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# Mechanism of Action of Streptogramins and Macrolides

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## Summary

Protein synthesis is catalysed by ribosomes and cytoplasmic factors. Bacterial ribosomes (70S) are made up of 2 subunits (50S and 30S) containing ribosomal RNA (rRNA) and ribosomal proteins: the 30S binds messenger RNA and begins the ribosomal cycle (initiation), whereas 50S binds transfer RNA (tRNA) derivatives and controls elongation. The key reaction, peptide bond formation, is promoted by the catalytic centre of 50S (the peptidyl transferase centre), and the growing peptide chain (peptidyl-tRNA) attached at the donor P site undergoes peptide linkage with an aminoacyl-tRNA at the acceptor A site. This reaction is inhibited by several antibiotics, the best known being chloramphenicol, and the macrolide-lincosamide-streptogramin (MLS) group. These inhibitors have a reversible action, except for streptogramins that are composed of A and B components, which are bacteriostatic alone, but bactericidal when combined.

The peptidyl transferase centre has been identified at the 50S surface, and the binding sites of inhibitors have been mapped within this domain: some of these sites overlap (e.g. those of macrolides, and type B streptogramins, which compete for binding to ribosomes). Chloramphenicol blocks the catalytic portion, and A streptogramins the substrate sites of the peptidyl transferase centre. Macrolides and type B streptogramins interfere with the formation of long polypeptides and cause a premature detachment of incomplete peptide chains. The synergism between types A and B streptogramins is due to induction by type A streptogramins of an increased ribosome affinity for type B streptogramins.

Microbial resistance to antibiotics mainly involves inactivation of inhibitors and modification of targets (mutations of ribosomal proteins or rRNA genes). Alterations of rRNA bases can induce resistance to a single inhibitor or to a group of antibiotics (e.g. MLS<sub>B</sub>). The impact of resistance in chemotherapy is less important for streptogramins than for other inhibitors, because the synergistic effect of A and B streptogramins also applies to strains resistant to the MLS<sub>B</sub> group. It is proposed that mutations and modifications of rRNA bases induce conformational ribosomal changes that prevent antibiotics binding to the target. Conformational changes are also triggered by type A streptogramins: they are responsible for their synergism with type B streptogramins.

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## 1. Antibacterial Action of Macrolide-Lincosamide-Streptogramin (MLS) Antibiotics

Antibiotics are classified according to their *in vivo* action on bacteria into the following 3 groups: (i) bacteriostatic, (ii) bactericidal, and (iii) bacteriolytic (see table I).<sup>11</sup> Most protein synthesis inhibitors belong to the first group. Since these antibiotics have a transient action on bacterial growth, prolonged therapy is required to avoid relapse. Antibiotics interfering with nucleic acid synthesis have an irreversible action, whereas those altering the metabolism of other polymers most often cause a reversible inhibition of bacterial multiplication. The last-mentioned group includes inhibitors of the formation of the cell wall (the structure that confers bacterial rigidity and resistance to lysis).

Among protein synthesis inhibitors belonging to the first group are streptogramin components, macrolides, lincosamides, chloramphenicol and others. Streptogramins are unique in that they consist of 2 types of components, A and B, which exhibit a synergistic inhibition of bacterial growth.<sup>12-41</sup> Two aspects of this synergism are noteworthy. The first is quantitative: B components (pristinamycin I; virginiamycin S) undergo a 100-fold increase in activity in the presence of A

components (pristinamycin II; virginiamycin M). The second is qualitative: single streptogramin components are bacteriostatic, whereas their mixture is bactericidal (table II). It has been shown that attachment of type A components to ribosomes induces a conformational change that increases particle affinity for type B components.

Macrolides, the 14-membered subgroup (e.g. erythromycin), and the 16-membered subgroup (e.g. carbomycin and spiramycin), as well as lincosamides (e.g. lincomycin) have a bacteriostatic action. Thus, bacterial protein synthesis is reversibly inhibited by macrolides and lincosamides, and irreversibly inhibited by streptogramins.

Macrolides, lincosamides, and type B streptogramins constitute a major antibiotic group (MLS<sub>B</sub>), because they share similar mechanisms of action and have a common resistance pattern (MLS<sub>B</sub><sup>R</sup>). Streptogramins differ in that the conformational change induced by the type A component increases ribosomal affinity for type B components.

## 2. Mechanism of Action of Streptogramins

Protein synthesis is catalysed by ribosomes, in conjunction with some cytoplasmic proteins.

