INHIBITORS OF PROTEIN SYNTHESIS

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1. Introduction

Studies on the mode of action of protein synthesis inhibitors can be chronologically divided into two periods. The first period covers the years 1951–1960 and the second from 1961 until 1973. We make this chronological distinction because specific inhibition of bacterial protein synthesis by antibiotics (chloramphenicol and chlortetracycline) was first described in 1951 [1,2] and confirmed in a ribosomal amino acid incorporation system directed by a specific synthetic polynucleotide as mRNA, in 1961 [3]. Since 1961 studies on the mechanisms of antibiotic action have been so widely developed [4–6; reviews] that it became practically impossible to cover the subject in a single contribution, and protein synthesis inhibitors have usually been dealt with for the last eight years either in independent contributions or even in entire volumes [7–12]. This study will be concerned mainly with advances in our knowledge of the mode of action, selectivity, and specificity of inhibitors of protein synthesis over the last ten years. However, a complete survey of the literature would not be possible in such a brief contribution as this.

The process of protein biosynthesis can be arbitrarily divided, for didactic purposes, into (a) steps taking place prior to translation and (b) steps in the translation mechanisms taking place at the ribosome level.

2. Inhibitors of steps taking place prior to translation

2.1. *Inhibitors of aminoacyl-tRNA formation*

The synthesis of aminoacyl-tRNA, catalysed by the specific aminoacyl-tRNA synthetase, is a complex reaction involving the steps of activation and transfer:

Activation:

$$\text{Amino acid}_1 + \text{Enzyme}_1 + \text{ATP} \underset{\longleftarrow}{\overset{Mg^{2+}}{\longrightarrow}} \text{Enzyme}_1 - \text{aminoacyl}_1\text{-AMP} + PP_i$$

Transfer:

$$\text{Enzyme}_1 - \text{aminoacyl}_1\text{-AMP} + \text{tRNA}_1 \rightleftarrows \text{aminoacyl}_1\text{-tRNA}_1 + \text{Enzyme}_1 + \text{AMP}$$

Inhibitors of these reactions have been recently reviewed [13]. A number of amino acid analogues have been reported as inhibitors of the activation reaction by the corresponding aminoacyl-tRNA synthetases. Included in this group are 7-azatryptophan, tryptazan, 6-fluorotryptophan and 5-fluorotryptophan [14], norvaline, α-amino-β-chlorobutyrate, α-aminobutyrate, selenomethionine, ethionine, norleucine [15–17], a number of tyrosine analogues [18], the methyl and ethyl esters of serine [19] and certain lysine analogues [20]. Some competitive inhibitors of certain amino acid(s) in the activation step become attached to tRNA in the transfer reaction and abnormal proteins are formed in subsequent incorporation. This has been observed with ethionine, norleucine [17,21,22], alloisoleucine, azetidinecarboxylic acid, canavanine, *N*-ethylglycine and *O*-methyl threonine [13]. On the other hand, a number of competitive inhibitors of amino acid activation block the formation of a specific aminoacyl-tRNA. This is so with the antibiotics borrelidin, which has been shown to prevent threonyl-tRNA formation in a number of bacterial species [23,27], and furano-

mycin, which inhibits isoleucyl-tRNA formation in bacterial and mammalian cells [25,26], whereas minosine likewise blocks phenylalanyl-tRNA synthesis [27]. Similarly, ten aminoalkyl-adenylates have been synthesized and shown to compete specifically with their corresponding amino acids and with ATP in the activation step, whereas their amino alcohols are, by themselves, inert competitive inhibitors of both amino acid activation and the transfer step [28,29].

An increasing number of oligonucleotides and polynucleotides have been used as analogues of some parts of the tRNA molecule to inhibit aminoacyl-tRNA formation since Hayashi and Miura [30] observed that some rRNA-derived oligonucleotides inhibit valyl-tRNA formation. There are abundant data in this field reporting competitive inhibition of specific aminoacyl-tRNA formation by certain polynucleotides. However, there are many contradictory reports, probably owing to uncertainty about the purity of nucleotides and aminoacyl-tRNA synthetases used, a point which is discussed elsewhere [13].

From the data presented above it is obvious that some of the known inhibitors of aminoacyl-tRNA formation may useful for studying individual reactions in some purified cell-free systems, but in most cases they have neither a specific effect on this step nor a selective action on certain types of cells, nor can they even be used with intact cells. Therefore their importance as protein synthesis inhibitors is not very great, with the possible exception of some amino acid analogues, minosine [27] and the antibiotics borrelidin [23,24] and furanomycin [25,26].

2.2. *Inhibitors of f-Met-tRNA$_F$ formation*

Biosynthesis of the initiator *N*-formyl-Met-tRNA$_F$ takes place according to the reaction:

Met-tRNA$_F$+N^{10}-formyl-H$_4$ folate$\rightleftarrows$*N*-formyl-Met-tRNA$_F$+H$_4$ folate

in which the α-NH$_2$ group of the methionine of Met-tRNA$_F$ is the acceptor group, the donor is N^{10}-formyl-H$_4$ folate and the enzyme catalysing the reaction is N^{10}-formyl-H$_4$ folate-methionyl-tRNA$_F$ transformylase. Hence, inhibition of f-Met-tRNA$_F$ formation can take place by blocking either N^{10}-formyl-H$_4$ folate formation or the transformylase.

N^{10}-Formyl-H$_4$ folate synthesis is inhibited by a number of dihydrofolate analogues such as aminopterin, amethopterin (synonym methotrexate) and pteroylaspartic acid. Aminopterin has indeed been shown to block the f-Met-tRNA$_F$ required for initiation of protein synthesis in mitochondria without affecting cytoplasmic protein synthesis in mammalian cells [31]. Because of the permeability barriers for these drugs in a number of organisms, simpler compounds have been synthesized, including pyrimethamine and trimethoprim [32]. Indeed, in *E. coli* trimethoprim blocks the synthesis of β-galactosidase and phage T$_4$ proteins.

Furthermore, extracts of trimethoprim-treated bacteria require the addition of f-Met-tRNA$_F$ to initiate protein synthesis [33,34]. 6-Chloro-8-aza-9-cyclopentylpurine and a number of related compounds are also inhibitors of N^{10}-formyl-H$_4$ folate formation, and therefore block DNA, RNA and protein synthesis in *E. coli* [35,36].

The catalytic action of the N^{10}-formyl-H$_4$ folate-methionyl-tRNA$_F$ transformylase can be inhibited by either N^{10}-formyl-H$_4$ folate analogues, of Met-tRNA$_F$ analogues, or compounds which deplete the pool of the substrates of the reaction. Indeed, a number of pteridine derivatives have been shown to block the formylation reaction by competing with N^{10}-formyl-H$_4$ folate [37,38].

Hydroxylamine is known to block DNA, RNA and protein synthesis, but when added at low concentrations to growing bacterial cultures, it specifically blocks the initiation phase of protein synthesis [34]. This is due to the reaction of hydroxylamine with the formyl residue of 5,10-methylene-H$_4$ folate, leading to the formation of a folmaldoxine and depleting the pool of N^{10}-formyl-H$_4$ folate which is the precursor of 5,10-methylene-H$_4$ folate [40].

Inhibitors of f-Met-tRNA$_F$ formation inhibit the initiation of translation in bacteria, blue green algae, mitochondria and chloroplasts, but not in eukaryotic systems, since f-Met-tRNA$_F$ is not an initiator in these systems. In any case, care has to be taken in the use of inhibitors of dihydrofolate reductase and N^{10}-formyl-H$_4$ folate transformylase in intact bacteria as specific inhibitors of protein synthesis, since they can also block many other cell processes.

