting profile of tmRNA is multiphase, with a first transition around 40°C corresponding likely to the unfolding of its tertiary structure; a series of smaller transitions, from 60°C to 90°C, correspond to the progressive unfolding of the secondary structure (not shown). Since the unfolding pathway of tmRNA is so intricate, no clear picture emerges, e.g. stabilization or destabilization, when aminoglycosides are present (not shown). For tmRNA-TLD, however, UV melting experiments have provided information about the effects of aminoglycosides on the RNA structure (Fig. 5). In the absence of aminoglycosides, tmRNA-TLD structure unfolds in two transitions; the first one is centred on a calculated melting temperature ($T_m$) of around 35°C; the second transition occurs at higher temperature, with a $T_m$ close to 80°C (Fig. 5A). That second transition is broad and non-cooperative, suggesting that several conformations of the RNA coexist in solution and unfold independently when temperature increases. 10 mM tobramycin does not affect the lower transition, whereas the $T_m$ corresponding to the second transition is considerably reduced (a decrease of 18°C), to 60°C (Fig. 5B). Also, the second transition becomes sharper and cooperative. A similar destabilizing effect on tmRNA-TLD structure is observed in the presence of 2 mM paromomycin, with no significant effects on the lower transition, but a 16°C drop of the $T_m$ corresponding to the second transition (Fig. 5C). As for tobramycin, the second transition, whereas shifted to a lower temperature, is nevertheless sharper and more cooperative, compared to tmRNA-TLD alone. Therefore, both aminoglycosides reduce the conformational freedom of tmRNA-TLD and stabilize a
conformation that is no longer aminoacllatable with alanine. In the presence of 500 µM neomycin B, the $T_m$ corresponding to the first transition is not affected, but the $T_m$ of the second increases of about 10 degrees, up to 90°C (not shown). Therefore, upon binding tmRNA-TLD, neomycin B stabilizes its conformation.

**Chemical footprints of tmRNA-TLD with neomycin B.** To gain structural information about the interaction between tmRNA-TLD and neomycin B, lead acetate footprints, in the absence and presence of increasing concentration of neomycin B, were performed (Fig. 3, bottom). Nine nucleotides (A8-U12, A15-C18) from the D-analog of tmRNA-TLD and nucleotides G31 and A32 are cleaved by lead, but become protected in the presence of increasing concentrations of neomycin B (Fig. 3, bottom). Six nucleotides involved in the three base pairs U6-A52, G7-C51 and G34-C50 are cleaved by lead in the absence of the aminoglycoside, suggesting that these pairs are unstable in tmRNA-TLD; when neomycin B is present, all the six cleavage sites disappear or are significantly reduced, suggesting that neomycin B, upon binding tmRNA-TLD, stabilizes the three pairings (result consistent with the increase of the $T_m$ corresponding to the second transition). There are no visible changes in the conformation of stem-loop H5, the five upper pairs in H1, the four upper pairs in the T-stem and the T-loop, with and without the aminoglycoside.

**Chemical footprints of tmRNA with paromomycin and tobramycin.** The phosphates (ENU) or nucleotides (lead) of tmRNA that are protected or that become accessible in the presence of the aminoglycosides are indexed on the right-hand sides of the four upper panels (A-D) in Figs 6 and 7. The chemical footprints of both aminoglycosides are summarized on the secondary structure of *E. coli* tmRNA. Mapping of ribose accessibility with Fe-EDTA shows no differences in the absence and presence of increasing concentrations of both aminoglycosides (Figs 6 and 7, panels E are representatives; additional experiments were performed with longer migration times and 3’-labeling of the RNA to get resolution of the
upper part of the gels), suggesting that they do not contact any sugars from tmRNA backbone.

