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Progress in targeting bacterial transcription

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The bacterial RNA polymerase (RNAP) is an essential enzyme that is responsible for making RNA from a DNA template and is targeted by several antibiotics. Rifampicin was the first of such antibiotics to be described and is one of the most efficient anti-tuberculosis drugs in use. In the past five years, structural studies of bacterial RNAP and the resolution of several complexes of drugs bound to RNAP subunits have revealed molecular details of the drug-binding sites and the mechanism of drug action. This knowledge opens avenues for the development of antibiotics. Here these drugs are reviewed, together with their mechanisms and their potential interest for therapeutic applications.

Introduction

RNA polymerase (RNAP) enzymes are responsible for making RNA from a DNA or RNA template by a process called transcription. These enzymes are vital to the bacteria and are present in most organisms, including many viruses, prokaryotes and eukaryotes. Despite structural and functional similarities, however, bacterial RNAPs do not share extensive sequence homology with eukaryotic RNAPs. This lack of homology explains why most of the antiviral nucleosides do not affect bacterial transcription and, inversely, why rifampicin, an antibiotic targeting bacterial RNAPs, does not target viral or eukaryotic RNAPs and can be used for months on individuals undergoing anti-tuberculosis treatment.

The prokaryotic RNAP is a large protein composed of core subunits (α_2, β and β′) and a σ factor that is required for specific recognition of the promoter site and the initiation of transcription. The large subunits, β and β′, comprise conserved domains and dispensable regions. The interplay between σ factors and the RNAP finely tunes gene expression. Many bacteria, particularly those with complex genomes, contain multiple σ factors that share sequence homology. σ^{70} is the best-studied of these factors and contains four conserved domains, numbered 1 to 4: region 1 antagonizes the DNA-binding activity of σ^{70}; region 2 is involved in binding to the core RNAP and recognition of the −10 promoter element; region 3 interacts with DNA upstream of the 'extended −10′ promoters and with the initiating nucleotide in the RNAP active site during initiation; and Region 4 is involved in binding to the −35 promoter element. The substitution of one σ factor for another can redirect the RNAP to activate the transcription of genes that would otherwise not be transcribed. Despite structural and functional similarity between eukaryotic and prokaryotic RNAPs, no eukaryotic proteins are homologous to σ factors.

The structures of the *Thermus aquaticus* and *Thermus thermophilus* RNAP cores and/or holoenzymes have been solved at high resolution [1–4]. The subunits of the RNAP assemble into the same hand-like structure, similar to that of DNA polymerases. The large subunits (β and β′) form an internal DNA channel, 10–12 Å wide, that is used by the incoming DNA, and the active site is located on the back wall of this channel (Figure 1 a,b). A Mg^{2+} ion essential to the catalysis is chelated in the active site. A secondary channel is presumably used by the incoming nucleotides. During elongation, the RNA–DNA hybrid extends through the DNA channel and then through the RNA channel underneath the flap, before finally exiting the RNAP.

It is clear that the σ factor is intimately associated with the enzyme and forms a V-shaped structure near the opening of the active site cleft. On formation of the holoenzyme, conformation changes in both the core subunits and the σ factor occur. Some regions move within the core subunits, whereas others that were disordered in the core RNAP structure become ordered, facilitating promoter recognition, promoter opening and initiation. The σ

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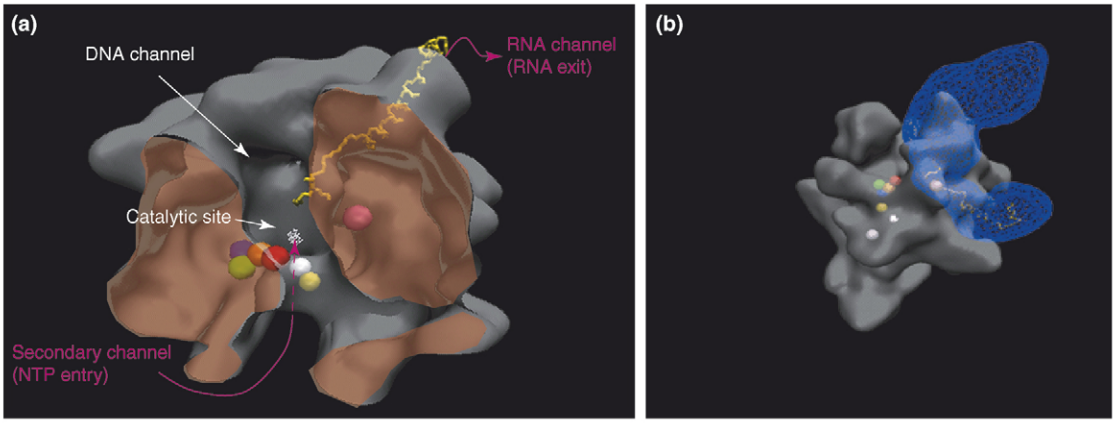

