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With 61 Figures

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List of Abbreviations

cAMP	Cyclic adenosin-monophosphate
RNA	Ribonucleic acid
mRNA	Messenger ribonucleic acid
MDMP	2-(4-methyl-2,6-dinitroanilino)-N-methyl-propionamide
-2'(3'),5'-ADP	2'(3'),5'-Adenosintriphosphate
PAP	Phytolacca americana protein
TPCK	1-Chloro-4-phenyl-3-tosylamido-2-butanone
PA toxin	Pseudomonas aeruginosa toxin
f-Met-$tRNA_F$ or fMet-$tRNA_F$	Initiator formyl-methionyl-tRNA of prokaryotes
Met-$tRNA_F$	Initiator methionyl-tRNA of eukaryotes
GTP	Guanosintriphosphate
IF-1	Bacterial initiation factor 1
IF-2	Bacterial initiation factor 2
IF-3	Bacterial initiation factor 3
eIF-1	Eukaryotic initiation factor 1
eIF-2	Eukaryotic initiation factor 2
eIF-3	Eukaryotic initiation factor 3
eIF-4A	Eukaryotic initiation factor 4A
eIF-4B	Eukaryotic initiation factor 4B
eIF-4C	Eukaroytic initiation factor 4C
eIF-5	Eukaroytic initiation factor 5
$m^7G(5')ppp$ or $m^7G5'ppp$	"cap" or 7-methylguanosine-5'-triphosphate
SV40	Simian virus 40
$m^7G5'p$	7-Methylguanosine-5'-monophosphate
tRNA	Transfer ribonucleic acid
rRNA	Ribosomal ribonucleic acid
EF-2	Elongation factor 2

ATP	Adenosin-triphosphate
Ac-Phe	Acetyl-phenylalanine
EF-G	Elongation factor G
GTPase	GTPasa
EF-Ts	Elongation factor Ts
Poly(U)	Polyuridilyc acid
DNA	Deoxyribonucleic acid
$m^7G^{5'}pp$	7-Methylguanosine-5'-diphosphate
AMP	Adenosin-monophosphate
GMPPCP or	Guanylyl methylenediphosphonate
GDPCP	
GDPNP	Guanylyl imidodiphosphate
EF-T	Elongation factor T
RF-1	Release factor 1
RF-2	Release factor 2
RF-3	Release factor 3
m^7G	7-Methylguanosine
$5'pm^7G$	5'-Phosphate-7-methylguanosine
Sm^D	Streptomycin dependent
EF-Tu	Elongation factor Tu
EF-1	Elongation factor 1
C. diphtheriae	Corynebacterium diphtheriae
NAD	Nicotinamide-adenine dinucleotide
PRT	Penicillium roqueforti toxin

Contents

Chapter 1
Protein Synthesis and Translation Inhibitors

1. Introduction

Studies concerning selectivity, site, and mode of action of translation inhibitors have been widely developed and the subject has been reviewed repeatedly in the last fifteen years (Gale, 1963; Newton, 1965; Newton and Reynolds, 1966; Gottlieb and Shaw, 1967a; Vázquez and Monro, 1967; Weisblum and Davies, 1968; Pestka, 1971; Muñoz, García-Ferrandiz and Vázquez, 1972; Gale et al., 1972; Kaji, 1973; Vázquez, 1974; Corcoran and Hahn, 1975; Pestka, 1977; Vázquez, 1978a). This study will be concerned mainly with the present state of the problem. A complete survey of the literature will not be possible in this contribution and the above reviews should be very useful to readers interested in different aspects of the problem. Furthermore, there are a number of reviews concerning the chemistry, biosynthesis, toxicology, inhibitory spectra of the different inhibitors (Korzybski, Kowszyk-Gindifer and Kurylowicz, 1967a, 1967b; Gottlieb and Shaw, 1967a, 1967b; Glasby, 1976) and mechanism of drug resistance (Benveniste and Davies, 1973a; Mitsuhashi, Rosival and Krčméry, 1975; Mitsuhashi, 1977; Mitsuhashi and Hashimoto, 1977) which should be very useful to readers who are concerned with these topics.