Mapping the phosphates from tmRNA by ENU in the absence of aminoglycoside shows that out of 363, 39 phosphates are cleaved by the probe and therefore accessible in the native conformation of tmRNA (Figs 6 and 7, panels A and B). Paromomycin induces 29 concentration dependent chemical footprints onto tmRNA structure, 15 against ENU and 14 against lead (Fig. 6, panels A-D). With lead, two positions that are not accessible in tmRNA structure, C44 in helix H2 and G150 in the helical portion of pseudoknot PK2, become reactive in the presence of paromomycin. Due to the conformational flexibility of tmRNA (22), several nucleotides located in helical portions within the RNA structure are cleaved by lead in the absence of the aminoglycoside. These nucleotides are U26-U27 (5’ to an internal bulge within H5), G31-A32 (between two internal bulges in H5, and G31 is involved in GA pair), U59 (in one stem of PK1), C353 (at one end of H1), U328-U330 (flexible GU pairs in H5), and G333 (at one end of H5). When paromomycin binds tmRNA, these accessible sites become protected (direct contact between the aminoglycoside and tmRNA, or indirect effect of paromomycin on the conformation of tmRNA). Increasing concentrations of paromomycin modify the reactivity of nucleotides or phosphates located in the tRNA-like domain, H5-PK4, PK1-H3-H4 and PK2, but not in H6 and PK3. Within the tRNA-like domain of tmRNA, seven positions (U9, U17, U328-U330, G333 and C353) become protected by paromomycin, as tobramycin also does but at much higher concentrations (see Fig. 7).

Tobramycin induces 31 concentration dependent chemical footprints onto tmRNA structure, 14 against ENU and 17 against lead (Fig. 7, panels A-D). As for paromomycin, C44, in helix H2, become reactive towards lead cleavage. Three nucleotides, G61 in PK1, G150 in PK2 and G288 in PK4 are protected against both lead and ENU cleavages (Fig. 7, black stars). The footprints are in the tRNA-like domain, PK1-H3-H4 and in PK2-PK3-PK4.
Paromomycin and tobramycin share 20 common footprints located, for the most part, in the tRNA-like domain, the tag reading frame and PK2. Out of the common footprints between tmRNA and both aminoglycosides, seven are in the tRNA-like domain, indicating that tight binding requires full-length tmRNA. This result is in agreement with a higher $K_i$ for tmRNA-TLD aminoacylation by both aminoglycosides, compared with tmRNA (Fig. 2 and Table I). However, there are significant differences between both aminoglycosides (underlined nucleotides in Figs 6 and 7): paromomycin, but not tobramycin, modifies specifically the reactivity of a cluster of nine nucleotides centred on an internal bulge in H5 and in PK4. Tobramycin, but not paromomycin, modifies specifically the reactivity of 11 nucleotides, in PK1, in and around the tag, in PK3 and PK4. Altogether, our probing data suggest that when aminoglycosides bind tmRNA, there is a significant conformational rearrangement of the RNA, as already suggested by our UV melting data collected on tmRNA-TLD.

**Discussion**

Functional and structural evidence are provided to demonstrate that aminoglycoside antibiotics interact *in vitro* with tmRNA from *E. coli*, modify its conformation in solution, resulting in its inability to be efficiently aminoacylated with alanine by alanyl-tRNA synthetase. tmRNA aminoacylation was most strongly inhibited by neomycin B and by paromomycin followed by, in descending order, gentamicin, amikacin, kanamycin A and tobramycin (Fig. 2). When tmRNA is reduced to its tRNA-like domain, the concentration of paromomycin, gentamicin and amikacin required for half inhibition of aminoacylation increases approximately two-fold (Table I), indicating that structural domains of tmRNA outside the tRNA-like core are required for optimal inhibition of aminoacylation. This functional result is confirmed by our structural analysis of tmRNA in complex with two different aminoglycosides, because paromomycin and tobramycin both induce chemical
Aminoglycosides impair trans-translation protections of accessible bases and phosphates located outside the tRNA-like domain (Figs 6 and 7). Therefore, with the exception of neomycin B, efficient binding of aminoglycosides requires full-length tmRNA. Neomycin B interacts specifically with yeast tRNA\(^{Phe}\) (17), rationalizing that the tRNA portion of tmRNA is sufficient for efficient binding.

