Review

Movement of the 3′-end of tRNA through the peptidyl transferase centre and its inhibition by antibiotics

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Abstract Determining how antibiotics inhibit ribosomal activity requires a detailed understanding of the interactions and relative movement of tRNA, mRNA and the ribosome. Recent models for the formation of hybrid tRNA binding sites during the elongation cycle have provided a basis for re-evaluating earlier experimental data and, especially, those relevant to substrate movements through the peptidyl transferase centre. With the exception of deacylated tRNA, which binds at the E-site, ribosomal interactions of the 3′-ends of the tRNA substrates generate only a small part of the total free energy of tRNA-ribosome binding. Nevertheless, these relatively weak interactions determine the unidirectional movement of tRNAs through the ribosome and, moreover, they appear to be particularly susceptible to perturbation by antibiotics. Here we summarise current ideas relating particularly to the movement of the 3′-ends of tRNA through the ribosome and consider possible inhibitory mechanisms of the peptidyl transferase antibiotics.

Key words: Peptidyl transferase; Hybrid tRNA site; Antibiotic; Inhibitory mechanism

1. Introduction

The concept of hybrid sites for tRNA binding on the ribosome has radically changed our view of how peptide elongation occurs. The early hypothesis that such sites can form [1–3] has received experimental support from footprinting of tRNA-ribosome complexes [4] and from kinetic studies on the puromycin reaction [5–7]. Their formation can also facilitate proof-reading of amino acid incorporation [8]. Hybrid tRNA sites form the basis of the 'hybrid state' model for elongation [4] that is illustrated, in modified form, in Fig. 1. This model provides a basis for reinterpreting the extensive early literature on the binding of various tRNA substrates to the ribosome and on the binding and mode of action of the peptidyl transferase drugs [9]. Here, we try to rationalise further the molecular bases of inhibition by these drugs. Particular attention is paid to the thermodynamics and kinetics of the elongation process and we refer primarily to the bacterial (*Escherichia coli*) ribosome.

2. tRNA binding sites

The strongest tRNA-ribosome interactions take place on the small ribosomal subunit. They are codon-dependent and occur at the acceptor A- and peptidyl P-sites within the mRNA binding centre [10,11]. Deacylated tRNA binds primarily to the exit (E) site on the large subunit [12–16]. The remaining tRNA-ribosome interactions, including those at the peptidyl transferase centre of the large subunit [17], are all weak [18]. Three different functional forms of tRNA, aminoacyl-tRNA, peptidyl-tRNA and deacyl-tRNA, can bind simultaneously to the bacterial ribosome where they attach, primarily and respectively, to the A-, P-sites on the small subunit and to the E-site on the large subunit; other sites on the large subunit are designated T, possibly A′, and P′ [10–15,18]. During the elongation cycle tRNAs can exist in various physical and functional states some of which are considered separately below.

2.1. The entry (A/T) site

The incoming aminoacyl-tRNA, complexed with EF-Tu.GTP in the ternary complex, binds initially at an entry site on the ribosome [19–21]. Experiments with ribosome-bound ternary complexes containing non-cleavable GTP analogues demonstrate that both aminoacyl-tRNA (Phe-tRNA) and the GTP analogue can readily exchange in this state [22]. The bound ternary complex protects some rRNA sites against chemical modification that are located exclusively on the 30S subunit [23]. This suggests that interactions on the large subunit occur partly via interactions with ribosomal proteins [24] that may include interactions between EF-Tu and the $(L12)_4$.L10 pentamer. This latter protein complex binds within a region of domain II of 23S rRNA that has been implicated in the ribosome-dependent GTP hydrolysis reactions [25], and it also constitutes the site at which bound EF-Tu has been mapped on the ribosome by immuno-electron microscopy [26].

In the ternary complex, the acceptor end of the aminoacyl-tRNA lies between two structural domains of the EF-Tu structure, in a constrained conformation [27]. However, the capacity of Phe-tRNA, carrying an azido group at the 2 position of the 3′-terminal A_{76} (2-azidoA_{76}Phe-tRNA), to cross-link within positions 1930–1980 of domain IV of 23S rRNA (fragment F5, Fig. 2), when bound in a ternary complex containing the non-hydrolysable GTP derivative GMP-PNP [28], suggests that 23S rRNA can gain access to this groove. On GTP hydrolysis, the 3′-end of the tRNA is released and is free to move across the 50S subunit into the peptidyl transferase centre [29].

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2.2. Pre-peptide bond forming state(s)

After release from the ternary complex, and prior to peptide bond formation, the 3′-end of the aminoacyl-tRNA enters the catalytic centre. This region of the ribosome corresponds to the A′-component of an A/A′-site that had a prominent role in an earlier model in providing a reciprocating binding site for aminoacyl- and peptidyl-tRNA. A consequence of the hybrid state model is that no discrete, relatively stable binding is required. This is a region through which the 3′-end of the tRNA must pass, transiently, before entering the transition state for peptide bond formation. At present, we have no estimate of how far the 3′-end of aminoacyl-tRNA moves in this step.

It is now clear, for many of the early experiments, that it was not an A′-like site that was primarily being investigated but rather the P′-component of the hybrid A/P′-site (see below). Included in this category are experiments in which (a) the P/P′-site was blocked with deacylated tRNA and an aminoacyl-tRNA, or derivative thereof, was cross-linked to, or probed on, the ribosome [30–32]; (b) a puromycin derivative was cross-linked to an unlocalised GUUCG (or GUCCG) sequence within the large subunit rRNA [33–35]; and (c) competitive binding studies between antibiotics and aminoacyl-oligonucleotides [36], since the latter, and at least some of the former, can bind in the P′-site [37].

Moreover, there is evidence that the region of the ribosome, through which the 3′-end of the aminoacyl-tRNA passes, is conformationally heterogeneous on the free ribosome [38]. Circumstantial evidence suggests that the acceptor substrate region of the tRNA interacts there [39,40] since, first, puromycin can bind and react with a donor substrate [41], second, both the accuracy and rate of elongation are affected in a systematic way by mutations in the 23S rRNA [42,43] and, third, peptidyl transferase antibiotics can modulate the affinity of aminoacyl-tRNA for the ribosome, after GTP hydrolysis and release of EF-Tu-GDP [44,45]. However, interactions appear to have a low degree of site specificity [46] and, moreover, mutational studies of the -CCA sequence of the acceptor substrate revealed a low level of base specificity, although changes in the 3′-terminal-CA sequence did produce some lowering of acceptor activities in the 'fragment' reaction [47].

Attempts to determine the protein environment of this ribosomal region yielded fairly complex protein patterns [24,48]. Parallel studies have been performed to characterise an rRNA component of this region. They include: (i) footprinting of tRNA-ribosome complexes on 50S subunits [49]; (ii) RNA footprinting of puromycin, and other supposed A′-site-specific drugs, on ribosomes [50–52]; (iii) site-directed mutagenesis of the peptidyl transferase loop [53], and (iv) affinity probing experiments between the 5′-end of ribosome-bound-tRNA and 23S rRNA [54]. None of these data are readily reconcilable with a discrete, relatively stable, A′-site on the 50S subunit.

One promising approach used to investigate this region of the 50S subunit was to fill the P/P′-site with AcPhe-$tRNA_{ox-red}$, for which the 2′-3′ bond of the 3′-terminal ribose is cleaved. This peptidyl-tRNA analogue lacks donor activity but binds with thermodynamic and kinetic parameters similar to those of AcPhe-tRNA [55,56]. Subsequently, Phe-tRNA is bound to the ribosome either within a ternary complex or alone. The former produces the more stable complex, as judged by its lower rate of exchange with unlabelled Phe-tRNA in solution and, consistent with this, the former also exhibits a slower dissociation rate [22,56]. The results were confirmed by binding in the presence of tetracycline, which perturbs tRNA binding on the 30S subunit; Phe-tRNA complexed via a ternary complex bound at a lower rate whereas free Phe-tRNA bound with a 10-fold lower apparent association constant. These differences are consistent with the 3′-end of the tRNA existing in different states immediately prior to peptide bond formation possibly reflecting that conformational changes are induced in the aminoacyl-tRNA and/or the ribosome as a result of EF-Tu-dependent GTP hydrolysis [57].

2.3. Hybrid A/P′ site

The activated complex decays after peptide bond formation and the peptidyl-tRNA enters the relatively stable A/P′-site. Moreover, when peptidyl-tRNA is added to ribosomes carrying deacylated-tRNA in the P/P′-site, it does not bind to an A/A′-site but rather enters the A/P′-site directly. The latter hypothesis was established using an in vitro system where translocation was inhibited by adding viomycin or kanamycin; both AcPhe-tRNA and AcPhePhe-tRNA reacted with puromycin when they were bound to ribosomes carrying deacylated-tRNA in the P/P′ site [5–7]. The result was confirmed by a cross-linking experiment in which $(2N_3A_{76})tRNA^{Phe}$, bound at either the P/P′-site or the presumed A/A′-site (with deacylated-tRNA in the P/P′-site), was shown to cross-link to the same two 23S rRNA fragments, F1 (positions 2570–2590) and F2 (positions 2500–2528) [28] (Fig. 2). This result is consistent with the affinity labelled A_{76} of the tRNA being located at closely similar positions within the P′-site implying, in turn, that the affinity labelled tRNAs occupy the P/P′ and A/P′-sites, respectively.