**Table I.** Antibiotic classification

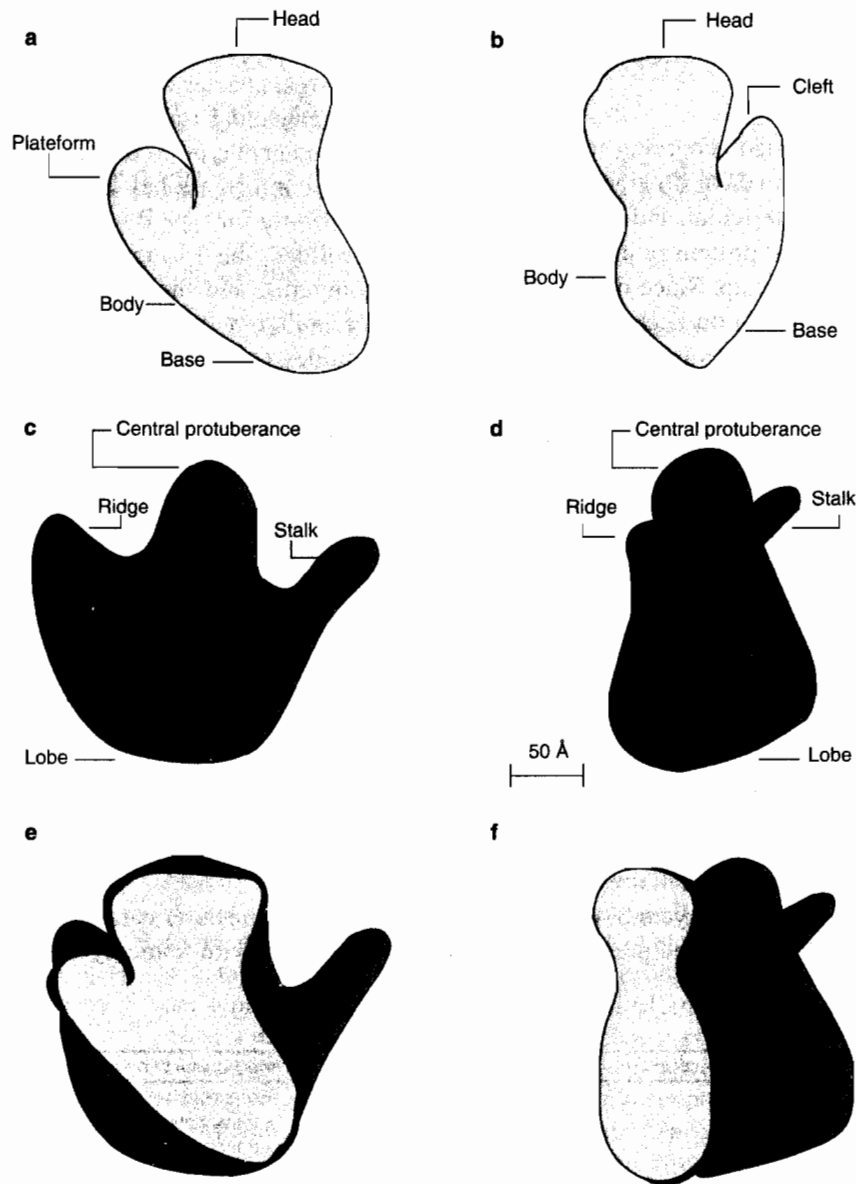
Group	Antibacterial action <sup>a</sup>	Target	Mechanism of action
I	Bacteriostatic	Cytoplasm	Reversible halt of <ul style="list-style-type: none"> <li>• transcription</li> <li>• translation</li> <li>• cell metabolism</li> </ul>
II	Bactericidal	Genome	Irreversible repression of gene replication
III	Bacteriolytic	Cell wall	Reduction of cell resistance to lysis

<sup>a</sup> Metabolic alterations involving (1) *Inhibition of polymer synthesis*: nucleic acids (DNA, mRNA, rRNA, tRNA), proteins, polysaccharides, lipids, glycolipids; and (2) *Inhibition of superstructure formation*: membrane, cell wall, peripheral polymers (peptidoglycan, arabinogalactan, lipoarabinomannan, lipopolysaccharide, teichoic acid).

**Table II.** *In vivo* synergistic actions of A and B streptogramin components

	Growth inhibition (MIC; mg/L)	Cell viability (CFU)	Inhibition of protein synthesis
Single components (A or B)	100	Unchanged	Reversible
Mixture of components (A+B)	1	Lowered (10 <sup>-2</sup> to 10 <sup>-3</sup> )	Irreversible

**Abbreviations:** CFU = colony forming units; MIC = minimum inhibitory concentration.



**Fig. 1.** Consensus models of ribosomes and subunits. Projections in 2 dimensions of the 30S (a, b) and 50S (c, d) ribosomal subunits, and of the 70S ribosomes (e, f) of *Escherichia coli*. Front (a, c, e) and side (b, d, f) views of the particles are presented. 1 Å = 0.1nm.

Bacterial ribosomes (70S) are composed of a small subunit (30S) and a large subunit (50S) [fig. 1].

The 2 subunits consist of an RNA core and a protein shell – in all some 50 ribosomal proteins and 3 ribosomal RNA (rRNA). 30S subunits contain 16S rRNA and some 20S proteins, whereas

5S and 23S rRNA together with 30L proteins are present in 50S subunits.