FIGURE 1

Three-dimensional structure of the RNA and compounds that bind the RNAP. **(a)** The structure of the *T. thermophilus* RNAP [9] is sliced to expose the DNA channel and the catalytic site. The catalytic site is shown as a white dot. The binding sites for MccJ25 (white) and tagetitoxin (yellow) are located at the surface of the secondary channel used by the NTP to reach the catalytic site. The other compounds that bind to the RNAP in the main channel (or DNA channel) are shown in blue (sorangicin), green (CBR), orange (rifampicin) and red (streptolydigin). The yellow ribbon represents region 3 of σ, which occupies the RNA channel during the initiation step. This RNA channel is the exit path used by the synthesized RNA. **(b)** The RNAP holoenzyme, showing its σ factor (blue frame) and the inhibitors listed in (a).

factor is released from the transcription complex shortly after transcription is initiated, and elongation proceeds in the absence of this factor.

As the above description demonstrates, the bacterial RNAP is a complex system made up of interdependent domains and proteins that requires particular arrangements at each step of transcription to function. The disruption of any one of these functions will severely affect the transcription process. Several structurally diverse antibiotics from natural or synthetic sources target the architecture of the enzyme complex. Many high-resolution X-ray structures of ligands bound to the RNAP have recently become available and provide a clear picture of how these molecules inhibit transcription. The availability of these structures, together with the entry of a new transcription inhibitor into a clinical trial, highlights the interest of the RNAP as an antimicrobial target and opens new opportunities for the development of antibiotics.

As we describe in this review, RNAP inhibitors have been classified into two categories depending on their mechanism of action and also on whether genetic and structural data support the observations made during *in vitro* transcription experiments.

Transcription inhibitors that bind characterized binding sites

The ansamycins

The ansamycins were initially discovered in a strain of *Nocardia mediterranei* (previously known as *Streptomyces mediterranei*) [5]. This family of antibiotics specifically inhibits bacterial transcription and is characterized by a planar naphtoquinone ring (Figure 2). Two positions (3 and 4) have been extensively modified by hemisynthesis to improve the pharmacological properties and to yield commercial antibiotics such as rifampicin. The crystal structure of the *T. aquaticus* core RNAP complexed with rifampicin has been recently solved.

From this structure, we have learned that the binding of such a large molecule directly involves 12 amino acid residues [6]. Because all of the rifampicin-resistant mutants have been mapped to the *rpoB* gene, rifampicin interacts only with the β subunit encoded by *rpoB*, and mutation of all but one of the 12 amino acids spontaneously generates a rifampicin-resistant phenotype. These mutations are clustered in a conserved region in the middle of the β subunit between regions C–D and E [7,8] (Figure 3).

Rifampicin is located in the DNA channel 12 Å away from the active site, and it has been suggested [9] that it acts by directly blocking the path of the elongating RNA when the transcript is 2–3 nucleotides long. A new mechanism of action, however, has been recently proposed [10] (Figure 4). Rif appears to be closer to the region 3 of σ than expected from the crystal structure of the Rif-core complex. This proximity can explain the σ–dependence of the inhibition of transcription by Rif, for example, the inhibition is stronger when the polymerase uses σ^{70} compared with σ^{32} [11]. Rifampicin could change the conformation of region 3 of σ, thereby inducing propagation of an allosteric effect along the DNA to the active site. This signal could also disfavor binding of the catalytic Mg^{2+} ion, resulting in spontaneous dissociation of the short and unstable DNA–RNA hybrid.

Regarding its antibacterial behavior, rifampicin is particularly active at low concentration (10 ng/ml) against Gram-positive bacteria including *Staphylococcus*, *Streptococcus*, *Bacillus*, *Corynebacterium* and *Mycobacteria*.

Streptolydigin

Streptolydigin, also known as portamycin, is an antibiotic produced by *Streptomyces lydicus* (Figure 2). It was isolated in 1955 from a culture broth by researchers at the UpJohn Company [12–14] and is a member of the acyl tetramic acids (3-acyl-2,4-pyrrolidinediones) group of natural products, which possess a broad spectrum of biological activity.