Between tmRNA-TLD and tRNA\(^{Ala}\) \(^{UGC}\), the concentration of paromomycin, gentamicin and amikacin required for half inhibition of aminoacylation increases also approximately two-fold (neomycin B is an extreme case, with a thirty fold difference). Specific sequences and/or structural features of tmRNA-TLD, that are not present in tRNA\(^{Ala}\), are responsible. These differences are located in the D-analog and the C21-G30 stem-loop of tmRNA-TLD; there are also a few sequence variations in the acceptor stem and the T stem-loop of canonical tRNA\(^{Ala}\), compared to tmRNA-TLD. Neomycin B protects accessible nucleotides in the D-analog of tmRNA-TLD against lead cleavages that are not present in tRNA\(^{Ala}\), rationalizing the binding specificity of the aminoglycoside on tmRNA-TLD. For an aminoglycoside to inhibit tmRNA aminoacylation, it could prevent the correct recognition of the limited set of nucleotides that specify the alanine identity, all located in the acceptor stem, by the AlaRS; alternatively, they could stabilize a non-functional state of tmRNA. Co-crystallization of yeast tRNA\(^{Phe}\) with neomycin B reveals that the aminoglycoside is positioned in the deep groove below the D-loop, possibly interfering with the interaction between PheRS and its cognate tRNA through its binding to major tRNA\(^{Phe}\) charging determinants (17). Structural data reported here indicate that neomycin B interacts with the D analog of the tRNA-like portion of tmRNA, but not in the upper portion of the acceptor stem where the major recognition determinant for alanylation, a GU pair, is located. Upon binding, neomycin B strengthens three base pairs at the junction between the acceptor and the T stems, perturbing the conformation of the acceptor stem that contains the aminoacylation signals. When tmRNA-TLD aminoacylation is performed at 20°C instead of 37°C, the \(K_i\) for
neomycin B increases about four fold (from 35 to 145 µM). Neomycin B is a better inhibitor when temperature increases, perhaps allowing the RNA to adopt an optimal conformation for binding.

The footprints of tobramycin and paromomycin on tmRNA and the fact that aminoglycoside binding can be described in two slopes (at least in some cases, Fig. 2), suggest that several antibiotic moieties bind to each tmRNA molecule. Both paromomycin and tobramycin induce the formation of a favoured conformation of tmRNA-TLD, but 7-fold lower concentrations of paromomycin are required, compared with tobramycin (Table I). Without aminoglycosides, the conformation of tmRNA-TLD is poorly defined in solution (Fig. 5), but aminoglycosides trap tmRNA-TLD into an inactive conformation that is either stabilized (neomycin B) or destabilized (paromomycin, tobramycin). Aminoglycosides displace a conformational equilibrium towards non-functional tmRNAs; once the conformational switch is triggered, increasing the magnesium concentration cannot restore the aminoaacylation capacities of tmRNA (Fig. 3).

tmRNA aminoaacylation is required for trans-translation. It is not known whether or not aminoglycosides bind tmRNA in vivo. trans-translation will be impaired in vivo only if the intracellular concentration of the aminoglycoside, in our case neomycin B, is sufficient to interact with some of the ~500 copies of tmRNA per cell (31), in addition to its other RNA targets. In bacteria, aminoglycosides impair various cellular processes: they mostly disturb ribosome decoding and cause misreading of the genetic code (32). Paromomycin binds RNA constructs containing the ribosomal A site with dissociation constants of ~1.5 µM (33), indicating that the major target of aminoglycosides is the ribosome. They also inhibit RNase P RNA cleavage in vitro. The strongest inhibitor of E. coli RNase P RNA cleavage in the presence of its associated protein is also neomycin B (16), with a 60 µM $K_i$ (paromomycin
has a $K_i$ value of 190 $\mu$M on RNAse P RNA). Therefore, the $K_i$ values of inhibition of RNAse P RNA cleavage and tmRNA alanylation by both neomycin B and paromomycin are similar.