3. Inhibitors of translation

3.1. *Translation of mRNA*

The translation of mRNA into protein, which takes place at the ribosome level, can be divided into three phases: initiation, elongation and termination. The overall reactions taking place in the process of translation by bacterial and eukaryotic ribosomes are shown schematically in figs. 1 and 2, according to the translocation mode with one single entry site on the small subunit.

A single entry site on the small ribosome subunit is favoured in studies with a number of protein synthesis inhibitors, since all these compounds preventing mRNA-directed f-Met-$tRNA_F$ binding to the small ribosome subunit (step (b)) are equally active in preventing aminoacyl-tRNA binding (step (d)). For the purpose of understanding the whole process, it can be divided into three phases: initiation, elongation and termination (figs. 1 and 2).

In the initiation phase we have to distinguish three steps: (a) recognition of initiation factors and mRNA by the small subunit, (b) binding of the initiator and (c) joining to the complex of the larger subunit (fig. 1).

This step (c) involves two reactions; in the first, the larger subunit is attached to the initiation complex (step (c_1)), and the other, requiring GTP hydrolysis (step (c_2)), in which the 3′ end of f-Met-$tRNA_F$ binds to the donor-site of the peptidyl-transferase centre and the antidocon interaction with the small subunit is interrupted, leaving the site open for the entry of aminoacyl-tRNA to start the elongation phase. The initiation phase has not been well resolved in mammalian systems, but for comparative purposes we have assumed that a similar sequence of reactions takes place (fig. 2). This might not be entirely correct, since the initiator appears to bind to mammalian ribosomes (step (b)) prior to mRNA recognition (step a(a), fig. 2) [41,42].

The elongation phase is composed of repeated cycles of elongation. Steps (d) EF T- (or EF 1)-dependent aminoacyl-tRNA binding, (e) peptide bond formation and (f) translocation, can be distinguished in each elongation cycle. In the translation process there are as many elongation cycles as there are peptide bonds to be formed in order to synthesize the protein (figs. 1 and 2). There is evidence for only a single entry site in the small subunit, and therefore for the codon–anticodon interaction of the incoming aminoacyl-tRNA (step (d)), the anticodon interaction of either f-Met-$tRNA_F$ or peptidyl-tRNA in the small subunit has to be interrupted. The specific step of peptide bond formation (step (e)) is catalysed by the peptidyl transferase centre, which is an integral part of the larger ribosomal subunit. The translocation step requires one of the elongation factors and involves movement of the peptidyl-tRNA from the A-site to the P-site, coupled with GTP hydrolysis and release of the uncharged tRNA.

The termination phase takes place when a chain-terminating codon (nonsense codon) (either UAA or UAG or UGA) is recognized and cleavage of the bond between the peptidyl and tRNA moieties takes place in a reaction catalysed by the ribosomal peptidyl transferase centre requiring the release factors (g).

3.2. *Selectivity of protein synthesis inhibitors*

Early studies on [^{14}C]chloramphenicol binding showed that this antibiotic binds to all classes of ribosomes of the 70 S type which were tested, but does not bind to any of the 80 S type ribosomes studied [43]. A similar finding was later observed with a number of antibiotics, whereas others have a much wider spectrum. Since there are at least two types of systems for protein synthesis, their inhibitors can be classified, according to their specificity, into those affecting systems of (a) the prokaryotic type, (b) the eukaryotic type and (c) the prokaryotic and the eukaryotic types (table 1). In general antibiotics affecting prokaryotic-type systems are active in bacteria, blue-green algae, mitochondria and chloroplast, whereas those acting selectively on eukaryotic-type systems are active in all types of cells having 80 S type ribosomes. However, there are a few important cases in which some antibiotics appear to have a narrower spectrum of selectivity. Thus, tenuazonic acid inhibits protein synthesis by mammalian but not by yeast ribosomes [44]; erythromycin, lincomycin and paromomycin inhibit protein synthesis by bacteria but not by rat liver mitochondria [391]; and although fusidic acid is active in prokaryotic and eukaryotic systems it has been reported to be inactive in *Neurospora crassa* mitochondria [45] and in protein-

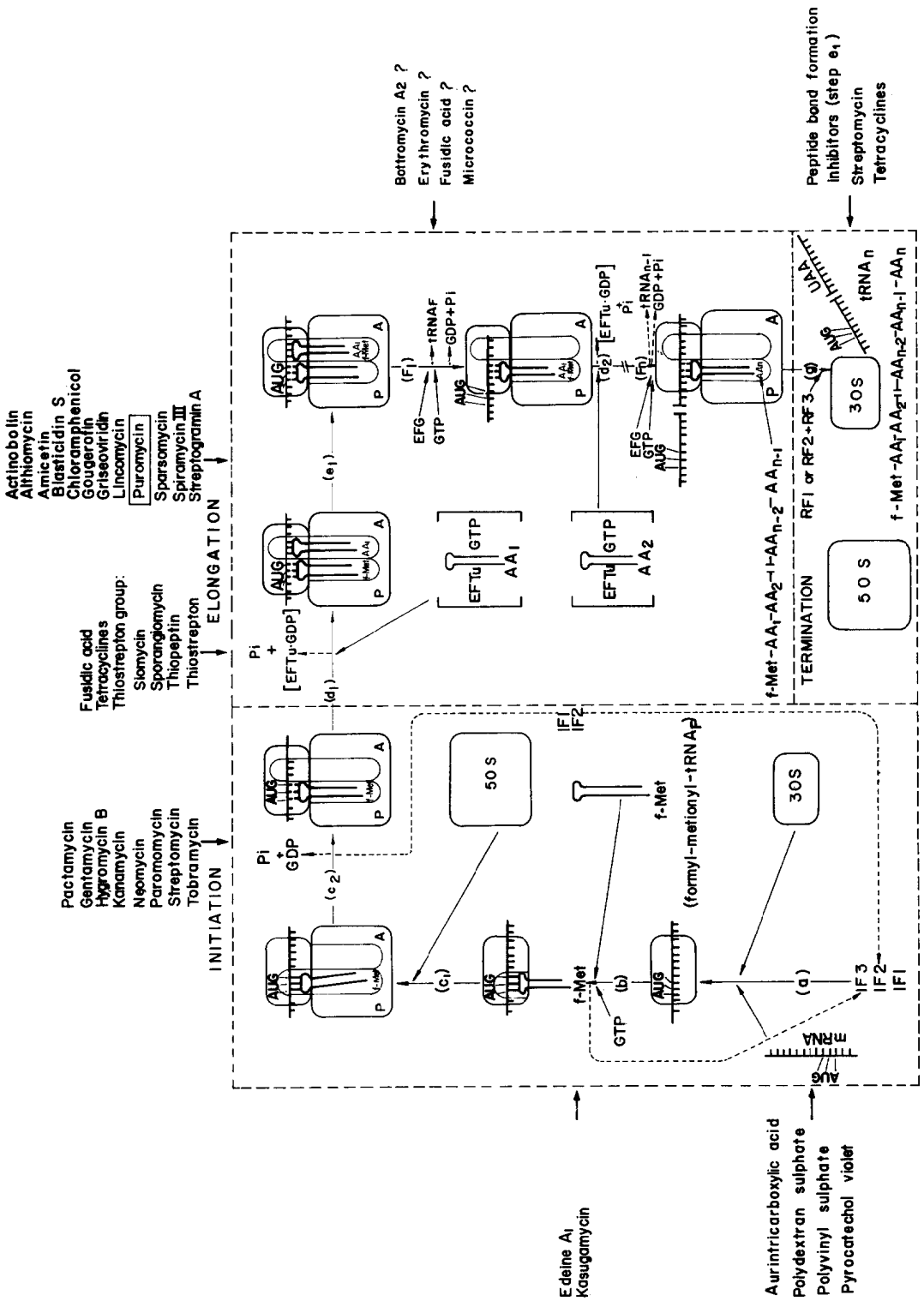
Actinobolin
Althiomycin
Amicetin
Blasticidin S
Chloramphenicol
Gougerotin
Griseoviridin
Lincomycin
Puromycin
Sparsomycin
Spiramycin III
Streptogramin A
ELONGATION
Fusidic acid
Tetracyclines
Thiostrepton group:
Siomycin
Sporangiomycin
Thiopeptin
Thiostrepton
Pactamycin
Gentamycin
Hygromycin B
Kanamycin
Neomycin
Paromomycin
Streptomycin
Tobramycin
INITIATION
Bottromycin A2 ?
Erythromycin ?
Fusidic acid ?
Micrococcin ?
Peptide bond formation inhibitors (step e1)
Streptomycin
Tetracyclines
Edeine A1
Kasugamycin
Aurintricarboxylic acid
Polydextran sulphate
Polyvinyl sulphate
Pyrocatechol violet
TERMINATION
RF1 or RF2+RF3
50 S
30S
IF1
IF2
IF3
GTP
EFG
tRNAF
f-Met
(formyl-methionyl-tRNAf)
f-Met-AA1-AA2-AAn-2-AAn-1
f-Met-AA1-AA2-AAn-2-AAn-1-AAn
tRNAn