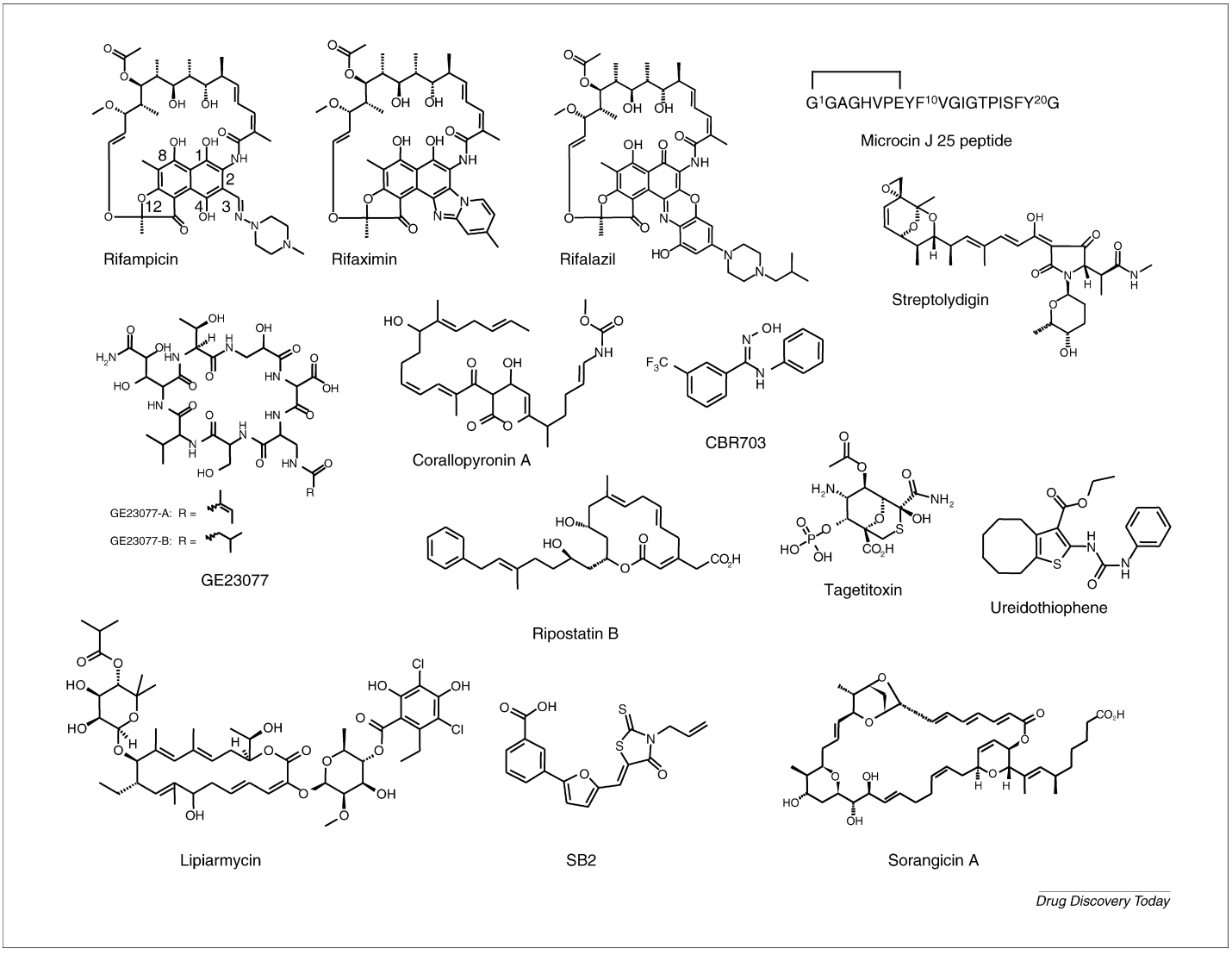

FIGURE 2

Structures of transcription inhibitors that target the bacterial RNAP.

RNA synthesis is the preferential target of streptolydigin and this antibiotic has been proposed to inhibit initiation, elongation and pyrophosphorolysis [15,16]. Resistance to streptolydigin can be conferred by alterations in both the β and β′ subunits (Figure 3). In the β subunit, these mutations map to a spacer region between the two principal rifampicin clusters and rifampicin cluster II [17,18] (Figure 3), whereas in the β′ subunit these mutations map to the highly conserved region F [17–19]. Streptolydigin has no effect on rifampicin binding to the RNAP, indicating that the binding sites of the two antibiotics are distinct [20]; however, some rifampicin-resistant mutants show weak resistance to streptolydigin, suggesting a proximity between the two ligands.

The location of these mutations on the three-dimensional structure of the *T. thermophilus* RNAP–streptolydigin complex suggests that streptolydigin binds to a site located 20 Å away from the active centre of the RNAP and encompasses three structural elements: the streptolydigin pocket; the 'bridge helix', a structure suspected to assist translocation; and the 'trigger loop', a highly mobile loop located in the secondary channel [21,22].

From these structural data, and in agreement with previous enzymatic studies [15,16], it can be concluded that streptolydigin might affect an unspecified step in the cycle of nucleotide addition (other than NTP binding) by blocking conformational cycling of the bridge helix or by blocking transfer of the NTP from the pre-insertion site to the insertion site [21,22].

Streptolydigin is active against aerobic Gram-positive bacteria such as *Staphylococcus*, *Streptococcus*, *Bacillus* and *Corynebacterium*, and against anaerobic Gram-positive bacteria such as *Clostridium*.