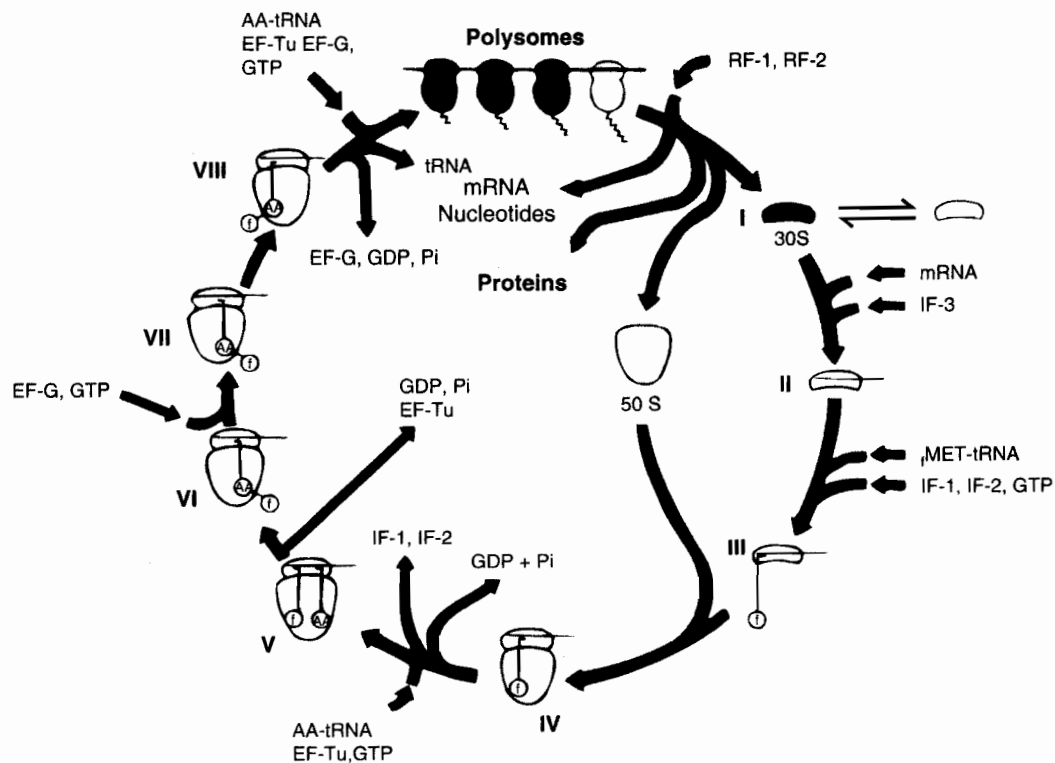
Ribosome function involves translating the genetic message conveyed by messenger RNA (mRNA) into proteins. In the course of this process, bacterial ribosomes undergo a periodical dissociation

tion into subunits and a re-association into complete (70S) particles (the ribosomal cycle).

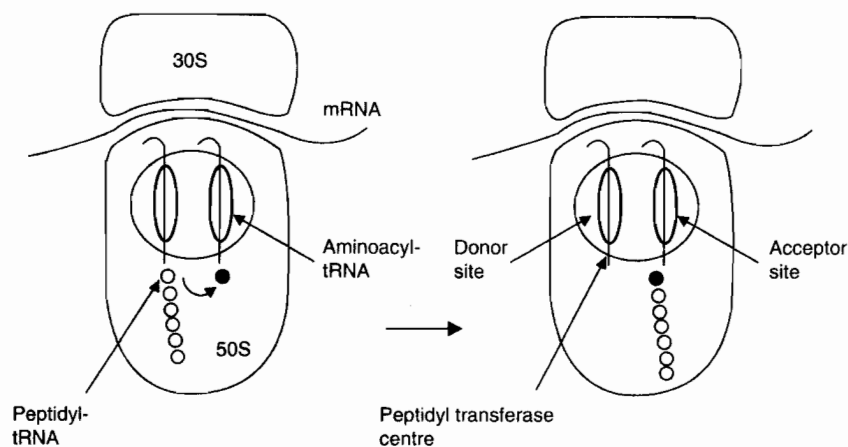
At the beginning of this cycle (initiation), the 30S subunit joins mRNA and initiator aminoacyl-transfer RNA (tRNA) carrying formyl methionine ( $\text{fmet-tRNA}$ ). This step is promoted by the 3 initiation factors (IF-1, -2 and -3), which are released when the 50S subunit joins. This leads to formation of the complete initiation complex, which contains, at the catalytic centre of the 50S subunit, 2 binding sites (A and P) for aminoacyl-tRNA derivatives.

In the second phase of protein synthesis (elongation), amino acids are joined to form a protein chain. Three steps are involved in the addition of each amino acid: (i) binding of the activated amino

acid form (aminoacyl-tRNA) at the A site; (ii) a peptidisation reaction between peptidyl-tRNA positioned at the donor P site and aminoacyl-tRNA at the acceptor A site; and (iii) displacement of peptidyl-tRNA from the A site to the P site (translocation). The elongation factors, EF-Tu and EF-Ts, promote step i, whereas EF-G catalyses translocation. Such a 3-step process is repeated for as many times as there are amino acid components of the protein. Upon completion, the protein chain is released, together with tRNA, mRNA and ribosomes (termination), and 70S particles dissociate into their subunits ready to initiate another cycle. The 3 phases of protein synthesis (initiation, elongation and termination) are depicted in figure 2. Inhibitors have been found for almost all steps of this meta-



**Fig. 2.** Metabolic pathway for protein synthesis and the ribosomal cycle. I: 30S initiation complex; II: 70S initiation complex; III: binding (elongation step i); IV: peptidisation (elongation step ii); V: translocation (elongation step iii). Steps VI, VII, and VIII represent the repetition of cycles III to V. *Abbreviations:* AA-tRNA = aminoacyl-transfer RNA; EF = elongation factor;  $\text{fMET-tRNA}$  = N-formyl-methionyl-tRNA; GDP = guanosine diphosphate; GTP = guanosine triphosphate; IF = initiation factor; Pi = phosphate; RF = release factor.



**Fig. 3.** Peptide bond synthesis promoted by the catalytic peptidyl transferase centre of 50S subunits involves the transfer of a peptidyl strand ( $n$  aminoacids) at the P site to the aminoacyl-transfer RNA at the A site: a 1-unit longer peptide chain ( $n + 1$  aminoacid) is thus formed.

bolic pathway. However, only a small number of them have had therapeutic applications.