2.4. The P/P′ site

The P/P′ site becomes filled by peptidyl-tRNA during translocation, a process involving movement of the anticodon arm-mRNA complex, relative to the 30S subunit [4–7] that is mediated by the [EF-G-GTP] complex that, alternatively, replaces ternary complexes on the ribosome [27,57–59]. The site can be filled specifically and efficiently by tRNA in vitro and, according to the hybrid state model, at least one component of the site (P or P′) is always occupied during the elongation cycle (Fig. 1). All three functional forms of tRNA (aminoacyl-tRNA, peptidyl-tRNA and deacylated tRNA) have a higher affinity for this site (10–50-fold, depending on the magnesium concentration) than for the A/P′-site [55,60–65]. The relative binding affinities are: deacylated tRNA > Phe-tRNA > Ac-Phe-tRNA [55,64] which also correlates with the observation that the 3′-pentanucleotide fragment CACCA-Phe binds at least as strongly as its acetylated form [37]. Importantly, the affinity of the 3′-end of the tRNA for this site on the free 50S subunit (for both Phe-tRNA and pentanucleotide fragments) is undetectable in aqueous solution but is enhanced by at least 3–5 orders of magnitude in the presence of 20–50% alcohol [15,66]. Thus, for yeast deacylated-$tRNA^{Phe}$, bound at the P/P′-site in the presence of poly(U), the CCA-end contributes only about 17% of the total standard free energy change of the tRNA-ribosome interaction (53.6 kJ/mol) divided as follows: A_{76} 2.4 kJ/mol, C_{75} 3.0 kJ/mol, C_{74} 3.9 kJ/mol [67], while for the peptidyl-tRNA, this contribution is likely to be even less because of its lower overall affinity for the P/P′-site

and since the peptide moiety is unlikely to weaken the binding.

Various lines of evidence indicate that the P′-site is composed primarily of rRNA. These include the inactivation of peptide bond formation by RNase T_1 treatment [68] and fluorescence quenching effects on dyes attached near the 3′-end of $tRNA^{Phe}$ [69]. Furthermore, studies on donor-substrate specificity have revealed a minimal requirement for an N-blocked CCA-aminoacyl fragment, although the very low activities observed for N-blocked di- and mononucleotide substrates [70,71] suggest that they carry residual structural and functional specificity. Interactions of the 3′-terminal CC sequence of the peptidyl-tRNA probably contribute to the overall specificity, since both cytosine residues are protected against chemical modification by the 50S subunit when deacylated-$tRNA^{Phe}$ is bound in the putative P/P′-site (see below) in the presence of poly(U) [30,72]. Moreover, the CCA sequence has been implicated by footprinting experiments in binding to two distinct sites on 23S-like rRNA: the dinucleotide G-G_{2253}, and nucleotides U_{2585}, U_{2584} and U_{2506} clustered at the base of the peptidyl transferase loop [49] (Fig. 2). The latter results are consistent with the "zero-length" cross-links induced by ultraviolet radiation between P/P′-site-bound $(2N_3A_{76})tRNA^{Phe}$ and 23S rRNA fragments F1 (positions 2570–2590) and F2 (positions 2500–2528) [28] (Fig. 2). For the G-G_{2253} sequence, evidence for a base-pairing interaction forming in vitro between C_{74} (in the conserved sequence C_{74}-C-A of P′-site-bound tRNA) and G_{2252} was obtained [73], and confirmed [74], using a compensatory base change approach. In contrast, no evidence was found for Watson-Crick pairing in the lower part of the peptidyl transferase loop, using either rRNA mutagenesis approaches [74,75] or a similar compensatory base change approach [74], although the latter data were compatible with a Hoogsteen pair forming between A_{76} of the tRNA and U_{2585} [74].

Interpretation of these results is complicated by the strong evidence for AcPhe-tRNA and deacylated tRNA binding in different states within the putative P/P′-site of ribosomes which is outlined below.

(1) Affinity constants and other thermodynamic and kinetic parameters are different for ribosomal binding of AcPhe-tRNA and deacylated tRNA, as is the Mg^{2+} concentration dependence of their binding in the absence or presence of mRNA [64]. For example, in the absence of poly(U), the slope of the dependence curve for log K_a (association constant for tRNA binding) versus log $[Mg^{2+}]$ was 8 for AcPhe-tRNA and 3 for deacylated-tRNA; while in the presence of poly(U) the slope was 4 for AcPhe-tRNA and 5 for deacylated-tRNA [64].

(2) Energy transfer measurements for different fluorescence probes attached to ribosome-bound tRNAs and fluorescein acceptor probes attached to proteins S21, L1 or L11, as well as differences in the quantum yields of fluorescence for the tRNA-bound probes, all indicate that AcPhe-tRNA and deacylated-tRNA are located in different structural environments [76–78]. Moreover, the results were similar when deacylated-tRNA was bound directly to the P/P′-site or when it entered this site via the peptidyl transfer reaction.

(3) AcPhe-tRNA carrying 8-N_3A at position A_{73}, which was bound at the P/P′-site, cross-linked primarily to 23S rRNA and to a lesser degree to proteins L2 and L27 on ultraviolet irradiation, whereas deacylated-tRNA carrying an 8-N_3-adenosine at either position A_{73} or A_{76} cross-linked exclusively to protein L27 [79].

(4) The chemical footprints produced by AcPhe-tRNA and deacylated-tRNA on 23S rRNA and 16S rRNA are not identical [4,49]. Thus, A_{2439} and A_{2451} were protected on 23S rRNA by P/P′-site-bound AcPhe-tRNA but not by deacylated-tRNA, while the reactivity of A_{2602} was enhanced only by AcPhe-tRNA binding [49]; these different reactivities may, or may not, be induced by the N-blocked aminoacyl-moiety. Similarly, for 16S RNA, deacylated-tRNA produced stronger protection of G_{1338} and higher reactivity at A_{702} compared with AcPhe-tRNA [4].

One interpretation of these differences is that the 3′-end of peptidyl-tRNA binds, as the donor substrate, in the P′-site while the 3′-end of deacylated tRNA occupies a site intermediate between the P′- and E-sites with the remainder of both tRNAs occupying the same, or strongly overlapping sites on the 30S subunit. This interpretation would also explain the observation that deacylated-tRNA (the product of peptidyl transfer) has a higher affinity than peptidyl-tRNA (the substrate of peptidyl transferase) for the presumed P/P′-site, i.e. the former is partially displaced from this site.

An alternative explanation is that the 3′-end of peptidyl-tRNA moves from the P′-site after the aminoacyl-tRNA is released from the ternary complex (or bound non-enzymatically), and immediately prior to peptidyl transfer, i.e. during formation of a short lived transition state for peptide bond formation (Fig. 3). This scenario would provide a rationale for the apparent inconsistency between the low contribution of C_{74} to the free energy of tRNA binding at the P/P′-site (<1 kcal/mol) [67] and the three hydrogen bonds ($\sim$4–5 kcal/mol) required for the above-mentioned putative Watson-Crick base pair interaction between C_{74} and G_{2252} [73]. Thus, the latter interaction may constitute a short-lived interaction important for the formation of the transition state. After peptide bond formation, the activated complex would decay leaving the peptidyl-tRNA in the A/P′-site and deacylated tRNA in the P/E-site.

2.5. Hybrid P/E-site

Substantial movement of the 5′-end of the peptidyl-tRNA occurs, relative to the ribosome, concomitantly with peptide bond formation [76,78,80] that corresponds to movement of the newly deacylated tRNA from the P/P′- to the P/E-site. This movement results in the disappearance of a P/P′-site footprint on 23S rRNA (strong protection of G_{2252}, G_{2253}, U_{2506}, U_{2584}, U_{2585}) and the appearance of an E-site footprint (strong protection of C_{2394}, weak protection of G_{2112}, and G_{2116}), while the corresponding footprint on the 16S rRNA remains unchanged (protection of G_{693}, A_{794}, C_{795} and G_{926}) [4].

2.6. The E-site

During translocation, the deacylated tRNA enters the E-site [12–14,81–83], which is specific for this tRNA and is located primarily, or exclusively, on the large subunit [13–15,84,85]. Deacylated-tRNA binds at least 2000 times more strongly to the E-site (on either 50S subunits or 70S ribosomes) than to the P′-site, on the free 50S subunit, where binding is only detectable in water-alcohol mixtures [15]. Moreover, it resides there transiently before leaving the ribosome after translocation [85,86]. The following circumstantial evidence suggests

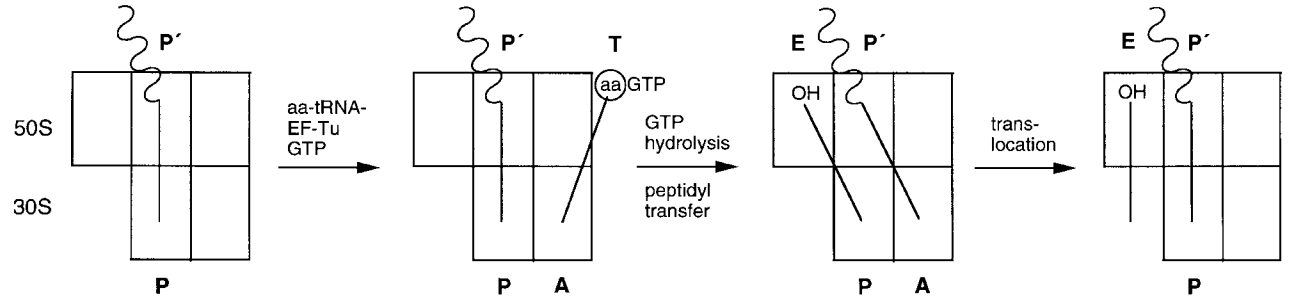


Fig. 1. The hybrid state model for peptide elongation is presented in a schematic form, modified from [4], where the sequential binding sites of tRNA during peptide bond formation are illustrated. This version of the model assumes that no major dislocation of the subunits occurs during the elongation cycle and a discrete A/A′-binding site is omitted (see text for discussion).

that the E-site substrate interacts with rRNA: (1) the 3′-terminal adenosine is important for ribosomal binding [13,86]; (2) chemical probing of E-site-bound deacylated tRNA revealed protection effects on the 23S rRNA (see above), and (3) deacylated $(2N_3A_{76})$tRNA^{Phe} bound to ribosomes programmed with poly(U) when the P/P′-site, and putative A/A′-site, are occupied by two copies of AcPhe-tRNA^{Phe}, cross-linked specifically to fragment F3 of 23S rRNA (positions 2300–2360, Fig. 2A) [28]. Since fragment F3 falls in the rRNA region where the 5S rRNA-L18,L5 complex attaches [87], and yields a footprint [88], on the 23S rRNA, the long sought after function of 5S rRNA may be to mediate movement of the 3′-end of tRNA.