tmRNA aminoacylation with alanine can be impaired by an aminoglycoside in the presence of all tRNAs from *E. coli* (Table III). In its natural context, tmRNA has specific ligands. Two proteins interact with tmRNA, small protein B (34) and elongation factor-Tu (35), stimulate tmRNA aminoacylation *in vitro* (28). When both proteins are added to deacylated tmRNA prior neomycin B, they prevent tmRNA charging from being inhibited by low concentrations of aminoglycoside (Fig. 4). Whereas SmpB has a small protecting effect, EF-Tu, in both the GDP and the GTP forms, has a significant protecting effect against the inhibition of tmRNA aminoacylation by neomycin B. Protection is maximal when both SmpB and EF-Tu are present, suggesting that tmRNA aminoacylation might not be impaired *in vivo*. RNase T$_1$ footprints of SmpB on a tmRNA transcript indicate that nucleotides G333 (G31 in tmRNA-TLD), G336 (G34 in tmRNA-TLD) are protected by SmpB against RNase T$_1$ cleavage (28). In presence of neomycin B, G31 from tmRNA-TLD is protected against lead cleavage and the G34-C50 pairing is reinforced, indicating that there are some overlaps between the binding sites of SmpB and neomycin B on tmRNA. Deacylated tmRNA forms a complex with either EF-Tu.GDP or EF-Tu.GTP, and two UV cross-links with EF-Tu.GDP are located outside the tRNA part, in H5 (U268) and PK4 (U308; 36). Binding sites between EF-Tu and neomycin B onto tmRNA might overlap. Alternatively, SmpB and EF-Tu could modify the conformation of deacylated tmRNA, with high efficiency when both proteins are present, such as tmRNA becomes an efficient substrate for aminoacylation but an inefficient ligand for neomycin B. In presence of equimolar ratio of purified ribosomal protein S1 that also interacts with tmRNA, the inhibition constant of tmRNA aminoacylation with neomycin B increases three-fold (not shown).
All the known 298 tmRNA gene sequences (20) possess the recognition determinants for efficient aminoacylation with alanine, suggesting that in all these species the first amino acid of the tag is alanine. The secondary and probably also tertiary structures of these tmRNA sequences might be sufficiently conserved to provide the proper recognition determinants for aminoglycoside binding. Therefore, aminoglycosides are probably also able to interfere with trans-translation in bacterial species other than *E. coli*. Mutating the acceptor stem of tmRNA to confer histidine acceptance retains its ability of protein tagging *in vitro*, suggesting that the first alanine of the tag can be substituted by another amino acid (37). Therefore, after specific mutations within its nucleotide sequence, tmRNA could potentially be chargeable by amino acids other than alanine. Paromomycin and tobramycin modify the reactivity of the tag reading frame towards structural probes, likely disturbing re-registration from the stalled ribosome to the tag and preventing tagging of the truncated proteins, even if aminoacylation can proceed. The overall pharmacological effect of aminoglycosides during the treatment of infectious diseases may result from a combination of actions prior and during protein synthesis, but also when protein synthesis has stalled or has been interrupted. Aminoglycosides primarily cause misreading of mRNA that leads to the synthesis of nonsense or truncated peptides. If tmRNA function is also impaired by aminoglycosides, the nonsense or truncated peptides will accumulate, speeding up cell death.

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**Supplemental data on the chemical footprints**