The key reaction of this pathway, peptide bond formation, is promoted by the catalytic centre of 50S (the peptidyl transferase centre). As already mentioned, such a reaction involves the formation of a peptide link between the growing peptide chain and an aminoacyl-tRNA molecule, which are linked to the donor and acceptor sites of the peptidyl transferase centre, respectively (fig. 3). The  $\text{MLS}_B$  group and related antibiotics (streptogramin A, chloramphenicol) inhibit protein synthesis by blocking the function of the peptidyl transferase centre.<sup>[5,6]</sup>

Each of these inhibitors has a single distinct binding site on the 50S. These sites are located together within the domains of the peptidyl transferase centre and some overlap (fig. 4).<sup>[7,8]</sup> The affinity level of each antibiotic for its binding site is defined by the kinetic constant of the reaction of the ribosome-antibiotic complex formation. While the association constant of type A streptogramins is unconfirmed, it is probably much higher than the formerly reported value. Two phenomena contribute to the long-lasting action of these drugs: (i) an unusually strong linkage, of a noncovalent nature,

with ribosomes;<sup>[9]</sup> and (ii) a persistent conformational change induced by the collision of type A compounds with 50S subunits.<sup>[10]</sup> The proof for such an inference is the persistence of the particle alteration after removal of the antibiotic. This alteration is nonetheless abolished upon the dissociation of 50S particles into their constituents, which is followed by reassociation.<sup>[6]</sup>

In spite of the proximity of their binding sites and their action on a single target,  $\text{MLS}$  antibiotics interfere in different ways with the peptidyl transferase centre. Type A streptogramins block substrate attachment to the donor and acceptor sites of the peptidyl transferase centre,<sup>[11]</sup> thus preventing the earliest event of elongation, which occurs just after completion of initiation. On the other hand, type B streptogramins and macrolides prevent the extension of protein chains and cause a release of incomplete peptide threads.<sup>[5]</sup> Compared with that caused by type A streptogramins, the metabolic block induced by type B streptogramins and erythromycin is located at a later step in protein synthesis, and the inhibitory effect on the completion of growing peptide chains is proportional to the thread length.<sup>[5]</sup> Moreover, type A streptogramins can bind only to aminoacyl-tRNA-free A and P

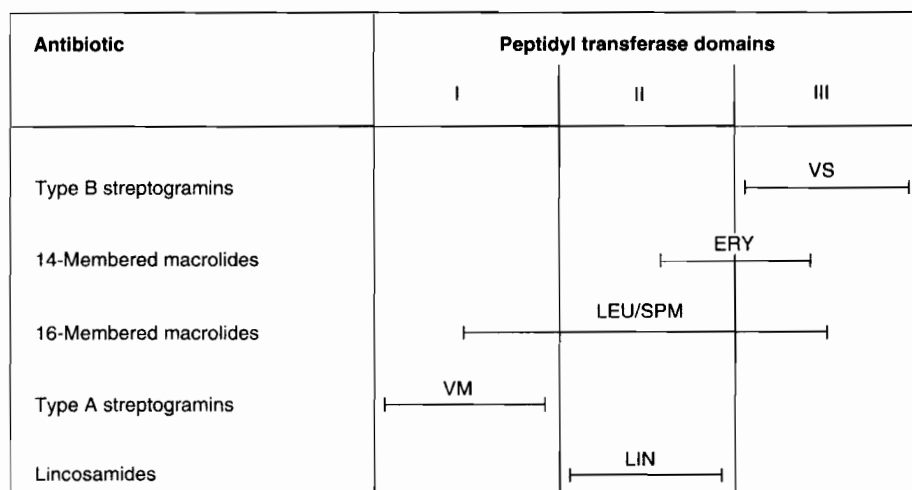
sites of 50S, since attachment of aminoacyl-tRNA to the substrate binding sites of the peptidyl transferase centre prevents fixation of these antibiotics to particles.<sup>[12]</sup> In contrast, type B streptogramins and macrolides can bind to ribosomes at any step of the cycle. When inhibition of initial steps (pristinamycin II) is associated with inhibition of more advanced steps (pristinamycin I), a double metabolic block is produced. This occurs when bacteria are incubated with a mixture of types A and B streptogramins – inhibitors that act sequentially in the protein synthesis pathway.

To reconcile the occurrence of a single binding site for type A streptogramins with their interference with both A and P ribosomal sites, a model has been proposed, whereby the 2 substrate binding sites of the peptidyl transferase centre exchange their conformations and functions at each elongation round.<sup>[12]</sup>