Surprisingly, when $(2N_3A_{76})$tRNA^{Phe} was bound directly to either 50S subunits or 70S ribosomes, where the P/P′-site was occupied by deacylated-tRNA^{Lys} coupled to poly(A), no cross-links to the 23S rRNA were detected. However, both complexes produced cross-links to protein L33, and it yielded additional cross-links between azidoadenosine, at position 37 (substituted wyosine), and protein S11 and the 3′-end of 16S RNA [28,89]. These complex cross-linking patterns may reflect binding to different states within the E-site or to sites intermediate between the P/P′- and E-sites.

3. The nascent peptide

Fluorescence data have provided strong evidence that the nascent peptide moves away from the catalytic centre concomitantly with each round of peptide bond formation [76,78]. The peptide passes along a passage that constitutes either a tunnel through the large subunit [90–92] or a surface channel [93], and the nascent protein assembles on the outer surface of the larger subunit [94,95]. Some progress has been made in mapping the sequential ordering of ribosomal components along the peptide passage; in particular, proteins L2 and L16 are located near the entrance to the passage ([96] and references therein). Some nascent homopolypeptides do not enter this passage and they include polyphenylalanine that forms a hydrophobic aggregate in the immediate vicinity of the peptidyl transferase centre [97–100]. This observation complicates the interpretation of in vitro assays for both peptide elongation and its inhibition by antibiotics.

4. The peptidyl transferase loop and its functional sub-sites

The peptidyl transferase centre on the large subunit [17] is rich in rRNA and includes the highly conserved central loop region in domain V of the 23S-like rRNA [101], although the G-G$_{2253}$ region of domain V [73] and a highly conserved region of domain IV [54,102–105] also contribute to the centre. There are also indications that 23S rRNA contributes directly to the catalytic process [106] and although no catalytic groups have been identified, the possible involvement of a 5′-terminal phosphate group [107] or a pseudouridine residue (e.g. at positions 2554 and 2580, Fig. 4A) has been considered (reviewed in [108]).

An attempt was made, recently, to define functional subsites within the peptidyl transferase loop region [51,53]. Data from various sources was utilised including: (a) footprinting data obtained from chemically altered tRNAs complexed with ribosomes [49]; (b) location of antibiotic sites within the peptidyl transferase centre [50–52], and (c) literature data on the modes of action of antibiotics [109–111]. The working model

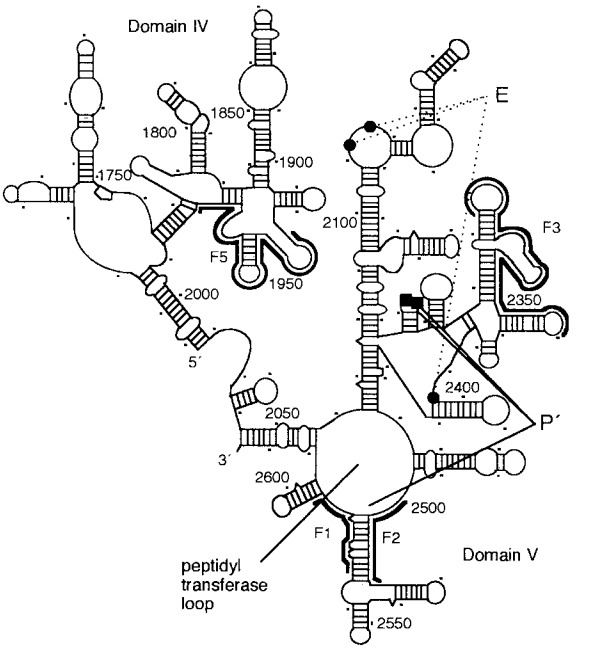


Fig. 2. Regions of 23S rRNA implicated in the binding and/or movement of the 3′-end of tRNA at the peptidyl transferase centre. An outline of the secondary structure of domains IV and V of *E. coli* 23S rRNA are shown that generate the peptidyl transferase centre. RNA regions that have been implicated in the binding of the 3′-ends of tRNAs bound at the P′-site or E-site are indicated [49], as are the rRNA fragments F1, F2, F3 and F5 that are cross-linked to the affinity labelled $(2N_3A_{76})$tRNA^{Phe} [28] bound in different tRNA sites (see text for details).

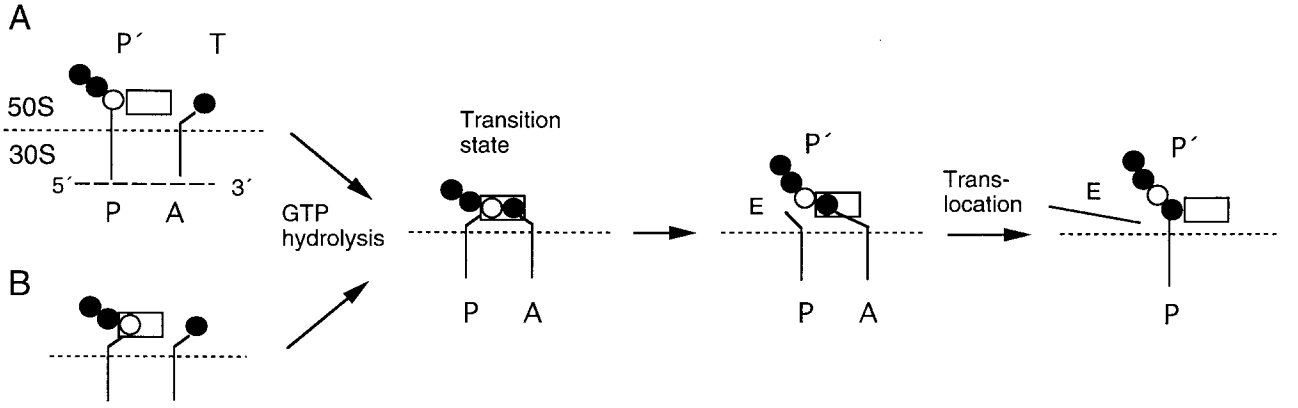


Fig. 3. Schematic representation of a model for different binding states of the 3′-end of the donor substrate within the P′-site, where a distinction is made between an initial binding state and an activated form in the transition state during peptide bond formation. In A the peptidyl-tRNA resides in an initial binding site and is activated on release of aminoacyl-tRNA from the ternary complex whereas in B the peptidyl-tRNA binds initially in the activated state. The A- and P-sites on the small subunit are indicated, as are the T-, P′- and E-sites on the large subunit (see also Fig. 1). Amino acids are represented by filled circles while the donor substrate aminoacyl residue is depicted as an open circle; the catalytic centre is boxed.

is presented in Fig. 4A alongside data relating to the binding of antibiotics in the peptidyl transferase centre (Fig. 4B). It remains tentative given the high degree of structural and functional cooperativity that occurs within this centre. The assigned sub-sites include the P′- and E-sites and the entrance to the peptide channel, mentioned above; others include (a) the assignment of A_{2439} close to the catalytic centre based on its likely protection by the aminoacyl residue of the peptidyl-tRNA [32,49] and its reactivity being affected by many peptidyl transferase antibiotics [51], and (b) a hydrophobic site for binding aromatic aminoacyl residues and possibly aromatic antibiotics (see below) centred on A-C_{2452} [51].

The peptidyl transferase centre may be physically shielded during peptide elongation in order to protect the ester bond, which links the nascent peptide to its tRNA, from hydrolysis by small nucleophilic molecules [9]. That such strong hydrolysis can occur was demonstrated for deacylated tRNA, or a -CCA fragment, bound to ribosomes carrying P/P′-site-bound fMet-$tRNA^{fMet}$, under the solution conditions of the 'fragment' reaction that includes 20% ethanol; fMet-ethyl ester was produced, presumably catalysed by the peptidyl transferase [112]. A similar reaction occurs for aminoacyl-oligonucleotides bound to free ribosomes [46]. In the former study, both the rate and yield of the reaction was strongly reduced in the presence of aminoacyl-tRNA (when peptide bond formation occurs), and by peptidyl transferase inhibitors including amicetin, chloramphenicol and sparsomycin [112]. This suggests that the aminoacyl moiety, and the antibiotics, can, directly or indirectly, protect the ester bond of the fMet-tRNA. These results provided the basis for a model [9] in which the 3′-end of the aminoacyl-tRNA, and some antibiotics, bind to the ribosome and induce opening of the catalytic centre, and facilitate their own access to the catalytic centre which then closes, thereby effecting protection of the ester bond. A more static view of the observed ethanolysis is that a deacylated tRNA, anchored at the A(30S)-site, stabilises an ethanol molecule in the acceptor substrate position that can hydrolyse the ester bond of the peptidyl-tRNA and that can also be displaced by adding aminoacyl-tRNA or certain antibiotics.