Fig. 1.

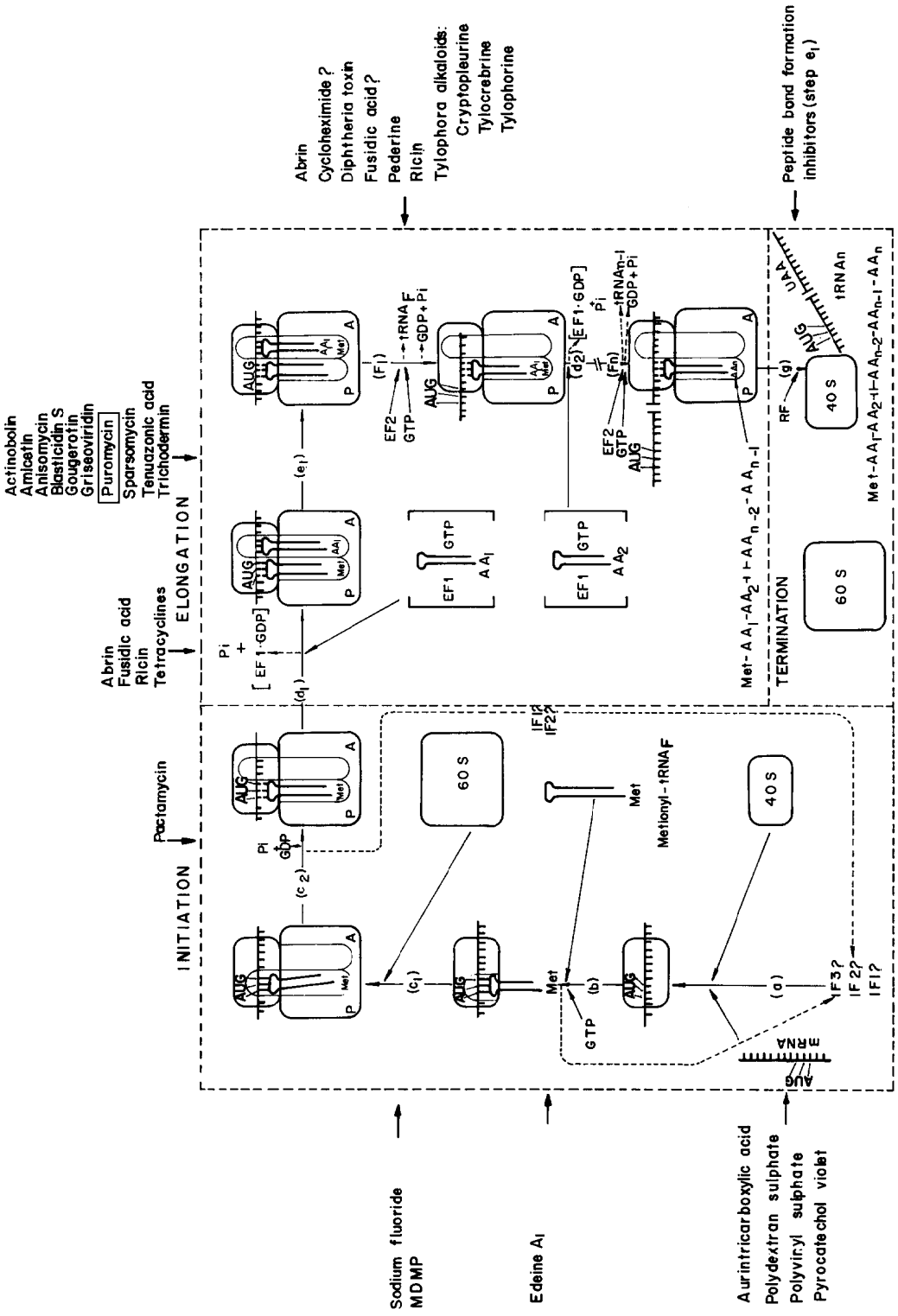
Abrin
Cycloheximide?
Diphtheria toxin
Fusidic acid?
Pederine
Ricin
Tylophora alkaloids:
Cryptopleurine
Tylocrebrine
Tylophorine
Peptide bond formation inhibitors (step e1)
Actinobolin
Amicetin
Anisomycin
Blasticidin S
Gougerotin
Griseoviridin
Puromycin
Sparsomycin
Tenuazonic acid
Trichodermin
Abrin
Fusidic acid
Ricin
Tetracyclines
ELONGATION
Pactamycin
INITIATION
Sodium fluoride
MDMP
Edeine A1
Aurintricarboxylic acid
Polydextran sulphate
Polyvinyl sulphate
Pyrocatechol violet
EF1
GTP
EF2
GDP+Pi
tRNA F
Met-tRNA F
Met
60 S
40 S
AUG
TERMINATION
RF
tRNAn
IF1?
IF2?
IF3?

Fig. 2.

Table 1
Inhibitors of protein synthesis

Acting on prokaryotic systems

- Althiomycin
- Bacteriocin DF_{13}
- Berninamycin
- Chloramphenicol group:
  - Chloramphenicol
  - D-AMP-3
  - d-Thiomycetin
  - D-Win-5094
- Colicin E_3
- Kasugamycin
- Lincomycin group:
  - Celesticetin
  - Clindamycin
  - Lincomycin
- Macrolides group:
  - Angolamycin
  - Carbomycin
  - Erythromycin
  - Forocidin
  - Lancamycin
  - Leucomycin
  - Methymycin
  - Neospiramycin
  - Oleandomycin
  - Spiramycin
  - Tylosin
- Micrococcin
- Multhiomycin
- Rubradirin
- Spectinomycin
- Streptogramin A group:
  - Ostreogrycin G
  - Streptogramin A
- Streptogramin B group:
  - Staphylomycin S
  - Streptogramin B
  - Viridogrisein
- Streptomycin group:
  - Gentamycin
  - Hygromycin B
  - Kanamycin
  - Neomycin
  - Paromomycin
  - Streptomycin
- Thiostrepton group:
  - Siomycin
  - Sporangiomycin
  - Thiopeptin
  - Thiostrepton
- Viomycin

Acting on prokaryotic and eukaryotic systems

- Actinobolin
- Adrenochrome
- Amicetin
- Aurintricarboxylic acid
- Blasticin S
- Nucleocidin
- Pactamycin
- Polydextran sulphate
- Polyvinyl sulphate
- Puromycin