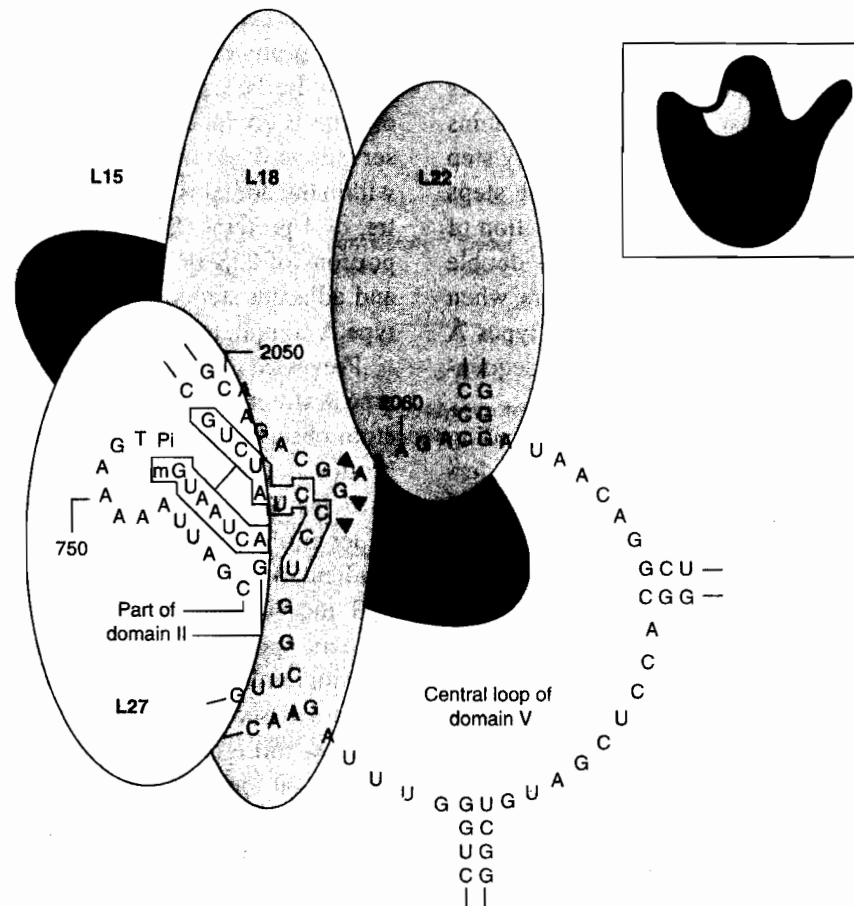
Since the receptors of types A and B streptogramins are located within the domains of the peptidyl transferase centre, their identification would allow the determination of the catalytic centre for protein synthesis on the ribosome surface. In a first biophysical approach,<sup>[13]</sup> the topological

site of this centre was established at the base of the central protuberance of the 50S subunit. In a further study, the L proteins present at the binding site of type B compounds were identified.<sup>[14]</sup> The observations made until now point to the presence, within the domains of the peptidyl transferase centre, of 4 proteins (L15, L18, L22, L27) and some portions of 23S rRNA (central loop of domain V and adjacent stems) [fig. 5]. The binding site for type A compounds has not yet been identified.

Prevention of tRNA binding to ribosomes by type A streptogramins accounts for a unique alteration observed in bacteria treated with these antibiotics – the appearance of pressure-sensitive particles. When the cytoplasm from cells growing under normal conditions is centrifuged in a density gradient, 3 peaks of ribosomes and subunits (70S, 50S and 30S) are observed: a fourth peak (60S) appears upon incubation of bacteria with type A streptogramins (fig. 6).<sup>[4]</sup> This additional peak is due to a dissociation of 70S particles, which occurs at a low milligram concentration and at a critical centrifugal speed. It appears that particles carrying aminoacyl-tRNA molecules attached to the substrate binding sites of the peptidyl transferase cen-



**Fig. 4.** Model of the peptidyl transferase domains on the large ribosomal subunit. The binding sites of 5 antibiotic families inhibiting peptidyl transferase have been located according to competitive (overlapping) and noncompetitive (nonoverlapping) interactions with ribosomes. Abbreviations: ERY = erythromycin; LEU/SPM = leucomycin/spiramycin; LIN = lincomycin; VM = virginiamycin M; VS = virginiamycin S (from Di Giambattista et al.,<sup>[18]</sup> with permission).



**Fig. 5.** Topological model of the binding site for macrolide, lincosamide and type B streptogramin (MLS<sub>B</sub>) antibiotics on the 50S ribosomal subunit. Ribosomal proteins (L15, L18, L22 and L27), which were affinity labelled by different MLS<sub>B</sub> antibiotics and located by immune electromicroscopy, and bases in domains II and V of 23S rRNA, which were altered by MLS<sub>B</sub> resistance (MLS<sub>B</sub><sup>R</sup>) mutations (solid triangles represent altered bases), are shown. The dotted area in the insert indicates the consensus topological site of the peptidyl transferase domain, which the MLS<sub>B</sub> binding site overlaps. Abbreviations: A = adenine; C = cytosine; G = guanine; mG = methyl guanine; Pi = phosphate; T = thymine; U = uracil.

tre are resistant to mechanical stress, whereas those lacking these substrates are pressure-labile. By preventing aminoacyl-tRNA interaction with the peptidyl transferase centre, type A streptogramins cause the appearance of 60S particles.<sup>[15]</sup>