5. The peptidyl-transferase antibiotics

Peptidyl transferase antibiotics are paradoxical in that they show a diverse range of chemical structures, yet appear to influence one catalytic centre on the large subunit [109–111]. However, the catalytic reaction is unusual; the substrates – the amino group of the incoming aminoacyl-tRNA and the carbonyl group of peptidyl-tRNA – are connected to complex tRNA molecules which have much stronger interactions with the ribosome-mRNA complex than do the substrates themselves at the catalytic centre [18]. Consequently, any antibiotic that interacts with any part of the tRNA – or interferes with the tRNA-ribosome interactions that are important for tRNA and/or ribosomal movement – can, in principle, produce distortions at the CCA-end of the acceptor or donor substrates and, thereby, inhibit the formation of a peptide bond. Thus, there could be as many different antibiotic binding sites, and inhibitory mechanisms, as there are different antibiotics.

Designating the inhibitory mechanisms of peptidyl transferase antibiotics has, to a large degree, been based on their capacity to prevent puromycin reacting with either *N*-acetylated aminoacyl-tRNA or, more commonly, the truncated 3′-terminal *N*-acetylated aminoacyl-CACCA fragment. However, this reaction, in itself, is problematic, first, because it is multi-step, both spatially and temporally [113] and, second, because binding of the donor fragment requires the presence of about 30% ethanol [36,114] which, in turn, weakens puromycin binding [115].

Another approach has been to study competitive binding between drugs and aminoacyl-CACCA fragments for a putative A′-site. For example, it was shown that many peptidyl transferase antibiotics inhibited binding of CACCA-Phe to ribosomes and they were designated A′-site inhibitors [113]. However, the pentanucleotide (and trinucleotide) fragments, carrying amino acids, can bind with an affinity similar to their *N*-acetylated derivatives to the P′-site [37] and, moreover, two copies of CACCA(3′NH)Phe were shown to bind cooperatively to the ribosome [116]. The latter observations invalidate earlier interpretations of the competition binding experiments between drugs and aminoacyl-CACCA fragments. A further

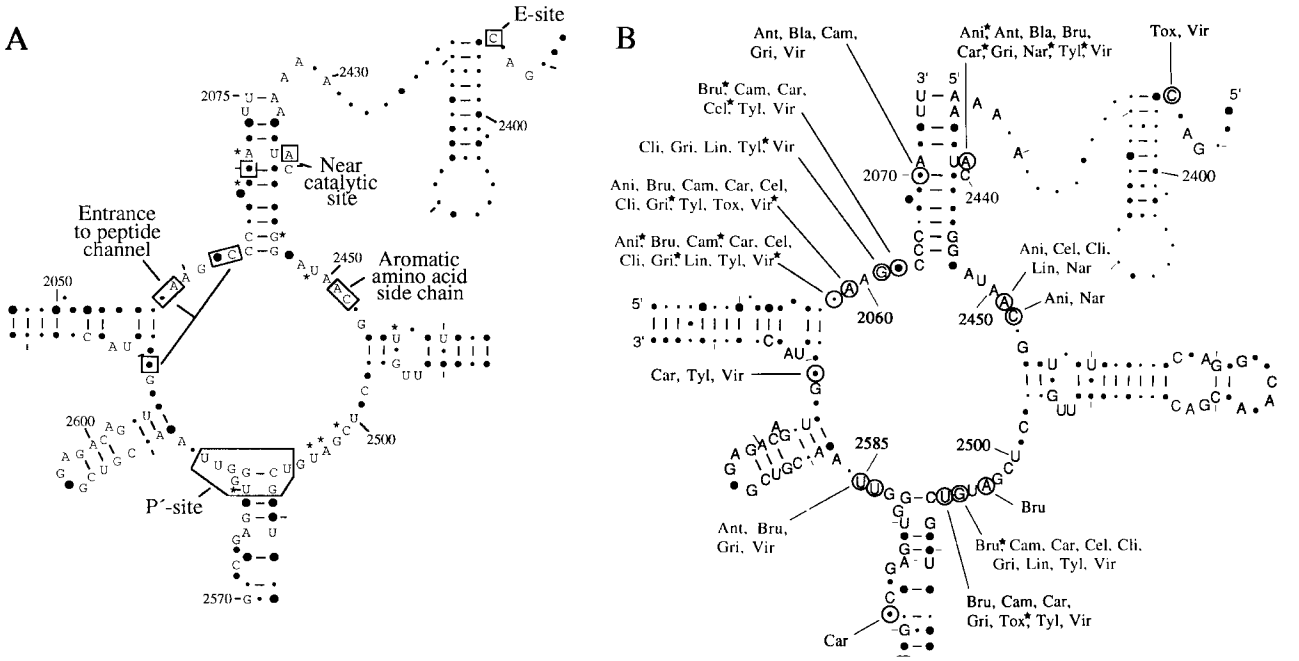


Fig. 4. A: Locations of putative sub-sites in the peptidyl transferase loop region, superimposed on a conservation plot of the secondary structure [51,53]. Assignments are based on the experimental and literature data that are cited in the text. Letters indicate universally conserved nucleotides, large filled circles denote highly conserved nucleotides and smaller ones indicate less conserved positions [51]. The nucleotide numbering system of *E. coli* is used and modified nucleotides, known to be present in *E. coli* 23S rRNA, including pseudouridines at positions 2457, 2504, 2580 and 2605, are indicated by asterisks. B: Summary of all the nucleotides that exhibit altered chemical reactivites in the presence of antibiotics within the peptidyl transferase loop region of *E. coli, Haloferax mediterranei* and/or *Saccharomyces cerevisiae* ribosomes [50,51,146–148]. Asterisks denote enhanced reactivities while all other effects correspond to reduced reactivities. Locations of the effects are indicated on a conservation plot of the secondary structure. Full names of the antibiotics are: Ami, amicetin; ani, anisomycin; ant, anthelmycin; bla, blasticidin S; bru, bruceantin; cam, chloramphenicol; car, carbomycin; cel, celesticetin; cli, clindamycin; ery, erythromycin; gri, griseoviridin; lin, lincomycin; tox, toxin T2; tyl, tylosin; vir, virginiamycin M_1.

complication, especially for the early experiments, is that substantial amounts of peptidyl-tRNA remained bound to ribosomes despite multiple high salt washes [46,117]. This can often be seen for antibiotic inhibition studies where sparsomycin was tested since it only binds to a ribosome when peptidyl-tRNA is bound [118]. Moreover, in many experi-

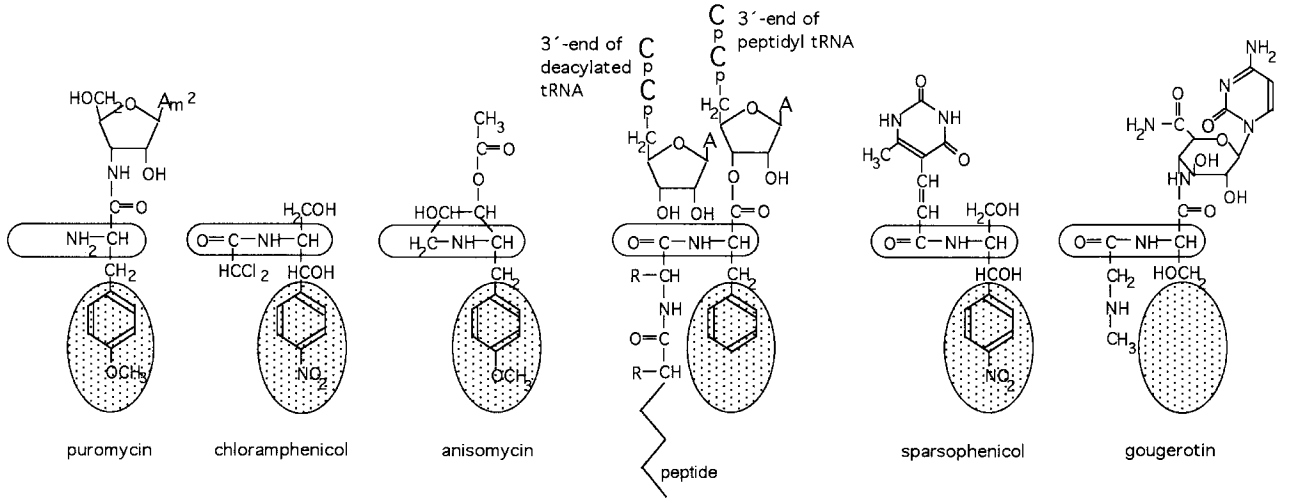


Fig. 5. Schematic representations showing how certain regions of the peptidyl transferase antibiotics may be iso-structural both with one another and with the 3′-end of the aminoacyl-tRNA in the transition state for peptide bond formation. Each of these drugs, except gougerotin, binds competitively to the ribosome with the other drugs and interferes with aminoacyl-tRNA binding and/or movement on the ribosome. Some of these structures were presented earlier [125,164]. For simplification some carbon-carbon bonds are shown as lines and their bound H-atoms are omitted. The shaded oval regions correspond in position to the aromatic ring of the phenylalanine component, and acceptor substrate, of Phe-$tRNA^{Phe}$ that is illustrated at the centre of the diagram; this aromatic moiety is common to all of the drugs shown except gougerotin. The latter is included as an example of a non competitive antibiotic that also impedes movement of the aminoacyl-tRNA into the catalytic centre [109–111]. The region corresponding to the peptide bond is also boxed. Sparsophenicol is a hybrid of sparsomycin and chloramphenicol and its antibiotic properties are described in [165].