Chemical footprints in solution provided direct evidence about the interaction between tmRNA and aminoglycosides. The base accessibility of tmRNA was monitored by lead acetate. Lead acetate cleaves single-stranded RNA, but with sensitivity to subtle conformational changes of the RNA chain. The accessibility of the riboses was assayed by Fe-EDTA and Ethyl-Nitroso Urea (ENU) mapped the phosphates. The reactivity towards these probes was monitored for each nucleotide of tmRNA, with and without increasing concentrations of either paromomycin (100 to 1000 μM, Fig. 6) or tobramycin (1 to 6 mM, Fig. 7). Altogether, 24 independent experiments were performed (Figs 6 and 7 are representative). Mapping the phosphates from tmRNA by ENU in the absence of aminoglycoside shows that out of 363, 39 phosphates are cleaved by the probe and therefore accessible in the native conformation of tmRNA (Figs 6 and 7, panels A and B). These accessible phosphates are scattered throughout the molecule (P7, P13-14, P19, P29, P43, P61, P64, P66, P87, P90, P97, P100, P108, P135, P151, P157, P195, P197, P200, P204-205, P223, P226, P228, P274, P288, P293, P297, P305, P308 and P326-333), especially in the upper portion of H5, around the resume codon and within the four pseudoknots. In the absence of aminoglycosides, lead acetate cleaves ~100 nucleotides of *E. coli* tmRNA (22). With ENU, several phosphates of tmRNA are not accessible without paromomycin, but become reactive in the presence of the aminoglycoside. These positions have been omitted, since all are already reactive in the control lanes without ENU. Nucleotides A69, A79, A86, C91, A94, G99, A106, A121, A124, U172, A175, C183, A185, A190, A192, A232, U236, A239-U240, C272, C279, U300 are subjected to cleavage in the presence of the higher concentration (6 mM) of tobramycin; most of them, however, are already present at weaker intensities in the
control lane with tmRNA alone (Fig. 7). This observation has also been noticed in the presence of paromomycin, at positions A45, A69, A79 and A174. Therefore, these cleavage sites are not specific to the interaction and suggest that aminoglycosides can accentuate cleavage of tmRNA at these sites. Other than these peculiar cases and among the 324 phosphates in tmRNA that are protected against ENU cleavages, none of them becomes accessible in the presence of tobramycin (Fig. 7).

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Fig. legends

**Fig. 1.** *E. coli* tmRNA, tmRNA-TLD (the tRNA-like portion of tmRNA) and the structures of the aminoglycosides tested in this study (A) Sequence and secondary structure of *E. coli* tmRNA (38), with emphasis to the tRNA-like domain; the additional domains containing four pseudoknots (PK1-PK4) and the tag reading frame are presented in outline (B) *E. coli* tmRNA-TLD; both RNAs have an alanine attached to their 3’-ends. Nucleotides specifying the alanine identity to both RNAs are circled; in grey is shown the GU pair, a major identity element for alanylation. (C) Structures of the six aminoglycoside antibiotics used in this study.

**Fig. 2.** Inhibition of tmRNA (gray circles) and tmRNA-TLD (black squares) aminoacylation with alanine by six aminoglycoside antibiotics. Aminoacylation plateaus as a function of increasing concentrations of neomycin B, paromomycin, gentamicin, amikacin, kanamycin A and tobramycin. For a direct comparison between the two RNAs, the percentage of charging by AlaRS of tmRNA and tmRNA-TLD without aminoglycosides was set to 100%, whereas tmRNA is aminoacylated up to 40% and tmRNA-TLD up to 90%. At low concentrations (100-200 $\mu$M) of kanamycin A, a beneficial effect on tmRNA aminoacylation was reproducibly observed.

**Fig. 3.** (A-B) Influence of the concentration of magnesium on the aminoacylation of tmRNA and tmRNA-TLD, in the presence (grey squares) and absence (black diamonds) of aminoglycosides. The MgCl$_2$:ATP is at constant ratio (5:1), while the magnesium concentration increases. (A) Aminoacylation of tmRNA-TLD in the presence of 500 $\mu$M paromomycin (10% of variance without and with the aminoglycoside). (B) Aminoacylation of tmRNA in the presence of 2 mM tobramycin (10% of variance without and with the aminoglycoside). (C-D) Lead acetate footprints of tmRNA-TLD with increasing concentrations of neomycin B. (C) Autoradiograms of 12% denaturing PAGE of
cleavage products of 3’-labeled RNAs. Lane C, incubation control; lanes G₁, RNase T₁ hydrolysis ladder; lanes A₁, RNase U₂ hydrolysis ladder. The RNA sequence is indexed on the left side. Gray nucleotides indexed on the right side are the identified protections. (B) Nucleotides from tmRNA-TLD with a decreased reactivity towards lead cleavage in the presence of neomycin B are in gray. The major recognition determinant for alanylation, a GU pair, is boxed.