Acting on prokaryotic and eukaryotic systems (continued)

- Bottromycin A_2
- Chartreusin
- Edeine A_1
- Fusidic acid
- Gougerotin group:
  - Bamicetin
  - Gougerotin
  - Plicamicetin
- Guanylyl-methylene-diphosphonate
- Pyrochatechol Violet
- Sparsomycin
- Tetracycline group:
  - Chlortetracycline
  - Doxycycline
  - Oxytetracycline
  - Tetracycline
- Tosylphenylalanylchloro-methane

Acting on eukaryotic systems

- Abrin
- Anisomycin
- Diphtheria toxin
- Emetine
- Enomycin
- Glutarimide group:
  - Actiphenol
  - Cycloheximide
  - Streptimidone
  - Streptovitacin A
- MDMP
- Pederine
- Phenomycin
- Ricin
- Sodium fluoride
- Tenuazonic acid
- Trichodermin group:
  - Crotocin
  - Crotocol
  - Fusarenon X
  - Nivalenol
  - Trichodermin
  - Trichodermol
  - Trichothecin
  - Verrucarin A
  - Verrucarol
- Tubulosine
- *Tylophora* alkaloids:
  - Cryptopleurine
  - Tylocrebrine
  - Tylophorine

synthesizing systems from sporulating *Bacillus subtilis* [45a].

3.3. *Inhibitors of the initiation phase*

Step (a)

Polydextran sulphate [46,47], polyvinyl sulphate [48], aurintricarboxylic acid [49,50] and Pyrochatechol Violet [51] used at low concentrations in cell-free systems interact specifically with the small ribosome subunit and prevent mRNA or synthetic polynucleotide binding to the subunit. Aurintricarboxylic acid has been widely used as an inhibitor of initiation and has been reported to block not only mRNA binding to the ribosomes [50,52,53], but also formation

of a ternary complex 1–GTP–Met-tRNA$_F$ [54], dissociation of free ribosomes into their subunits [55], EF G and ribosome-dependent GTPase [56] and the function of EF Ts [57]. Therefore aurintricarboxylic acid inhibits initiation and elongation. However, at low concentrations, this drug can be used as a selective inhibitor of the initiation phase in bacterial as well as eukaryotic systems [52, 58–61].

Step (b)

The main target of edeine A_1 in intact bacteria is DNA synthesis [62,63]. However, in cell-free systems edeine A_1 can be used as an instrument in protein synthesis since it interacts with both ribosomal subunits, but it has a preferential affinity for the small subunit [64–66]. The main effect of edeine is to prevent binding of the initiator substrate [65,67].

The aminoglycoside antibiotic kasugamycin does not possess the characteristic dihydrostreptamine or streptamine moiety which is common to all aminoglycoside antibiotics causing misreading. Therefore kasugamycin does not cause misreading, but acts on the 30 S ribosome subunit [68] by preventing the binding of f-Met-tRNA$_F$ [69]. Resistance to kasugamycin in *E. coli* is due to a lack of methylation of two adjacent adenine residues in the 16 S rRNA of the 30 S subunit [69,70]. Furthermore, an altered ribosomal protein (S4) has been observed in kasugamycin-resistant mutants [71].

Step (c)

Sodium fluoride causes polysomal dissociation and accumulation of ribosomes or ribosomal subunits [72–75]. The initiation complex with the 40 S ribosomal subunits appears to be normally formed in the presence of sodium fluoride, but this compound prevents the subsequent binding of the 60 S subunit [53,76]. A similar mechanism of action has been proposed for the herbicide MDMP (2-(4-methyl-2,6-dinitroanilino)-*N*-methylpropionamide) [77].

The antibiotic streptomycin affects a number of cell processes other than protein synthesis [7]. Nevertheless, we will restrict our discussion to the effects of the antibiotic as a known inhibitor of protein synthesis [78,79]. Streptomycin was found to cause misreading in translation early on in intact bacteria [80] as well as in cell-free systems [81]. The effect of streptomycin is located in the bacterial ribosome [81–84] in the 30 S subunit [85,86]. One of the 30 S ribosomal proteins (S4) is altered in streptomycin-resistant mutants [87] and leads to resistant ribosomes in reconstitution experiments. In fact, the antibiotic binds to sensitive ribosomes [88] and the 30 S subunit from sensitive [89–91] but not resistant ribosomes [91]. Misreading is not, however, the main effect of streptomycin in protein synthesis, since the antibiotic inhibits the translation process in the initiation phase [92–95]. Streptomycin does not prevent the joining of the 50 S subunit to the initiation complex formed on the small subunit (fig. 1c$_1$) but the initiation complex formed in the presence of streptomycin is unstable and the subsequent binding of aminoacyl-tRNA does not take place (step (d$_1$)) [96]. Therefore streptomycin appears to block reaction (c$_2$) (fig. 1). Streptomycin also inhibits elongation by preventing aminoacyl-tRNA binding to the A-site (step (d)) [95], and termination by preventing recognition of RF 1 or RF 2 [97]. These effects are evidently related to the same interaction of streptomycin with the ribosomal 30 S subunit which causes inhibition of initiation. However, many other effects of streptomycin ('stuck' 70 S ribosomes, inhibition of ribosome dissociation, killing effect, etc.) might or might not be related to the inhibitory effect of the antibiotic in the last reaction of the initiation phase [10–12; reviews].

A number of other aminoglycoside antibiotics (gentamycin, hygromycin B, kanamycin, neomycin and paromomycin) cause misreading in intact bacteria [98] and cell-free systems [99,100] and act on the 30 S ribosomal subunit at or near the streptomycin site [101]. Tobramycin also causes misreading in bacterial cell-free systems (San Millan, M.J. and Modolell, J., unpublished observations).

Pactamycin blocks protein synthesis by bacterial and mammalian systems [102,103] by interacting with the ribosomes [104] in one site on the smaller subunit [105,106]. Studies on bacterial and mammalian systems suggest that pactamycin is an inhibitor of the initiation phase [107–109]. Steps (a)–(c$_1$) are not inhibited by pactamycin, since the initiation complex is formed but the substrate is not reactive with puromycin, and therefore it is likely that the antibiotic blocks step (c$_2$) [110–112]. At higher

concentrations pactamycin also inhibits elongation, probably owing to a second site interaction of the antibiotic [106].

Step (d)

The tetracycline antibiotics block protein synthesis by bacterial and mammalian systems [113,114]. Early works on tetracycline binding showed many interaction sites of the antibiotic with both ribosomal subunits [115–117]. Since these antibiotics were found to prevent non-enzymic binding to bacterial ribosomes [118,119] and to their 30 S subunits [66,120,121], it was assumed that the main effect of these antibiotics was on the small ribosomal subunit. However, the EF T-dependent binding of aminoacyl-tRNA is inhibited by much lower concentrations of the tetracyclines than the non-enzymic binding [122–125]. The EF T-dependent GTP hydrolysis is not affected by the tetracyclines [93–95]. This enzymic binding is obviously the step inhibited by the tetracyclines. However, it is not yet clear whether the interactions of these antibiotics with the smaller or the larger ribosomal subunit are more relevant for their inhibitory effect on protein synthesis. Since EF T interacts with the 50 S subunit [123], and recent binding studies with tetracycline suggest that its interaction with the 50 S subunit might be more relevant for the antibiotic action [127,128]. Tetracycline has also been shown to block the termination phase of protein synthesis, probably by preventing the recognition of the release factors [129,130].