### 3. Mechanisms of Microbial Resistance to MLS<sub>B</sub> Antibiotics

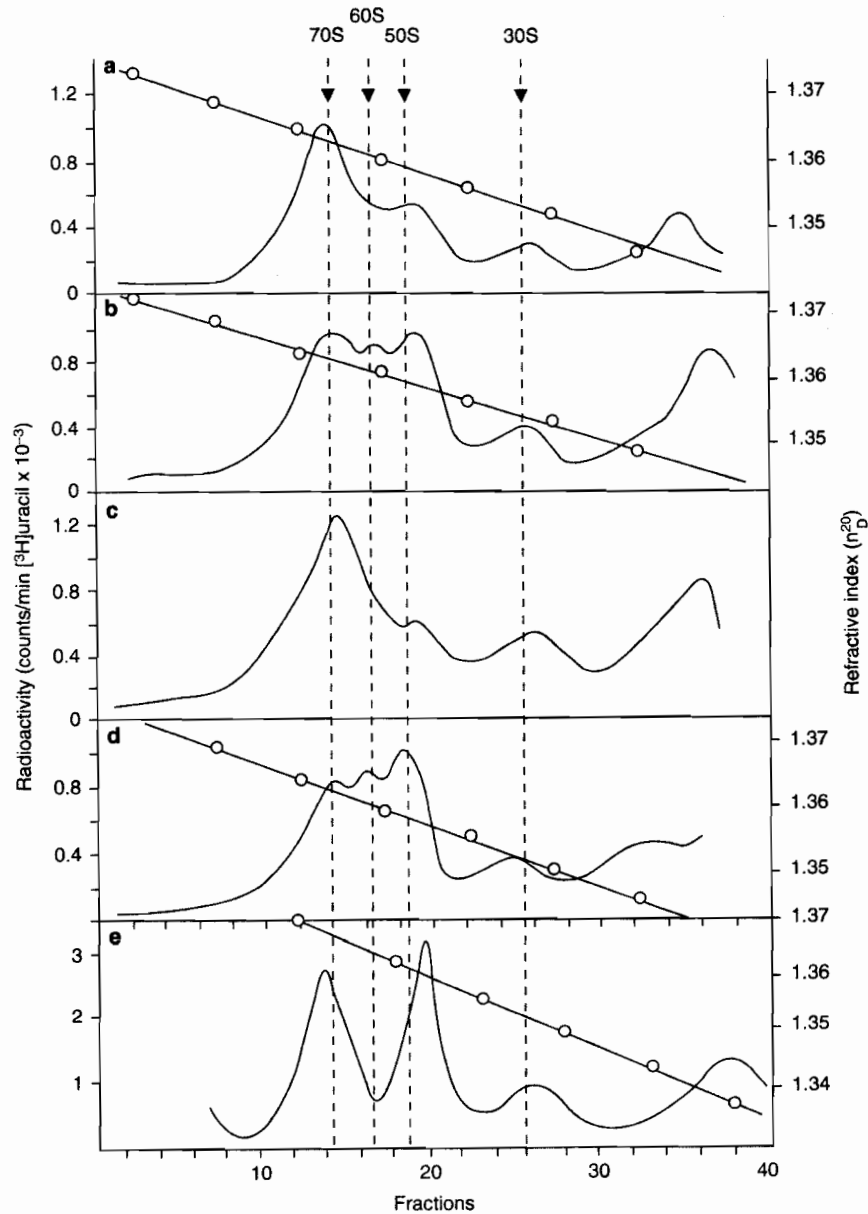
Under certain conditions, a sensitive bacterial strain may become insensitive to a given antibiotic. Three types of resistance mechanisms have been

identified: (i) lack of antibiotic penetration (alteration of transmembrane transport); (ii) failure of antibiotic attachment to the target (receptor alteration); and (iii) biochemical inactivation of the drug (by hydrolysis or by coupling) [table III].

From a genetic viewpoint, 2 kinds of resistance should be considered (table IV): (a) chromosomal resistance; and (b) plasmid borne resistance. The former type occurs, for example, upon mutation of ribosomal protein genes or of genes coding for membrane permeases that control the cytoplasmic

access of antibiotics. The latter form involves the acquisition of a plasmid (a minichromosome autonomously replicating in bacterial cytoplasm),

which carries genes causing resistance to a given antibiotic (single resistance). It may also contain several determinants conferring insensitivity to



**Fig. 6.** Production of pressure-labile ribosomes in the presence of type A streptogramins.  $^{3}\text{H}$ juracil-labelled bacteria are incubated with type A components (b), type B components (c), their mixture (d), or type B fixed with glutaraldehyde (e), before ribosome fractionation by density gradient centrifugation. (a) = control (no antibiotic). Whenever cells are incubated with type A components, pressure-labile ribosomes appear, unless the cells have previously undergone glutaraldehyde fixation.

**Table III.** Mechanisms of microbial resistance to antibiotics

Group	Antibiotic function altered	Biochemical mechanism
I	Penetration	Block of permease
II	Fixation to target	Receptor alteration
III	Biochemical inactivation	Hydrolysis or coupling

several groups of antibiotics. Plasmids can multiply and be transferred to other cells of related species, thus spreading the infectious process. Multiplication of resistant plasmids is favoured by the presence of suboptimal concentrations of antibiotics, a selective pressure allowing the accumulation of resistant clones.

### 3.1 Undissociated and Dissociated Resistance to MLS<sub>B</sub> Antibiotics

Antibiotics of the MLS<sub>B</sub> group are implicated in a peculiar phenomenon of multiple resistance, whereby bacteria incubated with one inhibitor become insensitive to all the other members of the group (undissociated resistance). It is noteworthy that other forms of insensitivity, restricted to single members of the MLS<sub>B</sub> group (dissociated resistance), have also been described. Surprisingly, a single base change in the main loop of domain V of 23S rRNA can cause the appearance of a mutant phenotype differing from that induced by the mutation of a nearby base.<sup>[9-13]</sup> Two apparently inexplicable observations have thus been made: (i) antibiotics with unrelated structures may have overlapping binding sites on the ribosome surface and a similar mechanism of action, and (ii) mutations of adjacent rRNA bases can cause different antibiotic-promoted alterations of ribosome functions, a situation apparently incompatible with the size of the inhibitor molecules. These phenomena

can only be accounted for by postulating that (a) antibiotic attachment causes a distortion of the particles, entailing functional alterations, and (b) base changes in rRNA induce other types of conformational changes, resulting in altered antibiotic binding.<sup>[16,17]</sup>