**Fig. 4.** Inhibition of tmRNA aminoacylation with alanine by neomycin B in the presence of purified SmpB, EF-Tu.GTP or both. Aminoacylation plateaus as a function of increasing concentrations of neomycin B of 1µM tmRNA (T, diamonds), 1µM tmRNA and 1µM SmpB (T+S, squares) 1µM tmRNA and 1µM EF-Tu.GTP (T+E, triangles) and 1µM tmRNA, 1µM SmpB and 1µM EF-Tu.GTP (T+E+S, circles). For a direct comparison, the percentage of charging by AlaRS of tmRNA with and without protein ligands in the absence of aminoglycoside was set to 100%. Inset table: charging levels and inhibition constants of aminoacylation of tmRNA with and without SmpB and/or EF-Tu in both the GDP and the GTP forms. The plots and values reported are averaged from four independent experiments for each condition.

**Fig. 5.** Thermal UV melting profiles tmRNA-TLD in the presence of various aminoglycosides. (A) Thermal UV melting profiles of tmRNA-TLD. The variance in the calculated \( T_m \) was calculated from 13 independent experiments; (B) UV absorbance melting profile of tmRNA-TLD in the presence of 10 mM tobramycin. The variance in the calculated \( T_m \) was calculated from 6 independent experiments. (C) UV absorbance melting profile of tmRNA-TLD in the presence of 2 mM paromomycin. The variance in the calculated \( T_m \) was calculated from 6 independent experiments. The first derivative of the UV absorbency as a function of temperature is also shown. The plots shown are averaged from three independent experiments.
**Fig. 6.** Chemical footprints of increasing concentrations of paromomycin with tmRNA using ENU (A, B), lead acetate (C, D) and Fe-EDTA (E). Autoradiograms of 8% denaturing PAGE of cleavage products of 5’ and 3’-labeled tmRNAs, with similar migration times. Lanes C, incubation controls; lanes G_L, RNase T₁ hydrolysis ladder; lanes A₁, RNase U₂ hydrolysis ladder. Sequence is indexed on the left sides: nucleotides with black and white triangles are Gs and As, respectively. Nucleotides indexed on the right sides of the autoradiograms are the identified footprints. Footprints of the aminoglycoside are indicated onto tmRNA secondary structure, shown schematically. Only the protections or enhancements of reactivity of nucleotides that vary according to the concentration of the aminoglycoside were considered as reliable data. Black dots are the phosphates protected against ENU cleavages in the presence of the aminoglycoside. Grey dots are the nucleotides protected against lead cleavage in the presence of the aminoglycoside. Black stars are the positions that are protected by both ENU and lead in the presence of the aminoglycoside. Grey squares are the nucleotides that become accessible to lead cleavage in the presence of the aminoglycoside. With ENU, some nucleotides become accessible in the presence of the aminoglycoside, but all these cleavage sites are already present in the control lane; thus, they were omitted on purpose. The footprints that are specific to the aminoglycoside are underlined (those that are common to paromomycin and tobramycin are not).

**Fig. 7.** Chemical footprints of increasing concentrations of tobramycin with tmRNA. The indications provided are identical to Fig. 6.
Table I. $K_i$ values* of the inhibition of aminoacylation of tmRNA, tmRNA-TLD and \(tRNA^{\text{AlaUG}}\) by six aminoglycosides.

| RNAs        | tmRNA | tmRNA-TLD | tRNA^{\text{AlaUG}} |
|-------------|-------|-----------|----------------------|
| **Aminoglycosides** |       |           |                      |
| Neomycin B  | 70 ±20| 35 ±15    | 1000 ±200            |
| Paromomycin | 225 ±75| 500 ±100  | 1000 ±100            |
| Gentamicin  | 400 ±100| 700 ±100  | 1500 ±100            |
| Amikacin    | 450 ±200| 900 ±100  | 1500 ±100            |
| Kanamycin A | 1400 ±500| n.m.      | n.m.                 |
| Tobramycin  | 1600 ±500| n.m.      | 2000 ±100            |

* $K_i$ (µM) is defined as the concentration resulting in half inhibition of aminoacylation.