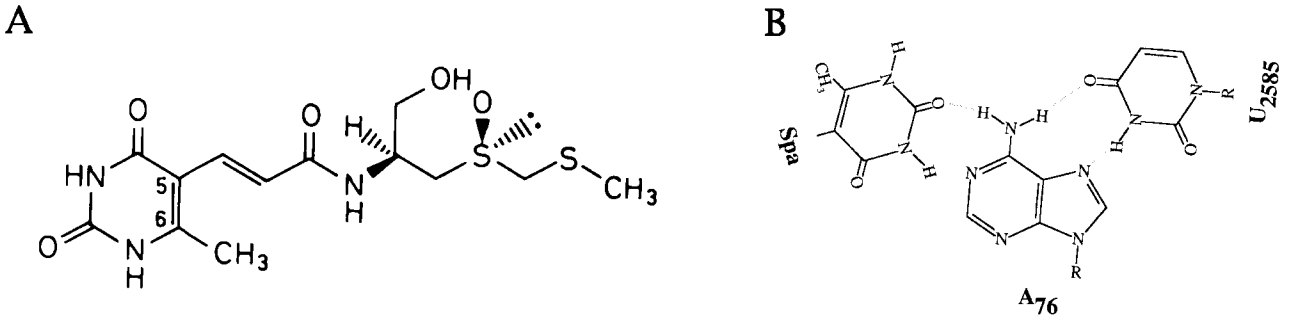
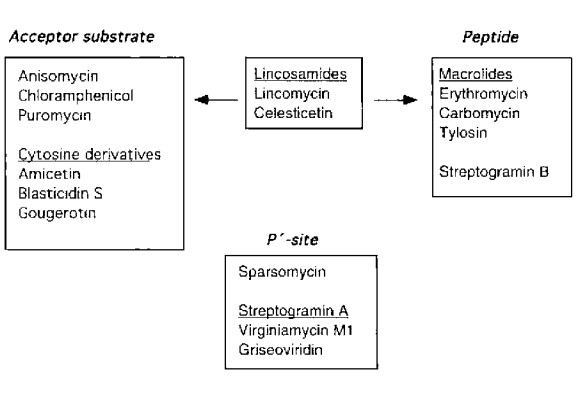


Fig. 6. A: Structure of sparsomycin; B: a model for how the modified uracil of sparsomycin may generate a base triple, together with the putative A_{76}-U_{2585} Hoogsteen pairing between peptidyl-tRNA and 23S-like rRNA [74], which could explain the drug-induced stabilization of the P′-site substrate within the peptidyl transferase centre.

ments, attempts were made to remove residual peptidyl-tRNA by incubating ribosomes with puromycin (e.g. [46]), however, this treatment may leave deacylated tRNA bound in the P/E-site and, possibly, peptidyl-puromycin chains trapped in the peptide passage. Therefore, few of these experiments are amenable to straightforward reinterpretation in terms of the hybrid state model.

Nevertheless, there are strong indications that peptidyl transferase antibiotics can be classified into a few major structural and functional categories. Two of the best characterised groups include (a) those drugs that are to some degree co-structural with the 3′-end of aminoacyl-tRNA and prevent it forming a peptide bond and (b) those that perturb the movement and/or positioning of the nascent peptide (see Table 1). In general, antibiotics can be assigned to one of these two groups on the basis of their capacity to bind to polysomes because, according to the hybrid state model, polysome-bound tRNAs occupy either the hybrid A/P′- and P/E-sites or the P/P′-site i.e. the P′-sites and some of the E-sites on the large subunits will be filled. Therefore, drugs that affect the region through which the acceptor substrate passes, prior to peptide bond formation, should bind while those that act primarily in the peptide binding region, or at the P′-site (and possibly the E-site), should neither bind to polysomes nor compete with puromycin. Examples of these two groups of drugs are given in Table 1.

Table 1
Examples of different antibiotics that perturb and/or inhibit the movement of the 3′-end of tRNA through the peptidyl transferase centre



The likely sites of perturbation, acceptor substrate, P′-site or nascent peptide are indicated in italics.

5.1. Antibiotics primarily affecting peptide bond formation

Many drugs, including puromycin, are to some degree co-structural with the 3′-end of aminoacyl-tRNA (Fig. 5) and have been considered to bind to the ribosome in competition with aminoacyl-tRNA analogues and some, but not all, bind competitively with one another [46,119]. They include anisomycin, chloramphenicol and the modified cytosine drugs althiomycin, amicetin, blasticidin S and gougerotin (Fig. 5), and they have generally been considered to block, directly or indirectly, the binding of the 3′-end of aminoacyl-tRNA to an A′-site [109–111,120–125]. More recently, in the context of the hybrid state model, it has been considered that they might act primarily by blocking movement of the 3′-end of the aminoacyl-tRNA immediately after release from the ternary complex [9,126]. This hypothesis is consistent with the observation that these drugs bind to, and inactivate, isolated polysomes where residual peptidyl-tRNAs are bound in the A/P′- or P/P′-sites with deacylated-tRNAs in the P/E-site or dissociated from the E-site, respectively [127].

5.1.1. Chloramphenicol. Chloramphenicol is generally represented as a competitor, and structural analogue, of puromycin (Fig. 5) and is assumed to interfere with the 3′-end of the acceptor substrate [41]. Various binding studies reinforce this view although the partial competition, often observed, leaves some doubt as to whether this is an exclusive inhibitory site for the drug [36]. It was demonstrated earlier that chloramphenicol has two binding sites on 50S subunits, a stronger one ($K_d = 2\times10^{-6}$ M) and a weaker one ($K_d = 2\times10^{-4}$ M) [36]. Although only the stronger bound drug molecule inhibits the fragment reaction between CACCA-Leu-Ac and puromycin [41]; this does not exclude the possibility that chloramphenicol can inhibit by binding at another site which overlaps with that of erythromycin. In favour of this view is (a) the observation that a G2057A mutation in the peptidyl transferase loop confers resistance against both chloramphenicol and erythromycin (but not other macrolides) [128], (b) the finding that erythromycin competes with chloramphenicol for binding to 70S ribosomes but cannot displace chloramphenicol from polysomes (to which only chloramphenicol binds) [127], (c) like erythromycin, chloramphenicol destabilises peptidyl-tRNA binding, enhancing the release of $Ac(Phe)_2$-tRNA and Lys_2-tRNA from the ribosome [129–131].

A possible explanation for these effects, given the lack of direct evidence for two different binding sites, is that chlor-

amphenicol can remain bound on the ribosome to alternative, and mutually exclusive, conformers within the peptidyl transferase loop region [66] and this could also explain: (a) why single-site mutations of at least 10 different nucleotides in the peptidyl transferase loop region can confer chloramphenicol resistance [101] and (b) why chloramphenicol produces partial protection of at least five nucleotides within this region against chemical modification (Fig. 4B) at positions that show minimal overlap with those produced by puromycin [52].

5.1.2. Sparsomycin. Sparsomycin is a highly potent, broad spectrum, antibiotic that strongly inhibits the 'fragment' assay for peptide bond formation [132]. It binds to the ribosome only when the P/P′-site is occupied by an N-blocked substrate, i.e. it does not inhibit formation of the first peptide bond on the eukaryotic ribosome [118]. It stabilises, and enhances, the binding of the *N*-blocked donor substrate in the P′-site [133–135] and destabilises, in some unknown way, binding of the ternary complex [113,136]. Binding studies with various sparsomycin derivatives revealed two important groups, at either end of a fairly rigid molecule (Fig. 6A), that interact with the ribosome-tRNA complex [137,138]; an S-CH_3 group and a methylated uracil residue that carries a pseudouridine-like linkage to a peptide-like grouping [139]. Although the modified uracil can potentially base pair with rRNA, in contrast to the results obtained for many other peptidyl transferase drugs, footprinting studies failed to reveal evidence of an rRNA binding site for the drug [51,135]. Evidence for an rRNA interaction is less direct and rests mainly on the observation that single-site mutations within the peptidyl transferase loop of archaeal 23S-like rRNAs can confer sparsomycin resistance [140,141]. These mutated sites correspond to lack of a methyl group on U_{2584} and a C2499U mutation, in haloarchaeal 23S rRNA, and they are located within, or near, the P′-substrate site (Fig. 4A) consistent with sparsomycin affecting primarily this site.

The turnover of aminoacyl-tRNA is increased in the presence of sparsomycin [136] and this may reflect hindrance, direct or indirect, of the binding of the ternary complex and/or access of the 3′-end of the aminoacyl-tRNA to the catalytic centre. The most likely interpretation of this is that it overlaps into the acceptor substrate domain of the peptidyl transferase centre, mimicking to some extent, the structure of the terminal adenosine and amino acyl residue (see sparsophenicol structure in Fig. 5). This is consistent with it binding to polysomes [66] and explains its competing surprisingly strongly with puromycin, as well as with chloramphenicol, lincomycin and the larger (16 carbon ring) macrolides such as tylosin [118] that appear to extend into the catalytic centre (see below).

The experimental evidence suggests that the drug has a fairly intimate interaction with the 3′-end of peptidyl-tRNA on the ribosome and the universality of the drug action points to highly conserved interactions. Both U_{2585} and some neighbouring nucleotides, as well as in the 3′-end of tRNA are universally conserved [74]. Whether the blocked amino group of the first amino acid is important for the drug interaction or for fixing the first aminoacyl residue in the catalytic centre is unclear. There is some evidence in support of the latter [141] that does not necessarily exclude the former. Moreover, the observation that an amicetin-resistant mutant, carrying the change C2438U, is also sparsomycin-resistant [9] may support this. A possible interaction between the methylated uracil residue, A_{76} of the peptidyl tRNA and U_{2585} of the 23S-like rRNA is presented (Fig. 6).