Sorangicin

Sorangicin is a macrolide antibiotic that is structurally unrelated to the macrolide family of translation inhibitors used in antibiotic chemotherapy (e.g. erythromycin). Isolation of sorangicin from the myxobacteria *Sorangium cellulosum* was first reported in 1985 by Jansen *et al.* [23] and its structure was elucidated in 1989 [24] (Figure 2). Approximately 100 analogs of sorangicin have been obtained by hemisynthesis, six of which possess improved antibacterial activity against *Staphylococcus aureus* [25].

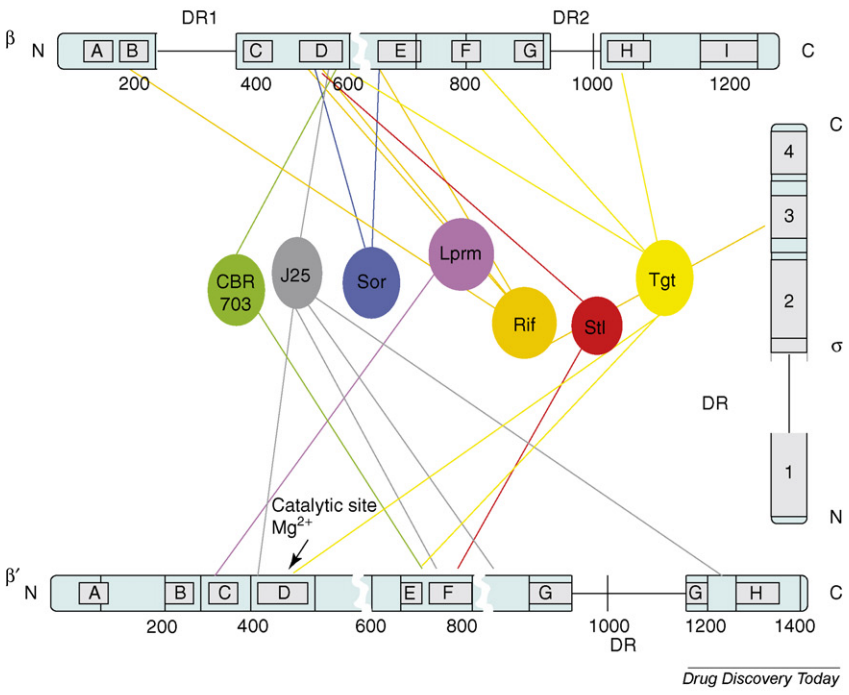

FIGURE 3

Positioning of the small-molecule transcription inhibitors on the structure of the RNAP. The *E. coli* RNAP comprises core subunits (α_2, β and β′) and a σ factor (note that only β, β′ and σ are shown). Compounds that bind the RNAP are shown in blue (sorangicin [Sor]), magenta (lipiarmycin [Lprm]), green (CBR [CBR703]), orange (rifampicin [Rif]), yellow (tagetitoxin [Tgt]), gray (Microcin J25 [J25]) and red (streptolydigin [Stl]). The areas of the protein proximal to the binding site, or proximal to the amino acids conferring resistance, are shown in accordance with the available data. Boxes A–I of the β and β′ subunits, and boxes 1–4 of σ^{70} represent conserved domains; DR, DR1 and DR2 represent dispensable regions of the enzyme. Most of the inhibitors are located in overlapping regions of this structure, in agreement with biochemical and structural data.

Binding of sorangicin and rifampicin to the RNAP is mutually exclusive [20]. Sorangicin has been co-crystallized with the *T. aquaticus* RNAP and resolved to 3.2 Å resolution [26]. This co-crystallization showed that sorangicin binds in the same β subunit pocket as rifampicin, with an almost complete overlap of RNAP binding determinants (Figure 2). This location explains why sorangicin behaves like rifampicin. Indeed, sorangicin blocks the synthesis of short transcripts [26] and is not inhibitory once RNA polymerization has started [27]. This observation is intriguing because rifampicin and sorangicin are not chemical analogs, although they share a similar overall shape.

The ability of an antibiotic to select resistance by mutation of the target varies among the molecules. For example, the frequency of spontaneous rifampicin-resistant mutants often reaches 10^{-7}. Spontaneous mutants of *Escherichia coli* that are resistant to sorangicin have been obtained as easily as mutants that are resistant to rifampicin. The sorangicin mutants are also all resistant to rifampicin. The RNAPs of these sorangicin-resistant mutants are much less sensitive to both inhibitors than are the RNAPs from wild-type strains; however, sorangicin retains good activity against most of the RNAPs of *E. coli* mutants that are resistant to rifampicin [20,28]. Campbell *et al.* [26] have suggested that the intrinsic conformational flexibility of sorangicin would favor its adaptation to amino acid modifications within the binding pocket of the β subunit, thereby explaining this difference.

Sorangicin is a potent inhibitor of bacterial but not eukaryotic RNAPs, and its main antibacterial activity is directed against Gram-positive species [27].