Since ribosomes are made up of ribosomal proteins and rRNA, and when the structures of these 2 polymers are considered, it would appear more reasonable to postulate antibiotic binding to the former rather than the latter compounds. However, most recent investigations point to an rRNA involvement.<sup>[18,19]</sup> It has thus been proved that methylation of an rRNA base hinders the attachment of antibiotics of the MLS<sub>B</sub> group to ribosomes. A plasmid coding a methyl transferase is responsible for such an alteration (undissociated resistance).<sup>[20]</sup> As has already been mentioned, the domain V of 23S rRNA and a few L proteins are basic components of the peptidyl transferase centre (fig. 5).<sup>[19,21]</sup> Alterations of rRNA bases in domain V accounting for both undissociated and dissociated types of resistance to MLS<sub>B</sub> antibiotics have been identified (table V).<sup>[17]</sup>

### 3.2 The Importance of Synergism

Although the phenomenon of resistance to antibiotics is a major concern for chemotherapy today, it is less so with streptogramins for the following reasons: (a) resistance to streptogramin A components is rarer than to MLS<sub>B</sub> or other protein synthesis inhibitors; (b) the presence of synergistically active streptogramin A and B components implies a far lower chance of the emergence of strains insensitive to both components (the frequency of single mutation is  $\approx 10^{-6}$ , whereas that of double mutation is  $\approx 10^{-12}$ ); and (c) the synergism of A and B

**Table IV.** Genetic elements involved in microbial resistance

Type of resistance (no.)	Genes involved		Resistance transfer	
	location	type	efficacy	species boundary
Chromosomal (single)	Genome	Receptors (rRNA, proteins)	Low	Intraspecific
Plasmid borne (multiple)	Plasmid	Enzymes (permeases, hydrolases, transferases, etc.)	High	Extraspecific

**Table V.** Base changes in 23S rRNA that confer resistance to different antibiotics

Microorganisms <sup>a</sup>	Mutations <sup>b</sup>			Phenotype <sup>c,d</sup>
	2058	2057	2611	
<i>Escherichia coli</i>				
Wild type	A	G	C	Sensitive
pE194	m <sub>2</sub> A	G	C	MLS <sub>B</sub> <sup>R</sup>
pERY	U	G	C	MLS <sub>B</sub> <sup>R</sup>
pDVE	G	G	C	MLS <sub>B</sub> <sup>R</sup>
pCAM	A	A	C	Cam <sup>R</sup> , Ery <sup>R</sup>
pBFL1	A	G	U	Ery <sup>R</sup>
<i>Saccharomyces cerevisiae</i>				
Wild type	A	G	C	Sensitive
Mutant	A	G	U	Spm <sup>R</sup>
Mutant	A	G	G	Spm <sup>R</sup> , Ery <sup>R</sup>
Mutant	G	G	C	Ery <sup>R</sup>

a *E. coli* strains with wild type and mutated plasmids. *S. cerevisiae* with wild type and mutated mitochondrial DNA.

b Base changes in the 23S rRNA gene.

c Antibiotic-resistant phenotypes.

d Superscript R denotes resistance pattern to different antibiotics.

Abbreviations: A = adenine; C = cytosine; Cam = chloramphenicol; Ery = erythromycin; G = guanine; m<sub>2</sub>A = dimethyl-adenine; MLS<sub>B</sub> = macrolide, lincosamide, streptogramin B group; Spm = spiramycin; U = uracil.

components is still expressed in MLS<sub>B</sub> resistant strains.

The synergism between types A and B streptogramins can be explained by a conformational change in ribosomes, which is caused by the attachment of type A components to particles. Erythromycin and type B components have the same mechanism of action and overlapping binding sites on the ribosome surface, hence their competitive binding to particles. Since ribosome affinity for erythromycin is higher than for type B compounds, ribosome-bound B streptogramin will be displaced from its complex by exogenous macrolides. However, there is evidence of an A component-promoted conformational change that results in a higher ribosome binding affinity for type B components, so that a displacement does not occur.

#### 4. Conclusion

The uniqueness of streptogramins among antibiotic inhibitors of protein synthesis can be defined as follows:

- A mixture of type A and B streptogramins has an inhibitory activity 100-fold higher than single components (no other antibiotic mixture has a comparable synergistic effect).
- Cell viability is suppressed by the 2 streptogramin components (no other inhibitor of the 50S subunit is bactericidal).
- Resistance to streptogramins is less common than to any known protein synthesis inhibitors.
- The synergistic effect of type A and B streptogramins also applies to clones resistant to the MLS<sub>B</sub> group.