Antibiotics of the thiostrepton group include thiostrepton (synonyms, bryamycin and thiactin) and the related compounds siomycin, thiopeptin and sporangiomycin, all of which appear to have a similar mode of action. Early genetic studies suggested that thiostrepton action might be located on the ribosome [131]. This was confirmed by biochemical studies and these antibiotics were shown to interact with the 50 S subunit [132–135]. These antibiotics inhibit translocation [133,136] and EF G-dependent GTP hydrolysis [134,137–139] in model systems by preventing interaction of EF G with the ribosome [134], and were therefore postulated as specific inhibitors of the translocation step. However, it was observed in further studies that the thiostrepton antibiotics inhibit both enzymic [126,138] and non-enzymic binding of aminoacyl-tRNA to the ribosomal A-site in cell-free systems [126,139]. Indeed, comparative studies showed that these antibiotics inhibit the EF G-dependent reaction and EF T-dependent binding of aminoacyl-tRNA in cell-free systems [126,139,140] and in intact bacteria [141] to a similar extent. Unlike the tetracyclines, the thiostrepton antibiotics block the GTP hydrolysis coupled to EF T-dependent aminoacyl-tRNA binding [126]. Since thiostrepton does not inhibit translocation in intact bacteria [141, 142], the main target reaction of the antibiotic is the aminoacyl-tRNA binding [126,143]. The non-enzymic binding of aminoacyl-tRNA to the A-site is sensitive to siomycin [126,139], whereas the uncoupled EF Tu-dependent GTP hydrolysis is insensitive to thiostrepton [144], indicating that the antibiotics block the A-site on the 50 S subunit competing with EF G and aminoacyl-tRNA. Furthermore thiostrepton enhances uncoupled EF T- or EF G-dependent GTP hydrolysis by 50 S-derived cores lacking the ribosomal proteins L7 and L12 [145], showing that these proteins are not directly involved in the binding of the antibiotic [146,147]. There is one report in which inhibition of IF 2-dependent GTP hydrolysis by thiostrepton was observed [148], which would favour the possibility of a single GTPase centre on the ribosome, as previously suggested [126,143].

Fusidic acid was shown to prevent translocation, EF G- and EF 2-dependent GTP hydrolysis by bacterial and mammalian cell-free systems [149–153]. However, EF G-dependent GTPase is resistant to the antibiotic in mutants resistant to fusidic acid [154–156]. EF G as a target for the antibiotic action was also suggested by preliminary binding experiments using the tritiated antibiotic [157]. In the presence of ribosomes, GTP and either EF G or EF 2, fusidic acid forms a stable complex fusidic acid–GDP–ribosome–EF G (or EF 2), which appears to account for the inhibitory effect of the antibiotic on translocation [158,159]. There is, however, increasing evidence that the primary effect of fusidic acid on protein synthesis in intact cells is not the inhibition of translocation: for fusidic acid-inhibited polysomes, formed on synthetic or viral RNA or an endogenous messenger, have their nascent peptides in the puromycin-reactive position [142,160–162]. Furthermore, formation of the complex GDP–ribosome–

fusidic acid–EF G [163–166] prevents aminoacyl-tRNA binding. Essentially similar results were obtained in studying aminoacyl-tRNA binding when the complex GDP–EF 2–ribosome–fusidic acid [167,168] was formed. Inhibition of aminoacyl-tRNA binding by fusidic acid appears to be a consequence of its effect in sequestring all the EF G or EF 2 available, owing to the formation of the GDP–ribosome–fusidic acid–EF G (or EF 2) complex, as postulated, [162,163] since the effect of the antibiotic in cell-free systems can be decreased or abolished by saturating concentrations of EF G [169] or EF 2 [167], but can be enhanced by increasing the concentration of free ribosomes [167].

Step (e)

Puromycin is an analogue of the 3′ terminal end of aminoacyl-tRNA [170]. However, puromycin lacks that part of the aminoacyl-tRNA molecule responsible for interaction with template and the small ribosomal subunit. Because of this, the use of puromycin provides a simplified method for the study of peptide bond formation in a reaction in which the α-NH_2 group of puromycin becomes linked to the C-terminal end of f-Met- or the peptidyl group ('puromycin reaction'). The product of the puromycin reaction (f-Met- or peptidyl-puromycin) is unable to take part in the next step of protein synthesis. However, all the evidence indicates that the formation of a peptide bond between puromycin and the f-Met or peptidyl group takes place by the same mechanism as peptide bond formation in protein synthesis [171,172]. Polyphenylalanyl-tRNA [172,173], polylysyl-tRNA [174,175], f-Met-$tRNA_F$ [176,177] and Ac-Phe-tRNA [152] are suitable donor substrates in the puromycin reaction. The terminal fragments CACCA–, AACCA–, ACCA– and CCA–Met-f from f-Met-$tRNA_F$ undergo a ribosome-catalysed reaction with puromycin to yield f-Met-puromycin in a simplified system known as the 'fragment reaction'. The reaction requires only the large ribosomal subunit, monovalent and divalent cations and either methanol or ethanol [178,179]. The small ribosomal subunit or mRNA is not required, but they can stimulate the peptidyl transferase activity of the large subunit in certain experimental systems [180]. The structural requirement for puromycin-like activity as an acceptor substrate has been studied by using a number of puromycin derivatives and aminoacyl-nucleosides [181–187]. In cell-free systems Phe-, Tyr- and Leu-adenosine are as active as puromycin as acceptor substrates in the peptide bond formation reaction [182–186]. The puromycin derivatives α-hydroxy- and demethoxy-α-hydroxypuromycin are of special significance. These compounds lack the α-amino group of puromycin and are therefore unable to form a peptide bond, but they form an ester bond with the f-Met moiety of f-Met-tRNA or CAACCA–Met-f in a reaction catalysed by the peptidyl transferase centre of the ribosome [188]. The kinetics of the puromycin reaction have been studied in the 'fragment reaction' assay and in a polysomal system [189, 190]. There is a weak interaction of puromycin with the acceptor site of the peptidyl transferase centre which is inhibited by Phe-adenosine [191]. Reports on affinity labeling studies of puromycin derivatives have appeared [192,193], one of them suggesting that Br-puromycin binds to the RNA of the larger ribosome subunit [193].

Inhibition of protein synthesis by chloramphenicol was shown in earlier work, in intact bacteria [1,2] and confirmed in different cell-free systems [3,194–196]. The antibiotic binds to ribosomes from bacteria [197], chloroplasts and blue-green algae [198,199] on the 50 S ribosome subunit [200]. One binding site for the antibiotic on the ribosome was initially proposed [201–203] and subsequently demonstrated [204], but two binding sites have also been proposed [202,205]. Chloramphenicol is an inhibitor of peptide bond formation, as is shown by studies in intact bacteria [103] and cell-free systems [206–211]. This was in fact observed in many systems, since chloramphenicol prevents the reaction of either polyphenylalanyl-tRNA [171–173], or polylysyl-tRNA [174,175,206,207], or f-Met-$tRNA_F$ [208], or Ac-Phe-tRNA [175,209–211], or dipeptidyl-tRNA [211], with puromycin. However, the most conclusive evidence for the inhibitory effect of chloramphenicol on the peptidyl transferase centre was observed in the 'fragment reaction' [212,213], since it is the most fully resolved assay for peptide bond formation. The inhibitory effect of chloramphenicol in this system was found to be a mixed competitive

effect for puromycin [189], which is in agreement with previous results on the puromycin reaction [206], although the same conclusions were not reached by another group [214,215]. Indeed chloramphenicol was found to have no effect on substrate binding (CACCA–Leu–Ac) to the donor site of the peptidyl transferase centre [213–215], but it inhibits binding of either puromycin [191] or aminoacyl-oligonucleotides to the acceptor site of the active centre [218–222]. Studies on reconstitution and affinity labeling suggest that protein L16 is involved in chloramphenicol binding [223,224].