5.1.3. Other peptide bond inhibitors. There are many other antibiotics that affect peptide bond formation, directly or indirectly, some of which are indicated in Fig. 4B, for which relatively little is known about their mechanism of inhibition. These include the alkaloid drugs bruceantin and narciclasine, as well as toxin T2. The streptogramin A drugs, however, constitute an important group. They inhibit elongation cooperatively with streptogramin B drugs which are related, in their mode of action, to the macrolides and lincosamides [142]. Paradoxically, prebound streptogramin B prevents binding of streptogramin A [110,111,142] which led to the suggestion that the latter may enter the catalytic cavity via the peptide passage [9]. Moreover, fluorescence measurements suggested that it acts primarily within the P′-site [143]. The RNA footprint produced by streptogramin A (virginiamycin M_1) and the related griseoviridin (Fig. 4B) are very complex suggesting that they produce substantial changes in the peptidyl transferase loop region. One of the sites affected by streptogramin A is exceptional in that it occurs at C_{2394} that has been implicated in the E-site (Fig. 4A). Thus, streptogramin A may act by preventing movement of peptidyl-tRNA from the P/P′-site to the P/E-site. Clearly, further experimental work is necessary on the mechanism of action of these drugs now that we have an improved model for peptide elongation.

5.2. Antibiotics primarily affecting the nascent peptide

Another large group of antibiotics, again with diverse structures, consists of the macrolides, lincosamides and streptogramin B (MLS) drugs. The macrolide and streptogramin B drugs, at least, appear to share overlapping binding sites near the entrance to the peptide passage (Fig. 4A), where they probably hinder movement, and/or perturb binding, of the peptide moiety of the peptidyl-tRNA, a process which may also lead to destabilisation of the peptidyl-tRNA bound in the P/P′-site. The primary evidence for their sharing common binding sites around nucleotides 2057–2062 and 2609–2611 at the start of the peptide passage is as follows. (1) Organisms producing macrolides and lincosamides protect their own ribosomes by N^6-mono- or di-methylated A_{2058} [25,144]. (2) Several single-site mutations in this region confer macrolide resistance (reviewed in [145]). (3) RNA modification studies yield altered reactivities in the presence of macrolides, especially in the region A_{2058} to A_{2062} [101,146–148] (Fig. 4B). (4) Finally, peptidyl-tRNAs carrying affinity-labelled peptides of length 1–4 amino acids cross-link to positions A_{2609} and A_{2062} [96]. These results are reinforced by footprinting of naturally occurring peptides that attenuate translation and bind competitively with erythromycin; protection was seen at positions 2058, 2059 and 2060 [149]. It is likely that A_{2058} occupies a key position in this site and since its reactivity is affected by both MLS antibiotics and by others (anisomycin, chloramphenicol) that probably do not act directly in the peptide channel, it is likely to be pivotal in determining the local conformation. It is also universally conserved amongst bacteria and only organisms from this domain of life are strongly susceptible to this group of drugs.

In general, the MLS drugs appear to inhibit movement of the nascent peptide after a few rounds of peptide bond for-

mation [150] but cannot inhibit when the peptide has reached a certain length in vivo [151]. Consistent with these observations, the drugs do not inhibit elongation on isolated polysomes where the A/P′- or P/P′-sites are filled, and where the start of the peptide passage is occupied by the nascent peptide [66,127,152]. However, the individual inhibitory mechanisms may be more complex and dependent, to some extent, on the size of the drug. The larger macrolides (e.g. carbomycin) are more effective inhibitors of the peptide-puromycin reaction than the smaller ones (e.g. erythromycin) [153], which may reflect the fact that they can extend into, and perturb, the catalytic centre. There are also strong indications that the lincosamides may behave similarly [142]. The primary inhibitory mechanisms of blocking movement of the peptide into the peptide passage would also provide an explanation for other inhibitory functions attributed to the drugs earlier including both destabilisation of P/P′-site bound peptidyl-tRNAs that have been observed both in vitro and in vivo [154,155] and the inhibition of the translocation reaction from A/P′- to P/P′-site. This inhibitory mechanism is consistent with the observation that only a small fraction of the total free energy of tRNA-ribosome interactions derives from binding of the 3′-end of the tRNA [67,156–158].

5.3. *Insight gained from kinetic studies of antibiotic-ribosome binding*

Comprehensive kinetic studies have been performed on the inhibitory mechanisms of many peptidyl transferase drugs. For example, the modified nucleoside antibiotics amicetin, blasticidin S and gougerotin, as well as chloramphenicol, produce biphasic curves for inhibition of peptide-bond formation when puromycin is used as an acceptor substrate in the presence of the donor substrate *N*-acetylated Phe-tRNA. An initial competitive step (high K_i) is followed by a mixed non-competitive step (low K_i) [66,159–162]. For chloramphenicol, the shape of the inhibition curve is sensitive to ionic strength and incubation temperature [163], which implies that competitive and non-competitive inhibition arise from conformational heterogeneity near the catalytic centre; this, in turn, could reflect the stabilising of different ribosomal conformers that form during the elongation cycle.

In general, these effects have been attributed to a two-step reaction between the drug and ribosome. This involves (i) initial, rapid binding of the drug, near the catalytic centre, producing a complex where the drug displays competitive inhibition kinetics of peptide bond formation, followed by (ii) a slow conformational change of the drug–ribosome complex, yielding an inactive ribosomal complex. In the latter step, the drug is considered to stabilise a ribosomal conformer that is non-functional because it is reached so slowly (i.e. with a half-time of several minutes).

This interpretation is consistent with the drugs binding initially at a site through which the acceptor end of the aminoacyl-tRNA passes on release from the ternary complex and prior to peptide-bond formation (Section 2.2). In this state, each drug can bind competitively with the acceptor substrate and with other drugs. The subsequent slow change that occurs may correspond to a drug-induced change in the conformation of the 23S rRNA, possibly involving an increased opening or accessibility of the catalytic centre. During this change the drug could move closer to, or enter, the catalytic centre, thereby producing an inactive ribosome and non-competitive kinetics of drug binding. The model [9] is consistent with the kinetic data, in that it is quite reasonable that the latter should be a slower process for a drug (a partial analogue) than for the aminoacyl-tRNA, and equally reasonable that many drugs of diverse structure could act in this way.

6. Conclusion

A model of elongation is emerging in which the ribosome sequentially assumes the correct topography for binding the different tRNA substrates. This process places fairly narrow limits upon the energy of binding at each site, and upon the freedom of movement of the bound substrate. Above, we have tried to summarise some of the interactions, movements and possible conformational changes that precede and proceed peptide bond formation and attempt to explain how some of them may be inhibited by drug action. A major unanswered question concerning the antibiotics is how many independent sites of action they have. We consider here two major regions of the peptidyl transferase centre where overlapping drug sites, and inhibition, putatively occur. (a) A site through which the 3′-end of aminoacyl-tRNA moves after release from the ternary complex and (b) the entrance to the peptide passage. Other sites remain to be designated more precisely including where the streptogramin A-type drugs bind. Nevertheless, for most peptidyl transferase drugs, whatever the detailed mechanism of inhibition, their primary mode of action is probably to prevent either movement of the 3′-ends of the tRNAs through the peptidyl transferase centre, at least partly by trapping a certain RNA conformer, or the movement of the nascent peptide away from the peptidyl transferase centre.

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References

[1] M. Bretscher, Nature 218 (1968) 675–677.
[2] A.S. Spirin, Dokl Akad Nauk 179 (1968) 317–318.
[3] A.S. Spirin, Cold Spring Harbor Symp Quant Biol 34 (1969) 197–207.
[4] D. Moazed, H.F. Noller, Nature 342 (1989) 142–148.
[5] Semenkov YuP, Shapkina TG, Kirillov SV. Leningrad Nucl Phys Inst Acad Sci USSR 1989; Reprint 1495.
[6] Yu.P. Semenkov, T.G. Shapkina, V.I. Makhno, S.V. Kirillov, FEBS Lett 296 (1992) 207–210.
[7] Yu.P. Semenkov, T.G. Shapkina, S.V. Kirillov, Biochimie 74 (1992) 411–417.
[8] C.G. Kurland, M. Ehrenberg, Q Rev Biophys 18 (1985) 423–450.
[9] B.T. Porse, C. Rodriguez-Fonseca, I. Leviev, R.A. Garrett, Biochem Cell Biol 73 (1995) 877–885.
[10] S.V. Kirillov, V.I. Makhno, Yu.P. Semenkov, Nucleic Acids Res 8 (1980) 183–196.
[11] V.I. Katunin, Yu.P. Semenkov, S.V. Kirillov, Nucleic Acids Res 8 (1980) 403–421.
[12] H.-J. Rheinberger, H. Sternbach, K.H. Nierhaus, Proc Natl Acad Sci USA 78 (1981) 5310–5314.
[13] R.A. Grajevskaja, Yu.V. Ivanov, E.M. Saminsky, Eur J Biochem 128 (1982) 47–52.
[14] S.V. Kirillov, E.M. Makarov, Yu.P. Semenkov, FEBS Lett 157 (1983) 91–94.
[15] D.V. Parfenov, E.M. Saminsky, Mol Biol 19 (1985) 1130–1136.
[16] R. Lill, J.M. Robertson, W. Wintermeyer, Biochemistry 23 (1984) 6710–6717.
[17] R.E. Monro, J Mol Biol 26 (1967) 147–151.