Each value reported is the average of 3 to 6 independent experiments, and were calculated from plots as shown in Fig. 2. n.m.: non measurable. Purified tRNA^{\text{Ala}} transcript is the isoacceptor with a UGC anticodon.
Table II. Kinetic parameters of tmRNA-TLD and tmRNA aminoacylation with alanine in the presence of aminoglycosides.

| RNAs          | $K_M$ (µM) | $V_{max}$ (nM s⁻¹) |
|---------------|------------|---------------------|
| tmRNA-TLD     |            |                     |
| alone         | 5.5 ±3     | 3 ±2                |
| + neomycin B  | 180 ±20    | 6 ±3                |
| + paromomycin | 23 ±5      | 4 ±3                |
| + tobramycin  | 26 ±5      | 3 ±2                |
| tmRNA         |            |                     |
| alone         | 6 ±3       | 0.3 ±.15            |
| + paromomycin | 13 ±3      | 0.3 ±.1             |
| + tobramycin  | 30 ±5      | 0.4 ±.1             |

Kinetic parameters represent an average of several experiments using independent enzyme purifications. The concentrations of the aminoglycosides correspond to their $K_i$ for tmRNA-TLD and tmRNA aminoacylation with alanine, respectively.
Table III. Inhibition of tmRNA aminoacylation with alanine by an aminoglycoside in the presence of all *E. coli* tRNAs.

| Paromomycin | - | + (250 µM) |
|-------------|---|------------|
| picomoles   |   |            |
| tmRNA       |   |            |
| 100         | 37 ± 1 | 21 ± .9 |
| *E. coli* tRNAs§ |   |           |
| 100 (6)     | 56 ± 8 | 51 ± 2.7 |
| 1000 (60)   | 52 ± 2 | 50.5 ± 1 |
| tmRNA with *E coli* tRNAs§ |   |           |
| 100 + 100 (6) | 38 ± .5 | 23 ± 1 |
| 100 + 1000 (60) | 41 ± 2 | 35 ± 2 |

*Charging levels were obtained for 30 min incubation in the presence of equivalent amounts of purified AlaRS; when both tmRNA and tRNAs were present, charging is calculated as the percentage of all RNAs aminoacylatable with alanine, without considering mischarging.

*§* *E. coli* tRNAs are all the 46 isoacceptors; the number in parenthesis corresponds to the amount of isoacceptors tRNA^Ala in the mixture (25) that can be aminoacylated with alanine.
Alanylation of tmRNA and tmRNA-TLD (%) vs. antibiotic concentration (µM)

- [Neomycin B] µM
- [Paromomycin] µM
- [Gentamicin] µM
- [Amikacin] µM
- [Kanamycin A] µM
- [Tobramycin] µM
Fig. 3, Corvaisier et al.
| Charging levels (%) | $K_i$ (µM) |
|---------------------|------------|
| T                   | 26 +/-5    | 70 +/-20  |
| T+S                 | 38 +/-6    | 115 +/-10 |
| T+EGTP              | 35 +/-5    | 370 +/-90 |
| T+EGDP              | 30 +/-2    | 180 +/-10 |
| T+EGTP+S            | 52 +/-6    | 780 +/-100|
| T+EGDP+S            | 48 +/-4    | 700 +/-100|

Fig. 4, Corvaisier et al.
Fig. 5, Corvaisier et al.

A

B

C

Normalized Absorbance

d OD/dT

T (°C)

34±6

78.5±2.3

36±5

60.6±1

32±5

62.1±1

0.004

0.003

0.002

0.001

0

0.004

0.003

0.002

0.001

0
Fig. 6, Corvaisier et al.
**Fig. 7, Corvaisier et al.**

**A**

![Image](A.png)

**B**

![Image](B.png)

**C**

![Image](C.png)

**D**

![Image](D.png)

**E**

![Image](E.png)
Inhibition of transfer-messenger RNA aminoacylation and trans-translation by aminoglycoside antibiotics
Sophie Corvaisier, Valérie Bordeau and Brice Felden

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