Specific inhibitory effects on bacterial protein synthesis by chloramphenicol and chlortetracycline, at their

minimal growth inhibitory concentrations, were first described by Gale and Paine (1950a, 1950b). At those concentrations the antibiotics did not affect respiration, fermentation, or amino acid accumulation (Gale and Paine, 1950b), but caused an immediate cessation of protein synthesis and an increase in the rate of nucleic acid accumulation in bacteria (Gale and Folkes, 1953). Similar effects were later observed in bacteria treated with a number of translation inhibitors (Gale, 1963; Newton, 1965; Newton and Reynolds, 1966; Gottlieb and Shaw, 1967a; reviews). The mechanism of protein synthesis remained obscure during the period 1950-1960, so that it was not possible to establish the site of action of the known translation inhibitors. A resolved cell-free system to study ribosomal amino acid incorporation directed by a synthetic polynucleotide such as mRNA was first described in 1961, and the specific inhibitory effect of chloramphenicol was confirmed in this system (Nirenberg and Matthaei, 1961). Model systems to study the individual reactions in protein synthesis were further developed in the following years and thus the specific steps blocked by different inhibitors of translation were elucidated.

Reports concerning inhibition of translation in higher cells did not appear until 1958, when the inhibitory effect of cycloheximide on protein synthesis in Saccharomyces carlsbergensis was described. Contrary to what was found in chloramphenicol-treated bacteria, the stringent control of nucleic acid synthesis in yeast was not abolished by cycloheximide and an inhibition of nucleic acid synthesis was also observed in the presence of the antibiotic (Kerridge, 1958). Similar results were later observed in higher cells treated with other translation

inhibitors (Newton and Reynolds, 1966; Gottlieb and Shaw, 1967a; reviews). Cell-free systems and model reactions to study protein synthesis have been developed in the last twenty years and the reactions blocked by the different inhibitors were elucidated.

For the sake of clarity we have adopted for the protein factors involved in translation the nomenclature developed at the International Symposia on Protein Synthesis held at the National Institutes of Health (Bethesda, USA) in 1971 and 1976 (Anderson et al., 1977). The nomenclature for the proteins of the bacterial and mammalian ribosomes is adopted from early studies on the subject (Kaltschmidt and Wittmann, 1970; Sherton and Wool, 1972).

2. Site of Action of Protein Synthesis Inhibitors

The process of protein synthesis can be arbitrarily divided into (a) steps taking place in early reactions in protein synthesis and (b) steps in the translation mechanisms taking place at the ribosome level. Following these criteria protein synthesis inhibitors of group (a) can be classified as indicated in Table 1. However, considering the specificity, selectivity, and permeability of the protein synthesis inhibitors, the most important compounds are undoubtedly those included within the ample group of translation inhibitors (see Tables 2, 3, and 4). Therefore we will now refer specifically to this group. The overall reactions inhibited by these compounds are indicated in Figures 1 and 2. However a number of inhibitors have pleiotropic effects on the ribosome and inhibit more than one reaction; in these cases we usually indicate the principal step(s) blocked by the inhibitors. A number of inhibitors are not presented in

Table 1. Inhibitors of protein synthesis acting on steps taking place prior to translation

Inhibitors of aminoacyl-tRNA formation		
Inhibitors of amino acid activation[a]	Inhibitors of amino acid transfer to RNA[a]	Inhibitors which are transferred to tRNA leading to synthesis of abnormal proteins[a]
7-Azatryptophan	Aminoalkyl-adenylates	Ethionine
Tryptazan	Borrelidin[b]	Norleucine
6-Fluorotryptophan	Furanomycin[b]	Alloisoleucine
5-Fluorotryptophan	Minosine[b]	Azetidine-carboxylic acid
Norvaline	4-Oxalysine	
α-Amino-β-clorobutyrate	2,6-Diamino-4-hexynoic acid	Canavanine
α-Aminobutyrate	Trans-4-dehydrolysine	N-Ethylglycine
Selenomethionine		O-Methyl threonine
Ethionine		2-Fluoro-L-histidine[c]
Norleucine		
Methyl-ester of serine		
Ethyl-ester of serine		
Aminoalkyl-adenylates		
Tiramine		
L-Tyrosinol		
L-Tyrosine amide		
L-Tyrosine methyl ester		

[a]Amino acid analogs specifically replace or compete with their corresponding amino acids.

[b]Borrelidin, furanomycin, and minosine specifically inhibit threonyl-, isoleucyl-, and phenylalanyl-tRNA synthesis respectively.

[c]Klein et al. (1977). Other data are taken from Vázquez (1974; review) and references therein.