Lipiarmycin

Lipiarmycin, also known as clostomicin and tiacumicin, is a member of a family of macrocyclic antibiotics first described in 1975 by Parenti and co-workers [29]. These antibiotics are produced by *Actinoplanes deccanensis* ATCC 21983, *Micromonospora echinospora* ssp. *armeniaca* ssp. *nov*. and *Dactylosporangium aurantiacum*. The structures of four members of this family, lipiarmycins A3, A4, B3 and B4, have been elucidated [30] (Figure 2).

Lipiarmycin is a specific inhibitor of transcription initiation. Lipiarmycin inhibits early transcription of the bacteriophage SPO1, which depends on the *Bacillus subtilis* transcription factor σ^A and the bacterial RNAP, but has no effect on middle or late transcription, which depend on two phage-encoded polypeptides, gp33 and gp34 [31], and the bacterial RNAP. We have recently isolated and characterized a lipiarmycin-resistant mutant of *B. subtilis*, carrying a mutation in the RNAP β′ subunit (R326L), that confers resistance to high concentrations of lipiarmycin [32] (Figure 3). The R326L mutation is located in the DNA channel, opposite to the rifampicin-binding site and proximal to region 3 of σ (Figure 1). Whether this mutation delineates the lipiarmycin-binding site is under investigation, but it suggests a new mechanism of inhibition: namely, lipiarmycin is the only inhibitor that