[18] S.V. Kirillov, Yu.P. Semenkov, J Biomol Struct Dynam 4 (1986) 263–269.
[19] A.-L. Haenni, J. Lucas-Lennard, Proc Natl Acad Sci USA 61 (1968) 1363–1369.
[20] B. Hardesty, W. Culp, W. McKeehan, Cold Spring Harbor Symp Quant Biol 34 (1969) 331–345.
[21] J.A. Lake, Proc Natl Acad Sci USA 74 (1977) 1903–1907.
[22] H. Yokosawa, M. Kawakita, K. Arai, N. Inoue-Yokosawa, Y. Kaziro, J Biochem 77 (1975) 719–728.
[23] T. Powers, H.F. Noller, Trends Genet 10 (1994) 27–31.
[24] S.N. Vladimirov, D.M. Graifer, G.G. Karpova, Yu.P. Semenkov, V.I. Makhno, S.V. Kirillov, FEBS Lett 181 (1985) 367–372.
[25] Cundliffe E. In: Hill W, Dahlberg A, Garrett RA, Moore PB, Schlessinger D, Warner JR, editors. The Ribosome: Structure, Function, and Evolution. Washington, DC: American Society for Microbiology, 1990:479–490.
[26] A.S. Girshovich, E.S. Bochkareva, V.D. Vasiliev, FEBS Lett 197 (1986) 192–198.
[27] P. Nissen, M. Kjeldgaard, S. Thirup, G. Polekhina, L. Reshetnikova, B.F.C. Clark, J. Nyborg, Science 270 (1995) 1464–1472.
[28] J. Wower, I.K. Wower, S.V. Kirillov, K.V. Rosen, S.S. Hixson, R.A. Zimmermann, Biochem Cell Biol 73 (1995) 1041–1047.
[29] Stöffler-Meilicke M, Stöffler G. In: Hill W, Dahlberg A, Garrett RA, Moore PB, Schlessinger D, Warner JR, editors. The Ribosome: Structure, Function, and Evolution. Washington, DC: American Society for Microbiology, 1990:123–133.
[30] S. Douthwaite, R.A. Garrett, R. Wagner, Eur J Biochem 131 (1983) 261–269.
[31] G. Steiner, E. Keuchler, A. Barta, EMBO J 7 (1988) 3949–3955.
[32] P. Mitchell, K. Stade, M. Osswald, R. Brimacombe, Nucleic Acids Res 21 (1993) 887–896.
[33] R.J. Harris, P. Greenwell, R.H. Symons, Biochim Biophys Res Commun 55 (1973) 117–124.
[34] R.J. Harris, J.E. Hanlon, R.H. Symons, Biochim Biophys Acta 240 (1971) 244–262.
[35] D.J. Eckermann, R.H. Symons, Eur J Biochem 82 (1978) 2256–3234.
[36] J.L. Lessard, S. Pestka, J Biol Chem 247 (1972) 6909–6912.
[37] S.B. Bourd, L.V. Victorova, M.K. Kukhanova, FEBS Lett 142 (1982) 96–100.
[38] J. Cerna, FEBS Lett 15 (1971) 101–104.
[39] L. Rychlik, S. Chladek, J. Zemlicka, Biochim Biophys Acta 138 (1967) 640–642.
[40] A.A. Krayevsky, M.K. Kukhanova, Prog Nucleic Acids Res Mol Biol 23 (1979) 1–52.
[41] R. Fernandez-Muñoz, D. Vázquez, Mol Biol Rep 1 (1973) 75–79.
[42] Bilgin N, Ehrenberg M. In: Nierhaus KH, Franceschi F, Subramanian AR, Erdmann VA, Wittmann-Liebold B, editors. The Translational Apparatus: Structure, Function, Regulation and Evolution, New York: Plenum Press, 1993:385–395.
[43] M. O'Connor, C.A. Brunelli, M.A. Firpo, S.T. Gregory, K.R. Lieberman, J.S. Lodmell, H. Moine, D.I. Van Ryk, A.E. Dahlberg, Biochem Cell Biol 73 (1995) 859–868.
[44] H. Hornig, P. Woolley, R. Lührmann, J Biol Chem 259 (1984) 563–5636.
[45] H. Hornig, P. Woolley, R. Lührmann, Biochimie 69 (1987) 803–813.
[46] J.L. Lessard, S. Pestka, J Biol Chem 247 (1972) 6901–6908.
[47] M. Tezuka, S. Chladek, Biochemistry 29 (1990) 667–670.
[48] G.G. Abdurashidova, I.O. Baskaeva, A.A. Chernyi, L.B. Kaminir, E.I. Budowsky, Eur J Biochem 159 (1986) 103–109.
[49] D. Moazed, H.F. Noller, Cell 57 (1989) 585–597.
[50] D. Moazed, H.F. Noller, Biochimie 69 (1987) 879–884.
[51] C. Rodriguez-Fonseca, R. Amils, R.A. Garrett, J Mol Biol 247 (1995) 224–235.
[52] Rodriguez-Fonseca C, Phan H, Amils R, Garrett RA. manuscript in preparation, 1997.
[53] B.T. Porse, R.A. Garrett, J Mol Biol 249 (1995) 1–10.
[54] S. Joseph, H.F. Noller, EMBO J. 15 (1996) 910–916.
[55] K.Sh. Kemkhadze, V.B. Odintsov, Yu.P. Semenkov, S.V. Kirillov, FEBS Lett 125 (1981) 10–14.
[56] Yu.P. Semenkov, E.M. Makarov, V.I. Makhno, S.V. Kirillov, FEBS Lett 144 (1982) 125–129.
[57] J.-R. Mesters, A.P. Potapov, J.M. de Graal, B. Kraal, J Mol Biol 242 (1994) 644–654.
[58] J. Czworkowski, J. Wang, T.A. Steitz, P.B. Moore, EMBO J 13 (1994) 3661–3668.
[59] A. Ævarsson, E. Brazhnikov, M. Garber, J. Zheltonosova, Yu. Chirgadze, S. Al-Karadaghi, L.A. Svensson, A. Liljas, EMBO J 13 (1994) 3669–3677.
[60] V.B. Odinzov, S.V. Kirillov, Nucleic Acids Res 5 (1978) 3871–3879.
[61] S.V. Kirillov, K.Sh. Kemkhadze, E.M. Makarov, V.I. Makhno, V.B. Odintsov, Yu.P. Semenkov, FEBS Lett 120 (1980) 221–224.
[62] S.V. Kirillov, V.I. Katunin, Yu.P. Semenkov, FEBS Lett 125 (1981) 15–18.
[63] S.V. Kirillov, Yu.P. Semenkov, FEBS Lett 148 (1982) 235–238.
[64] E.M. Makarov, V.I. Katunin, V.B. Odintsov, Yu.P. Semenkov, S.V. Kirillov, Mol Biol 18 (1984) 1090–1094.
[65] R. Lill, J.M. Robertson, W. Wintermeyer, Biochemistry 25 (1986) 3245–3255.
[66] S. Pestka, J Biol Chem 247 (1972) 4669–4678.
[67] D.V. Parfenov, E.M. Saminsky, Mol Biol 27 (1993) 507–510.
[68] J. Cerna, I. Rychlik, J. Jonak, Eur J Biochem 34 (1973) 551–556.
[69] W.D. Picking, O.W. Odom, B. Hardesty, Biochemistry 31 (1992) 12565–12570.
[70] J. Cerna, I. Rychlik, A.A. Krayevsky, B.P. Gottikh, FEBS Lett 37 (1973) 188–191.
[71] J. Cerna, I. Rychlik, A.A. Krayevsky, B.P. Gottikh, Acta Biol Med Germ 33 (1974) 877–883.
[72] D.A. Peattie, W. Herr, Proc Natl Acad Sci USA 78 (1981) 2273–2277.
[73] R.R. Samaha, R. Green, H.F. Noller, Nature 377 (1995) 309–314.
[74] B.T. Porse, H.P. Thi-Ngoc, R.A. Garrett, J Mol Biol 264 (1996) 472–483.
[75] C.M.T. Spahn, M.A. Schafer, A.A. Krayevsky, K.H. Nierhaus, J Biol Chem 271 (1996) 32857–32862.
[76] Hardesty B, Odom OW, Deng H-Y. In: Hardesty B, Kramer G, editors. Structure, Function and Genetics of Ribosomes. New York: Springer, 1986:495–508.
[77] O.W. Odom, B. Hardesty, Biochimie 69 (1987) 925–938.
[78] Hardesty B, Odom OW, Czworkowski J. In: Hill W, Dahlberg A, Garrett RA, Moore PB, Schlessinger D, Warner JR, editors. The Ribosome, Structure, Function and Evolution. Washington, DC: American Society for Microbiology, 1990:366–372.
[79] J. Wower, S.S. Hixon, R.A. Zimmermann, Biochemistry 27 (1988) 8114–8121.
[80] O.W. Odom, W.S. Picking, B. Hardesty, Biochemistry 29 (1990) 10734–10744.
[81] F.O. Wettstein, H. Noll, J Mol Biol 11 (1965) 35–53.
[82] S.V. Kirillov, Yu.P. Semenkov, Mol Biol 18 (1984) 1013–1025.
[83] J.M. Robertson, W. Wintermeyer, J Mol Biol 196 (1987) 525–540.
[84] M. Cannon, R. Krug, W. Gilbert, J Mol Biol 7 (1963) 360–378.
[85] R. Lill, W. Wintermeyer, J Mol Biol 196 (1987) 137–148.
[86] R. Lill, J.M. Robertson, W. Wintermeyer, EMBO J 8 (1989) 3933–3938.
[87] O. Dontsova, V. Tishkov, S. Dokudovskaya, A. Bogdanov, T. Döring, J. Rinke-Appel, S. Thamm, B. Greuer, R. Brimacombe, Proc Natl Acad Sci USA 91 (1994) 4125–4129.
[88] Phan H, Østergaard P, Garrett RA. manuscript in preparation, 1997.
[89] J. Wower, P. Scheffer, L.A. Sylvers, W. Wintermeyer, R.A. Zimmermann, EMBO J 12 (1993) 617–623.
[90] R.A. Milligan, P.N.T. Unwin, Nature 319 (1986) 693–695.
[91] A. Yonath, K.R. Leonard, H.G. Wittmann, Science 236 (1987) 813–816.
[92] J. Frank, J. Zhu, P. Penczek, Y. Li, S. Srivastava, A. Verschoor, M. Radermacher, R. Grassucci, R.K. Lata, R.K. Agrawal, Nature 376 (1995) 441–444.
[93] L.A. Ryabova, O.M. Selivanova, V.I. Baranov, V.D. Vasiliev, A.S. Spirin, FEBS Lett 226 (1988) 255–260.
[94] C. Bernabeau, J.A. Lake, Proc Natl Acad Sci USA 79 (1983) 3111–3115.
[95] C. Bernabeau, E.M. Tobin, A. Fowler, I. Zabin, J.A. Lake, J Cell Biol 96 (1982) 1471–1474.
[96] K. Stade, N. Jünke, R. Brimacombe, Nucleic Acids Res 23 (1995) 2371–2380.