Lincomycin inhibits protein synthesis by bacteria and bacterial cell-free systems [196,225,226], the antibiotic interacting with the larger ribosomal subunit [196,204,226,226a]. Lincomycin affinity to ribosomes from Gram-negative bacteria is very slight [226], but this affinity is considerably increased in the presence of ethanol [204]. Lincomycin inhibits peptide bond formation [213] and prevents binding of the 3′ terminal end of substrates to the donor [216–218] and to the acceptor [218,221,222] site of the peptidyl transferase centre.

Antibiotics of the streptogramin A group (streptogramin A, mikamycin A, virginiamycin M_1, vernamycin A, ostreogrycin A, synergistin A) inhibit protein synthesis by bacteria and bacterial cell-free systems [197,227–232], by preventing peptide bond formation, as shown in the 'fragment reaction' assay [213] and confirmed in other systems [209,233]. These antibiotics interact with the 50 S ribosome subunit [64,197,234] and prevent binding of CACCA–Leu–Ac to the donor-site [216,217] end of aminoacyl-oligonucleotides to the acceptor-site [219–222] of the peptidyl transferase centre. They have also been reported to inhibit EF T-dependent aminoacyl-tRNA binding without affecting the GTP hydrolysis [126,235] and non-enzymic binding of f-Met-$tRNA_F$ [236]. These effects might be due to the inhibition by these antibiotics of binding of the 3′ terminal end of the substrates, as indicated above. Indeed these results may also explain the reported polysome breakdown by these antibiotics [237,238], which is contrary to the results observed with most of the inhibitors of peptidyl transferase.

The macrolide antibiotic spiramycin III and its derived compounds neospiramycin III and forocidin III have been shown to block protein synthesis by intact bacteria [239] and bacterial cell-free systems [196,239]. There is ample evidence for the interaction of spiramycin III with the larger ribosomal subunit of sensitive bacteria [64,197,239–242]. Spiramycin-resistant mutants do not bind the antibiotic [242] and have an altered protein L4 [243]. Spiramycin III blocks peptide bond formation in different experimental systems [209,211,213,244,245], although it is a very poor inhibitor of the puromycin reaction when f-Met-$tRNA_F$ is used as the donor substrate [246]. Binding of both the donor [216–218] and the acceptor [219–222] substrate to the peptidyl transferase centre is inhibited by the antibiotic. Spiramycin III, like streptogramin A, causes polysome breakdown [237]. The macrolide antibiotics carbomycin, leucomycins, niddamycin, tylosin and relomycin appear to have a mode of action similar to that of spiramycin III [196,197,211,213,244, 246,247].

With the single exception of puromycin, the peptidyl transferase inhibitors mentioned above act selectively on ribosomes of the prokaryotic type, whereas amicetin [248–252], gougerotin [250,253–256], blasticidin S [251,257–259], actinobolin [251,252, 260] and sparsomycin [102,261,262] act on ribosomes of the prokaryotic and the eukaryotic type. Sparsomycin is the best known antibiotic of this group. It acts on the ribosome [263] in the larger ribosome subunit [213] and it has a reduced inhibitory effect on chloramphenicol binding [205,264]. Sparsomycin is an inhibitor of the peptide bond formation step since it inhibits the puromycin reaction in bacterial [206,265–267], yeast [251], mammalian [252] and mitochondrial ribosomes [268]. Furthermore, sparsomycin is a powerful inhibitor of the 'fragment reaction' assay by bacterial [213], yeast [251] and mammalian [252,269] ribosomes. Sparsomycin appears to act on the acceptor-site of the peptidyl transferase centre since it inhibits, to a certain extent, EF T-dependent binding of aminoacyl-tRNA, uncoupling GTP hydrolysis [270], and also prevents binding of CACCA–Phe [220,221,271]. On the other hand, sparsomycin enhances non-enzymic binding of Ac–Phe-tRNA to the donor site [272], even in the absence of any mRNA [273,274], and CACCA–Leu–Ac [275]. Amicetin, actinobolin, blasticidin S and gougerotin also inhibit peptide bond formation in the puromycin [209,249,265,276,277] and the frag-

ment [213,251,278] reaction assays. These antibiotics resemble sparsomycin in their mode of action, although they are less active. These antibiotics have some inhibitory effect on binding of the acceptor substrate [220,221,271,277,278], and enhance binding of the donor substrate [216,217,277,278] to the peptidyl transferase centre. Furthermore, blasticidin S binds to the larger ribosomal subunit; and this binding is inhibited by gougerotin [279] while, conversely, gougerotin binding to the ribosome is prevented by blasticidin S and also by actinobolin, amicetin and sparsomycin (Barbacid, M. and Vazquez, D., unpublished observations).

Anisomycin inhibits protein synthesis by eukaryotic systems [280,281] specifically blocking peptide bond formation in the 'fragment reaction' [179,251, 252,269] and in the puromycin reaction [252] assays. Anisomycin was reported to be a competitive inhibitor of puromycin [282]. Anisomycin specifically binds to the 60 S ribosome subunit; this binding is inhibited by trichodermin and tenuazonic acid (Barbacid, M. and Vazquez, D., unpublished observations).

Sesquiterpene antibiotics of the trichodermin group are also specific inhibitors of protein synthesis by 80 S type ribosomes [283–286]. These antibiotics inhibit the puromycin and the 'fragment reaction' assays [285]. Trichodermin binds to the 60 S ribosomal subunit and this binding is inhibited by anisomycin and tenuazonic acid (Barbacid, M. and Vazquez, D., unpublished observations). All the experimental evidence suggests that the trichodermin antibiotics act on the same ribosomal site as anisomycin.

Tenuazonic acid acts on the 60 S ribosome subunit at the same site as anisomycin and trichodermin. Therefore it is an inhibitor of mammalian protein synthesis [287], which specifically prevents peptide bond formation [44,252,269]. A very important characteristic of tenuazanic acid is the preferential inhibition in mammalian systems, since the antibiotic has less effect on plant ribosomes and no significant effect on yeast ribosomes, as has been determined by studying inhibition on protein synthesis [44] and on [^{3}H]anisomycin binding [288].

Griseoviridin is an interesting antibiotic which has been considered as an antibacterial agent since it is not active on any of the cells of the eukaryotic type

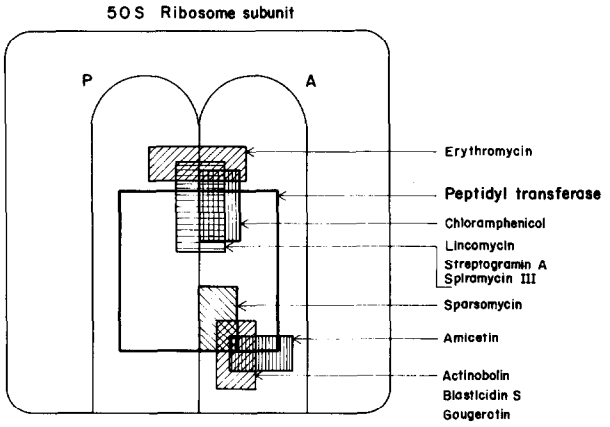

Fig. 3.

in which it has been tested 289]. However, griseoviridin is an active inhibitor of protein synthesis in mammalian systems [288]. This antibiotic prevents peptide bond formation by bacterial [290] and mammalian ribosomes [288]. Furthermore, griseoviridin has a unique synergistic effect with gougerotin at the ribosome level, since it enhances the binding of this antibiotic in cell-free systems up to 400% (Barbacid, M. and Vazquez, D., unpublished observations).