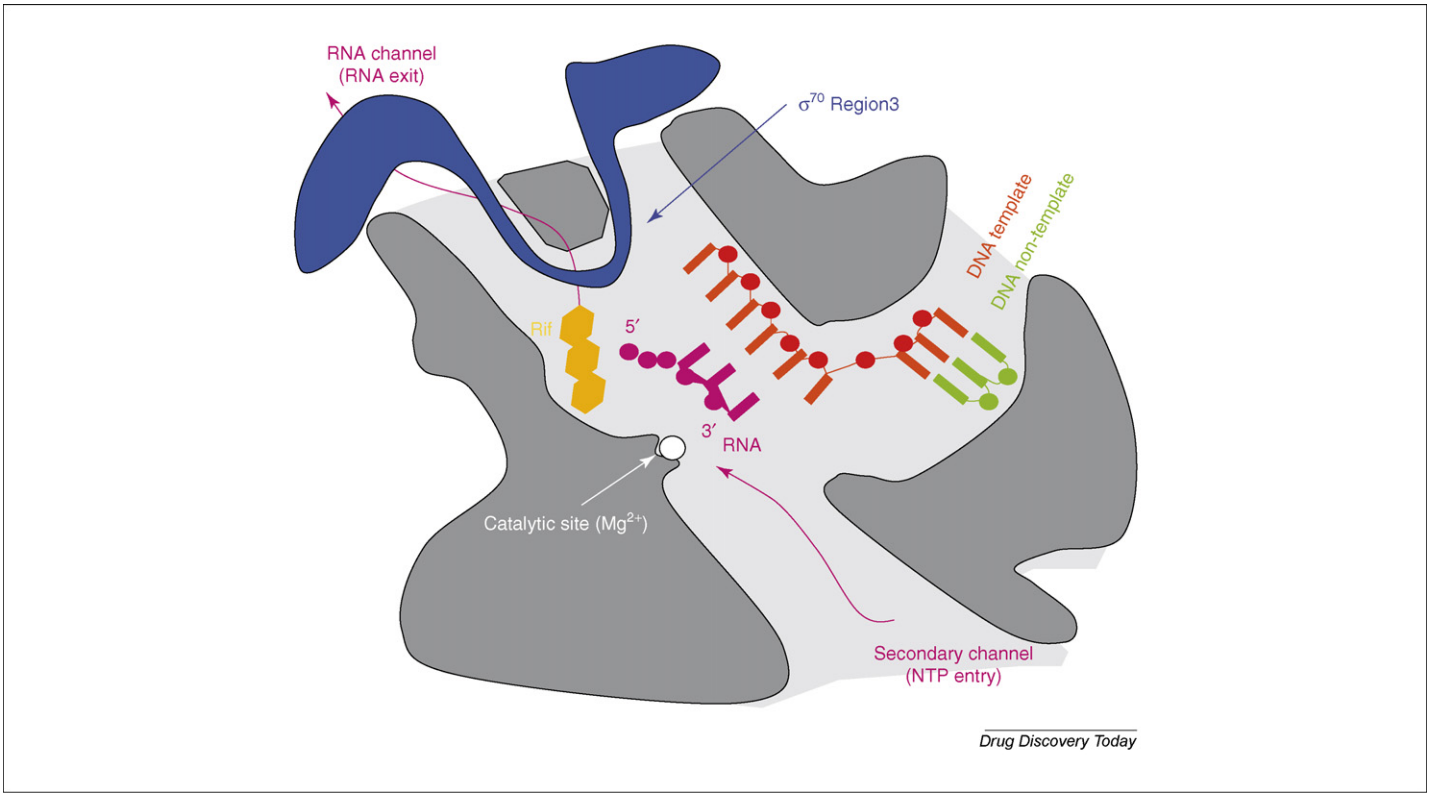

FIGURE 4

Topology of the rifampicin-binding site. Shown is a summary of the topology of the rifampicin-binding site and its proximity to region 3 of σ. The RNAP comprises the core (gray) and σ (blue). The promoter DNA (template strand, red; non-template strand, green) and the nascent RNA (magenta), which is hybridized to the template DNA, are also shown. The 3′-hydroxyl of the RNA is positioned in the catalytic site of the enzyme (white circle), and the 5′-triphosphate points toward the RNA channel. Rifampicin is shown in orange. The proximity between rifampicin and region 3 of σ could enable rifampicin to generate allosteric effects on region 3 that propagate along the DNA to the active site. Such a signal could disfavor binding of the catalytic Mg^{2+} ion. Also shown is how the nucleosides enter into the catalytic site through the secondary channel, and how the elongated RNA is extruded through the RNA channel. Figure adapted from [65].

affects transcription differentially depending on the σ factor; moreover, region 3 is one of the most diverging domains of σ.

Lipiarmycin shows activity against a broad spectrum of Gram-positive bacteria [30,33] and is particularly active against various *Clostridium* [34] species and other Gram-positive anaerobic bacteria.

CBR703 series

The CBR703 (CBR) series of antibiotics are the first synthetic inhibitors of the RNAP (Figure 2). They were discovered by screening a large library of compounds in a classical transcription assay [35]. The best molecules in the series are reversible inhibitors of *E. coli* transcription that act *in vitro* in the submicromolar range. They inhibit nucleotide addition but do not dissociate the transcript elongation complex nor do they inhibit translocation, and they seem to be specific to bacterial RNAPs because no inhibition of other classes of nucleic acid polymerases of bacterial, viral or mammalian origin has been observed.

CBR-resistant strains of *E. coli tolC*, a strain deficient in a multidrug efflux transporter, have been selected. The mutations are clustered in the middle of the *rpoB* and *rpoC* genes encoding the β and β′ subunits, respectively (Figure 3). The location of the mutations suggests that CBR antibiotics could bind at a surface-exposed groove at the junction of the β′ bridge helix and the β subunit. Because this location is away from the active site where the nucleotide is added, CBR antibiotics might be allosteric inhibitors. This new family of synthetic molecules is promising because further medicinal chemistry can be performed to improve their activity.

CBR antibiotics have moderate antibacterial activity against *S. aureus*, *B. subtilis* and *E. coli tolC* (~15 μg/ml) and optimized analogs have activity against wild-type strains of *E. coli*. Surprisingly, some CBR compounds show a greater level of activity on living bacteria than on the purified enzyme *in vitro* [36]. Possible explanations could be that some analogs are more permeable or have alternative targets, or that the molecules are chemically modified in the bacterium to yield more potent inhibitors.

Microcin J25

Microcins are classical bacteriocins produced by Enterobacteriaceae that inhibit *E. coli, Salmonella* and *Shigella* strains [37]. Microcin J25 (MccJ25) inhibits transcription [38] and is the only cyclic microcin described so far. It was isolated from the culture supernatant of *E. coli* MC 4100 (pTUC202) and its structure was assigned first to cyclo(-V^{1}GIGTPISFY^{10}GGGAGHVPEY^{20}F-) and then to a lassoed tail structure [39,40] (Figure 2).

Amino acid substitutions that confer resistance to MccJ25 have been mapped to the β and β′ subunits (Figures 1,3), and clusters of mutations are located in the inner surface of the secondary chan-

nel [41,42]. It is striking that the extensive regions conferring MccJ25 resistance overlap with those conferring streptolydigin resistance. This indicates that these two molecules could act through identical mechanisms: namely, either interfering with the conformational changes of the bridge helix and altering the nucleotide addition cycle, or blocking nucleotides from entering the enzyme catalytic site [41].

The antibiotic activity is mainly directed against Enterobacteriaceae, including several pathogenic species of *Escherichia*. At concentrations above the minimum inhibitory concentration (MIC: 1–100 nM), MccJ25 induces cell filamentation in sensitive *E. coli* cells [38].

Tagetitoxin

Tagetitoxin is a phytotoxin (Figure 2) produced by *Pseudomonas syringae* pv. *tagetis* that causes chlorosis of plant leaves. It was first described in 1981 by Mitchell and Durbin [43]. Tagetitoxin inhibits RNA synthesis directed by bacterial RNAPs, eukaryotic RNAP III from yeasts, insects and vertebrates, and chloroplast RNAPs, indicating that its binding site might be evolutionarily conserved. Single-subunit phage RNAPs and nuclear RNAPs I and II are either not or only mildly affected by this toxin [44,45].