Table 1 (continued)

Inhibitors of f-Met-$tRNA_F$ formation		
Inhibitors of N^{10}-formyl-H_4 folate synthesis	Inhibitors depleting the pool of N^{10}-formyl-H_4 folate	Analogs of N^{10}-formyl-H_4 folate
Aminopterin	Hydroxylamine	Pyrimidine analogs
Amethopterin (synonym methotrexate)		Pteridine analogs: tetrahydropteroate
Pteroylaspartic acid		N^5-formyl-H_4 folate
Trimethoprim		N^5-methyl-H_4 folate
6-Chloro-8-aza-9-cyclopentylpurine		tetrahydrohomofolate
		tetrahydrohomopteroate

Table 2. Inhibitors of translation acting on prokaryotic systems

- Althiomycin
- Avilamycin
- Berninamycin
- Bottromycin A_2
- Chloramphenicol group:
  - Chloramphenicol
  - D-AMP-3
  - D-Thiomycetin
  - D-Win-5094
- Cloacin DF13
- Colicin E3
- Griseoviridin
- Kasugamycin
- Lincomycin group:
  - Celesticetin
  - Clindamycin
  - Lincomycin
- Macrolide antibiotics:
  - Carbomycin group:
    - Carbomycins
    - Josamycin
    - Leucomycins
    - Niddamycins
  - Erythromycin group:
    - Erythromycins
    - Neospiramycins
    - Oleandomycin
  - Lancamycin group:
    - Chalcomycin
    - Kujimycin A
    - Lancamycin
  - Methymycin group:
    - Forocidins
    - Methymycin
    - Narbomycin
    - Neomethymycin
    - Picromycin
- Micrococcin
- Negamycin
- Rubradirin
- Spectinomycin
- Streptogramin A group:
  - Ostreogrycin G
  - Streptogramin A
- Streptogramin B group:
  - Staphylomycin S
  - Streptogramin B
  - Viridogrisein
- Streptomycin group:
  - Amikamycin
  - Gentamicin
  - Kanamycin
  - Neomycin
  - Paromomycin
  - Sisomicin
  - Streptomycin
  - Tobramycin
- Streptothricins
- Thermorubin
- Thiostrepton group:
  - Siomycin
  - Sporangiomycin
  - Thiopeptin
  - Thiostrepton
- Viomycin group:
  - Capreomycin
  - Viomycin

Table 2 (continued)

Spiramycin group:
Angolamycin
Relomycin
Spiramycins
Tylosin

Table 3. Inhibitors of translation acting on eukaryotic systems

Abrin	Narciclasine group:
Alpha sarcin	Haemanthamine
Anisomycin	Narciclasine
5-Azacytidine	Pretazettine
Bruceantin	PAP
Crotins	Pederine
Curcins	Phenomycin
Diphtheria toxin	Ricin
Emetine group:	Sodium fluoride
Emetine	Tenuazonic acid
Tubulosine	Trichotecene antibiotics:
Enomycin	Trichodermin group:
Glutarimide group:	Fusarenon-X
Actiphenol	Trichodermin
Cycloheximide	Trichodermol
Streptimidone	Trichothecin
Streptovitacin A	Verrucarin A group:
Harringtonine group:	Deacetoxyscirpenol
Harringtonine	Nivalenol
Homoharringtonine	Toxin T-2
Isoharringtonine	Verrucarin A
Lycorine group:	Tylophora alkaloids:
Lycorine	Cryptopleurine
Pseudolycorine	Tylocrebrine
MDMP	Tylophorine

Table 4. Inhibitors of translation acting on prokaryotic and eukaryotic systems

Actinobolin	Hygromycin B
Adrenochrome	Nucleocidin
AHR-1911	Pactamycin
Amicetin group:	Polydextran sulphate
Amicetin	Polyvinyl sulphate
Bamicetin	Puromycin
Plicamicetin	Pyrochatechol violet
Anthelmycin	Showdomycin
Aurintricarboxylic acid	Sparsomycin
Blasticidin S	Tetracycline group:
Chartreusin	Chlortetracycline
Edeine A_1	Doxycycline
Fusidic acid	Oxytetracycline
Gougerotin	Tetracycline
Guanylyl-methylene-diphosphate	Tosylphenylalanylchloromethane
Guanylyl-imido-diphosphate	

Fig. 1. Translation process in bacteria. Site of action of translation inhibitors. ▶
* Do not interact with polysomes. Therefore bind to free ribosome subunits and prevent only the first few rounds of peptide bond formation. ** Is an inhibitor of aminoacyl-tRNA binding in intact cells or in integrated systems in which elongation is proceeding in the presence of EF-Tu and EF-G. Does not inhibit aminoacyl-tRNA binding in resolved systems in the absence of EF-G. Can inhibit translocation in cell-free systems. *** Can also inhibit translocation in cell-free systems. **** Does not inhibit peptide bond formation in resolved assays. However, it blocks this step in intact cells and integrated systems by preventing the release of EF-Tu·GDP bound to the ribosome. ***** Do not interact with polysomes. Do not inhibit translocation in many model systems. Bind to free ribosome subunits and prevent elongation of the nascent polypeptide chain when it reaches a certain size, before polysome formation