[97] B. Hardesty, W.D. Picking, O.W. Odom, Biochim Biophys Acta 1050 (1990) 197–202.
[98] W.D. Picking, O.W. Odom, T. Tsalkova, I. Serdyuk, B. Hardesty, J Biol Chem 266 (1991) 1534–1542.
[99] O.W. Odom, W.D. Picking, T. Tsalkova, B. Hardesty, Eur J Biochem 198 (1991) 713–722.
[100] W.D. Picking, W.L. Picking, O.W. Odom, B. Hardesty, Biochemistry 31 (1992) 2368–2375.
[101] Garrett RA, Rodriguez-Fonseca C. In: Zimmermann RA, Dahlberg A, editors. Ribosomal RNA: Structure, Evolution, Processing and Function in Protein Biosynthesis. Boca Raton, FL: CRC Press, 1995:329–357.
[102] J. Wower, S.S. Hixson, R.A. Zimmermann, Proc Natl Acad Sci USA 86 (1989) 5232–5236.
[103] P. Mitchell, M. Osswald, R. Brimacombe, Biochemistry 31 (1992) 3004–3011.
[104] I. Leviev, S. Levieva, R.A. Garrett, Nucleic Acids Res 23 (1995) 1512–1517.
[105] M. O'Connor, A. Dahlberg, J Mol Biol 254 (1995) 838–849.
[106] H.F. Noller, V. Hoffarth, L. Zimniak, Science 256 (1992) 1416–1419.
[107] R.A. Garrett, P. Woolley, Trends Biochem Sci 7 (1982) 385–386.
[108] B.G. Lane, J. Ofengand, M.W. Gray, Biochimie 77 (1995) 7–15.
[109] Pestka S. In: Weissbach H, Pestka S, editors. Molecular Mechanisms of Protein Biosynthesis. New York: Academic Press, 1977:467–553.
[110] Vázquez D. Inhibitors of Protein Biosynthesis. New York: Springer, 1979.
[111] Gale EF, Cundliffe E, Reynolds PE, Richmond RA, Waring MJ, editors. The Molecular Basis of Antibiotic Action. London: Wiley and Sons, 1981.
[112] E. Scolnick, G. Milman, M. Rosman, T. Caskey, Nature 225 (1970) 152–154.
[113] S. Pestka, Proc Natl Acad Sci USA 64 (1969) 709–714.
[114] S. Pestka, T. Hishizawa, J.L. Lessard, J Biol Chem 245 (1970) 6208–6219.
[115] R. Fernandez-Muñoz, D. Vázquez, Mol Biol Rep 1 (1973) 27–32.
[116] S.B. Bourd, M.K. Kukhanova, B.P. Gottikh, A.A. Krayevsky, Eur J Biochem 135 (1983) 465–470.
[117] V.I. Makhno, N.N. Peshin, Yu.P. Semenkov, S.V. Kirillov, Mol Biol 22 (1988) 528–536.
[118] E. Lázaro, L.A.G.M. Van den Broek, A. San Felix, H.C.J. Ottemheiljm, J.P.G. Ballesta, Biochemistry 30 (1991) 9642–9648.
[119] T. Kinoshita, N. Tanaka, H. Umezawa, J Antibiot 23 (1979) 288–290.
[120] R.J. Harris, R.H. Symons, Bioorg Chem 2 (1973) 266–285.
[121] B.V. Cheney, J Med Chem 17 (1974) 590–599.
[122] F.W. Lichtenthaler, G. Trummlitz, FEBS Lett 38 (1974) 237–242.
[123] R.H. Symons, R.J. Harris, P. Greenwell, D.J. Eckermann, E.F. Vanin, Bioorg Chem 4 (1978) 409–436.
[124] P. Bhuta, H.L. Chung, J.S. Hwang, J. Zemlicka, J Med Chem 23 (1980) 1299–1305.
[125] B. Vester, R.A. Garrett, EMBO J 7 (1988) 3577–3587.
[126] I. Leviev, C. Rodriguez-Fonseca, H. Phan, R.A. Garrett, G. Heilek, H.F. Noller, A. Mankin, EMBO J 13 (1994) 1682–1686.
[127] S. Pestka, Antimicrob Agents Chemother 5 (1974) 255–267.
[128] M. Ettayebi, S.M. Prasad, E.A. Morgan, J Bacteriol 162 (1985) 551–557.
[129] G.R. Julian, J Mol Biol 12 (1965) 9–16.
[130] K. Tanaka, H. Teraoko, M. Tamaki, FEBS Lett 13 (1971) 65–67.
[131] H.-J. Rheinberger, K.H. Nierhaus, Eur J Biochem 193 (1990) 643–650.
[132] R.E. Monro, D. Vázquez, J Mol Biol 28 (1967) 161–165.
[133] R.E. Monro, M.L. Celma, D. Vázquez, Nature 222 (1969) 356–358.
[134] Goldberg IH. In: Horne R, editor. Antibiotics, Vol. 1: Mechanism of Action of Antibacterial Agents, 1979:264–271.
[135] D. Moazed, H.F. Noller, Proc Natl Acad Sci USA 88 (1991) 3725–3728.
[136] H. Hornig, P. Woolley, R. Lührmann, FEBS Lett 156 (1983) 311–315.
[137] H.C.J. Ottenheijm, L.A.G.M. van den Broek, J.P.G. Ballesta, Z. Zylicz, Prog Med Chem 23 (1968) 219–268.
[138] E. Lázaro, L.A.G.M. van den Broek, A. San Felix, H.C.J. Ottenheijm, J.P.G. Ballesta, Biochimie 73 (1991) 1137–1143.
[139] E. Lázaro, C. Rodriguez-Fonseca, B. Porse, D. Ure-a, R.A. Garrett, J.P.G. Ballesta, J Mol Biol 261 (1996) 231–238.
[140] G.T. Tan, A. BeBlasio, A.S. Mankin, J Mol Biol 261 (1996) 222–230.
[141] A. Panet, N. De Groot, Y. Lapidot, Eur J Biochem 15 (1970) 222–225.
[142] M.D. Giambattista, G. Chinali, C. Cocito, J Antimicrob Chemother 24 (1989) 485–507.
[143] O.W. Odom, B. Hardesty, J Biol Chem 267 (1992) 19117–19122.
[144] M.J. Calcutt, E. Cundliffe, J Bacteriol 172 (1990) 4710–4714.
[145] S. Douthwaite, J Bacteriol 174 (1992) 1333–1338.
[146] J. Egebjerg, R.A. Garrett, Biochimie 73 (1991) 1145–1149.
[147] S. Douthwaite, Nucleic Acids Res 20 (1992) 4717–4720.
[148] S. Douthwaite, C. Aagaard, J Mol Biol 232 (1993) 725–731.
[149] Z. Gu, R. Harrod, E.J. Rogers, P.S. Lovett, Proc Natl Acad Sci USA 91 (1994) 561–5616.
[150] A. Contreras, D. Vázquez, Eur J Biochem 74 (1977) 539–547.
[151] S. Andersson, C.G. Kurland, Biochimie 69 (1987) 901–904.
[152] S. Pestka, H. Rosenfeld, R. Harris, H. Hintikka, J Biol Chem 247 (1972) 6895–6900.
[153] J.C.H. Mao, R.R. Robishaw, Biochemistry 10 (1971) 2054–2061.
[154] J.R. Menninger, R.A. Coleman, Antimicrob Agents Chemother 37 (1993) 2027–2029.
[155] D.J.W. Burns, E. Cundliffe, Eur J Biochem 37 (1973) 570–574.
[156] S.K. Dube, P.S. Rudland, B.F.C. Clark, K.A. Marker, Cold Spring Harbor Symp Quant Biol 39 (1969) 161–166.
[157] S.J. Rose, P.T. Lowary, O.C. Uhlenbeck, J Mol Biol 167 (1983) 103–117.
[158] D.V. Parfenov, E.M. Saminsky, Mol Biol 25 (1991) 516–521.
[159] D.L. Kalpaxis, D.A. Theocharis, C. Coutsogeorgopoulos, Eur J Biochem 154 (1986) 267–271.
[160] D. Drainas, D.L. Kalpaxis, C. Coutsogeorgopoulos, Eur J Biochem 164 (1987) 53–58.
[161] D.A. Theocharis, D. Synetos, F.L. Kalpaxis, D. Drainas, C. Coutsogeorgopoulos, Arch Biochem Biophys 292 (1992) 266–272.
[162] D.A. Theocharis, C. Coutsogeorgopoulos, Biochemistry 31 (1992) 5861–5868.
[163] D.L. Kalpaxis, C. Coutsogeorgopoulos, Mol Pharmacol 36 (1989) 615–619.
[164] Ballesta JPG, Lázaro E. In: Hill W, Dahlberg A, Garrett RA, Moore PB, Schlessinger D, Warner JR, editors. The Ribosome: Structure, Function, and Evolution. Washington, DC: American Society for Microbiology, 1990:502–510.
[165] J. Zemlicka, A. Bhuta, J Med Chem 25 (1982) 1123–1125.