Tagetitoxin has been recently co-crystallized with the *T. thermophilus* RNAP and resolved to 2.4 Å resolution [46]. Structural elucidation of the complex has enabled suggestions to be made about its mechanism of action. Tagetitoxin binds to the base of the RNAP secondary channel, close to the active site of nucleotide addition, and its binding does not induce significant conformational changes. Tagetitoxin does not prevent NTP binding in the active site of RNAP, but rather interacts with the incoming NTP, possibly altering substrate loading or stabilizing the enzymatic complex in an inactive state. Tagetitoxin also inhibits the translocation and transcript cleavage reactions. Recombinant RNAP mutated in the tagetitoxin-binding site has been engineered and is resistant to the inhibitor. This observation indicates that tagetitoxin targets the RNAP *in vitro*, but it does not prove that the RNAP is its unique target in bacteria.

Tagetitoxin is active on a broad range of RNAP from different species, but it does not inhibit the growth of any bacteria [44].

Transcription inhibitors without identified binding sites

Among the many putative inhibitors are molecules that have proven inhibitory effects on transcription *in vitro* but lack the ultimate genetic proof that they are true transcription inhibitors; in other words, there are no data demonstrating that mutations on the relevant RNAP genes render the resulting mutants resistant to the inhibitors.

Ripostatins

Ripostatin A and B are macrolides produced by the myxobacteria *Sorangium cellulosum* [47] (Figure 2). Ripostatins seem to target the RNAP, mainly at the level of chain initiation rather than chain elongation, but the target and mechanism have not been studied in detail. Ripostatin does not share any cross-resistance with rifampicin or sorangicin [47,48].

Ripostatin B is slightly more active than ripostatin A, and its spectrum of activity is limited to few bacteria, such as *S. aureus* (MIC = 1 μg/ml) and *E. coli tolC* (MIC = 1.25 μg/ml), Ripostatin B is also active against some yeast and fungi. Both molecules have been found to be highly cytotoxic against mouse fibroblasts. Efflux and influx pumps often decrease the activity of antibiotics on living bacteria, and these permeability phenomena can account for the resistance of most bacteria to this antibiotic.

SB series

The SB series of antibiotics is a new class of synthetic bacterial RNAP inhibitors with antibacterial activity [49] (Figure 2). This series was discovered by screening a library of synthetic compounds using an *E. coli* core RNAP–σ^{70} factor dissociation assay [50]. The RNAP–σ^{70} interface is highly conserved and required among bacteria.

SB-type compounds dissociate the core–σ complex and inhibit transcription *in vitro* in the micromolar range. They are bactericidal agents with activity against Gram-positive bacteria (*Staphylococcus*, *Bacillus*, *Streptococcus*), but not against key Gram-negative bacteria (*E. coli*, *Pseudomonas aeruginosa*). Interestingly, SB-type molecules have no effect on the growth of eukaryotic cells (e.g. Chinese hamster ovarian cells, *Candida albicans* and *Aspergillus fumigatus*).

CBR-type and SB-type molecules share limitations owing to their relatively high hydrophobicity as compared with antibacterial agents used in chemotherapy. Indeed, it is highly probable that these molecules strongly bind to serum albumin, preventing them from reaching their target. Such hydophobicity is a common problem for molecules issued from combinatorial or parallel synthesis, but medicinal chemistry can be used to improve the physico-chemical properties of these molecules and might be easier to perform on them than it is on natural chemical products.

GE23077

GE23077 is a novel inhibitor of bacterial RNAP that was recently discovered in culture broths of an *Actinomadura* species [51]. GE23077 is a cyclic heptapeptide consisting of common and unusual amino acids (Figure 2). GE23077 was isolated as a complex of four factors: A1, A2, B1 and B2. The A and B forms differ slightly in a side chain of the cyclic peptides: A1 and A2 are epimers, as are B1 and B2 [52]. The chemical structures of the complex components have been elucidated, and the absolute stereochemistries of five out of ten chiral centers have been assigned [53].

GE23077 is a potent inhibitor of *E. coli* and *B. subtilis* RNAPs with an half-maximal inhibitory concentration (IC_{50}) in the 10 nM range [53]. GE23077 acts at the level of initiation of transcription and does not interfere with the interaction between the RNAP and DNA. These characteristics closely resemble those of rifampicin, but the two molecules probably possess distinct binding sites on the RNAP, as indicated by the fact that rifampicin-resistant RNAP mutants remain sensitive to GE23077 [53]. Despite its activity on purified RNAP, GE23077 has a narrow spectrum of antimicrobial activity, which is essentially limited to *Moraxella catharrhalis*. This narrow spectrum is presumably due to the permeability of the drug across bacterial membranes – a property suggested by experiments using permeabilized *E. coli* [53]. In the future, the

co-crystallization of GE23077 with its target enzyme will provide a good basis for further rational design of new derivatives and analogs of this antibiotic.