In view of what is known about the mode of action of antibiotics acting on the peptidyl transferase centre and the different studies on antibiotic competition for binding sites, the site of action of these antibiotics on the larger subunit can be represented schematically in bacterial (fig. 3) and mammalian (fig. 4) ribosomes. Although erythromycin is not

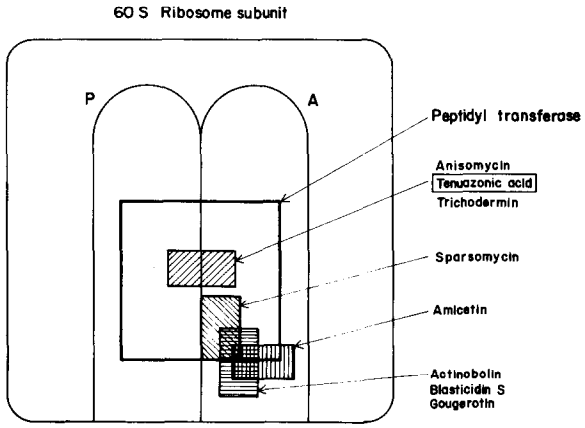

Fig. 4.

considered as an inhibitor of peptide bond formation, it is presented as overlapping the binding sites of chloramphenicol and lincomycin, since it blocks binding of these antibiotics [197,264] and prevents their effect on the peptidyl transferase centre [216, 226a,291].

Step (f)

Erythromycin inhibits protein synthesis in intact bacteria and bacterial cell-free systems [196,292, 293]. One molecule of erythromycin binds to sensitive ribosomes on the larger subunit [292,294–297]. It prevents chloramphenicol [197], lincomycin [226a] and spiramycin III [239] binding and vice versa [204]. These results and the reported inhibition of erythromycin on the puromycin [210,211,244,245, 298] and the fragment [247] reaction assays when certain donor substrates are used, suggest that erythromycin is an inhibitor of peptide bond formation. However, erythromycin does not inhibit peptide bond formation in most of the model systems. Moreover, some studies in intact cells [103] and cell-free systems [141,299,410] suggest that erythromycin is an inhibitor of translocation although it does not inhibit this step in most of the model systems. Clearly, the problem of erythromycin as an inhibitor of peptidyl transferase or translocation is still unresolved. Some *E. coli* mutants resistant to erythromycin have been observed to have an altered L4 protein and do not bind the antibiotic [300–302]. However, in other resistant mutants protein L22 is altered, and yet erythromycin binds to the ribosome [303]. On the other hand, induced and constitutive resistance to the antibiotic has been associated with the modification of 23 S rRNA in which formation of dimethyladenine has been observed [304,305].

Although cycloheximide was earlier found specifically to block eukaryotic protein synthesis by intact cells [306] and cell-free systems [104,307–310], little is known about its controversial site and mode of action. Apart from protein synthesis, cycloheximide has an inhibitory effect on DNA and RNA synthesis [8] in certain intact cells. Furthermore, inhibition by cycloheximide of RNA polymerase I from some fungi has been reported [311,312]. Certain results, initially obtained in hybridization experiments with cycloheximide-treated and untreated fractions, suggested the supernatant fraction as the site of cycloheximide action [104,313], whereas similar experiments in more resolved systems suggested the ribosome as the cycloheximide target [251]. Indeed, the ribosome is now widely accepted as the site of interaction of cycloheximide after hybridization experiments using strains resistant to the antibiotic [314–317]. It was concluded that the 60 S subunit, but not the 40 S, is responsible for the resistance to the antibiotic [316,317]. There is also disagreement concerning the reaction in protein synthesis inhibited by cycloheximide. Cycloheximide inhibits not only breakdown, but also reassembly of the polysomes [102,318–320], and this suggests that it might inhibit more than one reaction. In fact cycloheximide, at low concentrations, prevents polysome formation, but inhibits translocation at higher concentrations [321–323]. Inhibition of peptidyl-tRNA translocation by cycloheximide has been observed in certain cell-free systems [324,325], but the specific step blocked by the antibiotic in the initiation phase is still undetermined [325], although it inhibits reassociation of the ribosome subunits [326]. Despite numerous reports indicating that cycloheximide does not inhibit peptide bond formation [179,251,269, 324,325] there is a recent study postulating that peptidyl transferase is the target of cycloheximide [282]. Furthermore, the antibiotic has even been postulated as an inhibitor of chain termination [327].

Diphtheria toxin is a protein (mol.wt. 62 000) which, upon reduction of a disulphide bridge and hydrolysis of a peptide bond, leads to two polypeptides (mol.wt. 24 000 and mol.wt. 38 000). This hydrolysis can be carried out in cell-free systems, but it also takes place when the diphtheria toxin enters a eukaryotic cell. The largest polypeptide fragment is involved in the penetration of the toxin into the cell, whereas the small one inhibits protein synthesis [11]. Diphtheria toxin inhibits amino acid incorporation by mammalian cell-free systems [328]. NAD is absolutely required for the inhibitory action of the toxin [329], which takes place by inactivation of EF 2 [330,331] and therefore prevents translocation [252,332]. The catalytic inactivation of EF 2 by diphtheria toxin takes place according to the reaction:

$$\text{EF 2}+\text{NAD}^+ \rightleftharpoons \text{ADP-ribose-EF 2-nicotinamide}+\text{H}^+$$

[333,335]

Diphtheria toxin has been reported to inhibit formation of the ribosome–EF 2–GTP complex [336,337] and EF 2-dependent hydrolysis of GTP [333,336]; but inhibition by the toxin of either ternary complex formation [338] or GTP hydrolysis [339] has not been confirmed by other workers. Although there are numerous reports showing a lack of effect of diphtheria toxin on bacterial systems [11], one group has repeatedly reported an effect of the toxin in bacterial cell-free systems [340].

Pederine inhibits protein synthesis in eukaryotic cells [341–344] and cell-free systems [343,345]. The site of action of this compound is located on the ribosome [343,345]. Pederine at low concentrations causes reticulocyte polysome disaggregation, whereas at high concentrations prevents polysome breakdown and only partially inhibits release of growing peptides by puromycin [344]. It was, therefore, suggested that pederine might act at more than one site on the ribosome, by inhibiting mainly initiation and to a lesser extent translocation [344]. Studies on model systems have indeed clearly demonstrated an inhibition of translocation even by low concentrations of pederine [252].

The *Tylophora* alkaloids cryptopleurine, tylocrebrine and tylophorine are closely related in their chemical structure and mechanism of action. They are active in blocking protein synthesis by eukaryotic cells [345] and cell-free systems [346]. These alkaloids bind to eukaryotic ribosomes [346] on their 60 S subunit [251] but were reported not to affect peptide bond formation [251]. These alkaloids have been shown to block the translocation step in cell-free systems from rabbit reticulocytes [347] and human tonsils (Carrasco, L. and Vazquez, D., unpublished results). In another work, however, it is reported that cryptopleurine is an inhibitor of peptide bond formation [282]; but this might be an artefact of the system since cycloheximide is reported in the same study, to prevent peptide bond formation, and this finding contradicts all the previous observations by other workers.

Step (g)

As a consequence of their interaction with the ribosome, streptomycin, and tetracycline have been reported to block the termination phase of protein synthesis by bacterial ribosomes since these antibiotics inhibit the release factor(s)-dependent codon recognition [11,97,130,348,349]. Similarly thiostrepton is also an inhibitor of termination in bacterial systems probably by preventing the interaction of the release factor(s) with the ribosome [350].

Peptidyl transferase is widely accepted to be involved not only in peptide bond formation (step (d)), but also in the peptidyl-tRNA hydrolysis reaction required for the termination phase. In fact all the peptidyl transferase inhibitors which have been tested, block termination in bacterial [11,97,130,348, 349,351] and in mammalian [11,349,351,352] systems, with the same specificity shown in peptide bond formation (figs. 1–4).