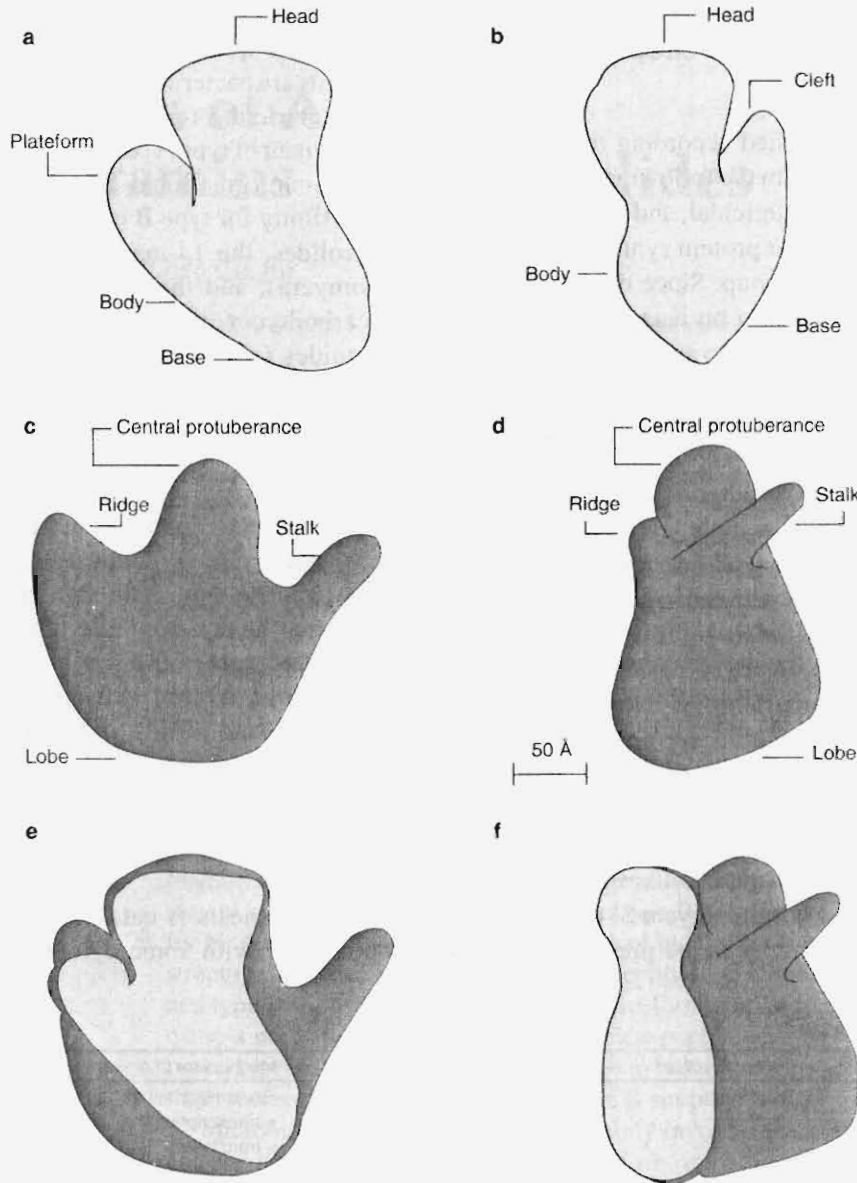
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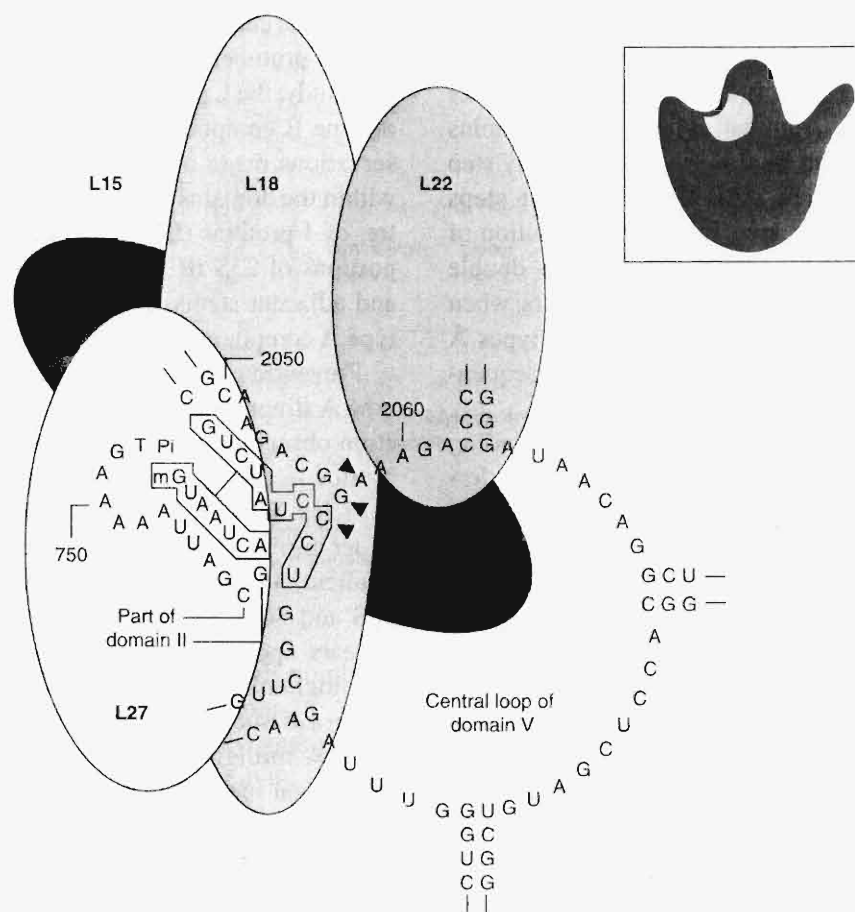
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Under certain conditions, a sensitive bacterial strain may become insensitive to a given antibiotic. Three types of resistance mechanisms have been

identified: (i) lack of antibiotic penetration (alteration of transmembrane transport); (ii) failure of antibiotic attachment to the target (receptor alteration); and (iii) biochemical inactivation of the drug (by hydrolysis or by coupling) [table III].

From a genetic viewpoint, 2 kinds of resistance should be considered (table IV): (a) chromosomal resistance; and (b) plasmid borne resistance. The former type occurs, for example, upon mutation of ribosomal protein genes or of genes coding for membrane permeases that control the cytoplasmic