Ureidothiophene

A family of low micromolar inhibitors based on a 2-ureidothiophene-3-carboxylate scaffold (Figure 2) was identified when a library of commercially available compounds targeted against the *S. aureus* RNAP holoenzyme was screened by using a functional assay to measure the incorporation of radiolabeled nucleosides [54]. These ureidothiophene molecules are active on some but not all *S. aureus* strains. The compounds have a high propensity to select spontaneous resistant mutants and maintain their antibacterial activity against rifampicin-resistant strains of *S. aureus*, suggesting that they have an alternative mechanism of action or a different binding site on the RNAP holoenzyme.

Corallopyronins

Corallopyronins are 2-pyrone-containing molecules produced by the myxobacteria *Corallococcus coralloides* [55] (Figure 2). They are closely related to myxopyronin produced by *Myxococcus fulvus* [56], and both types of molecule specifically inhibit the bacterial RNAP and are inactive on the eukaryotic enzyme.

Corralopyronins block the growth of Gram-positive bacteria with an MIC value between 0.1 and 10 μg/ml, but not affect Gram-negative bacteria, yeast or fungi.

Transcription as an antimicrobial drug target

Bactericidal drugs present notable advantages compared with bacteriostatic ones in several well-defined pathologies (reviewed in Ref. [57]). Indeed, rifampicin is generally classified as a bactericidal antibiotic and the molecular basis of its bactericidal effects has been recently identified as the induction of programmed cell death [58].

Ansamycines were introduced in 1962 to treat Gram-positive bacterial infections and subsequently tuberculosis. Currently, short-course chemotherapy comprising rifampicin, isoniazid, pyrazinamide and ethambutol–streptomycin still constitutes the cornerstone treatment for tuberculosis. Despite its excellent efficacy against Gram-positive bacteria, rifampicin has a high propensity to generate resistant mutants, which greatly limits its use as a therapeutic. For this reason, rifampicin is generally given in combination with another antibiotics and its use is restricted to well-defined pathologies. In addition to its use in tuberculosis, rifampicin is a first choice antibiotic for the prevention of meningitis and the treatment of brucellosis. In combination with quinolones, minocycline, co-trimoxazole, fusidic acid or vancomycin, rifampicin has also a major part in the treatment of biofilm-related infections [59].

More recently, two new ansamycines have been commercialized under the names of rifalazil and rifaximin [60,61]. Rifalazil has potential to treat the intracellular pathogen *Chlamydia trachomatis* and rifaximin to treat infectious diarrhea.

Apart from ansamycines, lipiarmycin is the only transcription inhibitor that is currently in clinical trials under the names of OPT-80 (Optimer Pharmaceuticals) and Par-101 (Par Pharmaceuticals) for the treatment of diarrhea associated with *Clostridium difficile* [62]. OPT-80 has been granted fast-track status by the Food and Drug Administration and a phase 3 trial started in April 2006.

Conclusion

Bacteria have become resistant to many existing antibiotics, making infections increasingly difficult to treat. New antibiotics are lacking, and the number of validated antibiotic targets being identified is fewer than expected.

The RNAP is the target for an astonishingly large number of antimicrobial compounds, including rifampicin – a commercial antibiotic that has been used for decades. This enzyme is a large and complex protein, and the consequence of this complexity is that a number of antibiotics with different mechanisms of action target this enzyme; however, most of these molecules are not good enough for clinical application and must be modified. Recently, high-resolution structures of several binding sites of these transcription inhibitors have become available, explaining how some of these molecules bind to the RNAP, how they exercise their inhibitory effects on transcription, and how resistance to a drug is selective or shared with other inhibitors. We can now speculate about how, based on these new structural data, these drugs might be improved or modified to improve their pharmacological properties.

Surprisingly, most of the inhibitors bind in the same spatial neighborhood, and a greater challenge will be to identify and to target as yet unexploited sites. The RNAP is a large flexible protein that interacts with many other transcription factors and thus with different potential drug-binding pockets. The situation becomes even more complex if we consider the links between transcription, translation, replication and DNA repair. It is increasingly clear that the complex machines involved in transcription and translation communicate directly with one another and share proteins [63]. So far, we do not know whether the sites targeted by the natural transcription inhibitors are the only effective ones or whether new sites remain to be discovered. The evolution process cannot always select compounds with the highest efficacy, broadest selectivity and lowest propensity to select resistant mutants because the antibiotic producers have to survive, and thus be resistant to, their own antibiotics.

The recent screening of large chemical libraries for synthetic inhibitors will probably provide at least some answers to these questions. If the evolution process is responsible for the close spatial proximity of natural inhibitor binding sites, then the screening of highly diverse chemical structures issued from the human mind will probably select molecules that target sites that are distributed more evenly at the surface of the enzyme.

As our knowledge of the transcription machinery increases, more inhibitors are likely to be characterized. Several potential binding pockets without identified ligands exist at the surface of the RNAP, and it is probable that the implementation of affinity-based screening [64] instead of transcription-based screening will generate novel effectors that affect the fidelity of the transcription, that target other transcription steps such as termination or pauses, or that possibly affect the coupling between transcription and other process. These features make the RNAP a validated but still attractive target for the next generations of antibiotics.

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