There is a report claiming that trichodermin might be a specific inhibitor of termination in eukaryotic protein synthesis [353]. However, it has been shown that trichodermin inhibits peptide bond fomation as well as termination [255,354]. Therefore specific inhibitors of the termination phase are not yet known.

3.4. *Miscellaneous inhibitors of translation*

A number of aminochromes [355] and catechols (homogentisic acid and related compounds) [356] have been reported to inhibit poly U-directed phenylalanine incorporation by mammalian cell-free systems. Adrenochrome is also active in *E. coli* cell-free systems at the ribosome level [357]. It inhibits aminoacyl-tRNA binding to human tonsil ribosomes [252]. However it is not yet known if this inhibition is due to an effect of adrenochrome in the codon–anticodon interaction. Therefore adrenochrome, besides inhibiting step (d), might be an inhibitor of the initiation phase.

Althiomycin inhibits protein synthesis by bacterial systems blocking the peptide bond formation step as shown in the puromycin [358] and the 'fragment reaction' assays (Cabrer, B. and San Millan, M.J., unpublished observations), and in the reaction of ribosome attached peptides with CACCA–Phe [359]. Althiomycin also blocks peptide bond formation in the intact bacteria [360].

Bacteriocin DF_{13} is a protein, (mol.wt. 56 000) produced by *Enterobacter cloacae,* the primary effect of which is the inhibition of protein synthesis in

intact bacteria [361]. Inhibition of MS2-RNA-directed amino acid incorporation by bacteriocin DF_{13} has also been obserbed in cell-free systems [362].

Berninamycin is a peptide antibacterial antibiotic which inhibits protein synthesis in intact bacteria and bacterial cell-free systems [363]. Chartreusin is an antibiotic active in bacterial and eukaryotic systems. It prevents the enzymic binding of aminoacyl-tRNA to the ribosome [364].

Bottromycin A_2 inhibits protein synthesis in intact cells and cell-free systems [365,366]. Bottromycin A_2 acts on the larger ribosome subunit but has no effect on [^{14}C]chloramphenicol binding [367]. The step mainly inhibited by bottromycin A_2 is not yet known, although there are data suggesting an inhibition by the antibiotic of translocation [368] and step (c_2) in initiation [369]. Bottromycin A_2 has also been reported to inhibit the puromycin [359] and the fragment [369] reactions but only to a limited extent.

Adsorption of colicin E_3 by sensitive bacteria results in a specific inhibition of protein synthesis [370] by inactivating the 30 S but not the 50 S ribosomal subunit [371]. This inactivation of the 30 S subunit in intact bacteria is due to a specific cleavage of the 16 S rRNA near the 3′ terminal. Studies on cell-free systems have confirmed the cleavage by purified preparations of colicin E_3 of the 16 S ribosomal RNA in the same position as it was previously observed working in intact cells [372,373]. Both the 30 S and the 50 S ribosome subunits are required for this inactivation [374] which is inhibited by streptomycin, by tetracycline [375] and by an immunity factor prepared from extracts derived from colicinogenic cells [376]. Bacteria other than *Enterobacteriaceae*, are resistant to colicin E_3 but their ribosomes are sensitive in cell-free system [377]. Furthermore 80 S type ribosomes from mouse ascites cells are sensitive to colicin E_3 in cell-free systems [378]. Both the 40 S and the 60 S subunit are required for this inactivation, but only the 60 S subunit is damaged by colicin E_3 [378]. Colicin E_3 damaged ribosomes are inactive in EF 2-dependent binding of aminoacyl-tRNA [378].

Emetine is an inhibitor of protein synthesis by 80 S type ribosomes. Emetine was proposed early on as a structural analogue of cycloheximide [379] but this was never confirmed by experimental evidence. Contrary to cycloheximide, direct interaction of emetine with the ribosomes has never been observed [251,380]. Enomycin inhibits protein synthesis by eukaryotic systems [251,381]. The antibiotic binds to the 40 S and the 60 S subunits [382].

The locus concerned with resistance to micrococcin was mapped early on and shown to be located in a region of the *Bacillus subtilis* genome containing a number of ribosomal proteins, suggesting that the antibiotic might be an inhibitor of protein synthesis [383]. Micrococcin has indeed been shown to prevent protein synthesis. The antibiotic has been proposed as an inhibitor of translocation [360].

Multhiomycin is a sulphur containing antibiotic. It is an antibacterial compound which interacts with the ribosome and inhibits EF T-dependent aminoacyl-tRNA binding and GTP hydrolysis [384,385]. All the evidence suggests that multhiomycin can be considered as a member of the thiostrepton group but the molecule is certainly smaller. Phenomycin is a basic polypeptide antibiotic very active as an inhibitor of protein synthesis on eukaryotic cells and cell-free systems. Little more is known about the mode of action of this antibiotic [386].

Antibiotics of the streptogramin B group (viridogrisein, staphylomycin S, streptogramin B and synonyms) inhibit protein synthesis by bacterial cell-free systems [197,229–231]. Although it was shown early on that these antibiotics act on the 50 S ribosome subunit [197,264,386a], little is known about the step specifically inhibited by these compounds. There is a study showing stimulation of f-Met-puromycin and inhibition of f-Met-Ala-puromycin by streptogramin B [386b] and therefore suggesting that formation of the first and the second peptide bond might be somehow different.

Tubulosine is an alkaloid which specifically prevents protein synthesis by eukaryotic cells and cell-free systems [387]. Although it has been initially proposed that tubulosine might have a similar mode of action to cycloheximide, further studies do not support this hypothesis [316].

Spectinomycin is an antibacterial aminoglycoside antibiotic which inhibits protein synthesis but, contrary to those of the streptomycin group, does not cause misreading [388,389]. Resistance to spectinomycin resides on the 30 S ribosome subunit

[388,390] and is due to an alteration of protein S5 [302,390,392]. Since protein S5 is somehow involved in the EF T- and EF G-dependent GTPase, spectinomycin has been proposed as an inhibitor of translocation [393]. Recent results show that spectinomycin is indeed an inhibitor of translocation in intact bacteria [360].

The compound *N*-tosyl-L-phenylalanylchloromethane is an effective inhibitor of bacterial cell-free systems and has a selective action on EF T [394, 395]. Ricin and abrin are two phytotoxin proteins (mol.wt. 65 000) of different origin which appear to have a similar mode of action [396,397]. Both toxins are inhibitors of protein synthesis by eukaryotic cells [398] and cell-free systems [399]. Although a preliminary report has suggested that the action of ricin was probably located at the ribosome level [400] recent studies in more resolved systems have shown that both ricin and abrin inactivate EF 1 and EF 2 and therefore inhibit binding of aminoacyl-tRNA as well as translocation (Carrasco, L. and Vazquez, D., unpublished observations).

Showdomycin is a maleimide derivative which reacts with sulphydryl groups. Therefore this antibiotic inhibits numerous reactions and is not a selective agent in intact cells. However showdomycin has been very useful in the study of the role of –SH groups, of EF 2 and the 60 S ribosome subunit, in elongation [401,402]. Other little studied inhibitors of protein synthesis are viomycin [403,404], kirromycin [405] and rubradirin [406].

The GTP analogue guanylyl methylenediphosphonate has a methylene group in place of oxygen between the β and γ phosphorus atoms thus preventing enzymic cleavage at this position without major alteration of configuration. The structure of this analogue was designed to test the nature of the reactions involving GTP hydrolysis in protein synthesis. Since this GTP analogue was first shown to block poly U-directed polyphenylalanine synthesis [407] it has been repeatedly used in studies on protein synthesis in the different steps of the initiation, elongation and termination phases in which GTP hydrolysis is involved [11,408,409; reviews].

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