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Rifampicin-resistance, *rpoB* polymorphism and RNA polymerasegenetic engineering

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A B S T R A C T

Following its introduction in 1967, rifampicin has become a mainstay of therapy in the treatment of tuberculosis, leprosy and many other widespread diseases. Its potent antibacterial activity is due to specific inhibition of bacterial RNA polymerase. However, resistance to rifampicin was reported shortly after its introduction in the medical practice. Studies in the model organism *Escherichia coli* helped to define the molecular mechanism of rifampicin-resistance demonstrating that resistance is mostly due to chromosomal mutations in *rpoB* gene encoding the RNA polymerase β chain. These studies also revealed the amazing potential of the molecular genetics to elucidate the structure–function relationships in bacterial RNA polymerase. The scope of this paper is to illustrate how rifampicin-resistance has been recently exploited to better understand the regulatory mechanisms that control bacterial cell physiology and virulence, and how this information has been used to maneuver, on a global scale, gene expression in bacteria of industrial interest. In particular, we reviewed recent literature regarding: (i) the effects of *rpoB* mutations conferring rifampicin-resistance on transcription dynamics, bacterial fitness, physiology, metabolism and virulence; (ii) the occurrence in nature of "mutant-type" or duplicated rifampicin-resistant RNA polymerases; and (iii) the RNA polymerase genetic engineering method for strain improvement and drug discovery.

1. Introduction

Bacterial RNA polymerase (RNAP) is a well established and still an attractive target for antibiotic therapy. RNAP is an essential enzyme with an impressive degree of structural conservation in the three domains of life despite a relatively low sequence identity, and with high degree of both structural and sequence conservation within each domain. These features account for the efficacy, selectivity and broad-spectrum activity of antibiotics targeting bacterial RNAP such as rifamycins (Chopra, 2007; Darst, 2004; Villain-Guillot et al., 2007).

Rifampicin, a semisynthetic rifamycin, is one of the most potent and broad-spectrum antibiotics against bacterial pathogens. The history of this antibiotic (also called rifampin in the United States) dates back to 1957 when a soil sample from a pine arboretum near the beach-side town of St Raphael in southern France was brought for analysis to the Lepetit Pharmaceutical research lab in Milan, Italy. There, a research group isolated an interesting bacterium, currently classified as *Amycolatopsis mediterranei* (Lechevalier et al., 1986), capable of producing a mixture of molecules with antibiotic activity. These related compounds were called "rifomycins" A, B, C, D, E (later changed to "rifamycins") (Aronson, 1999). The only component of this mixture sufficiently stable to be isolated in a pure form was rifamycin B, which unfortunately has only a very modest antibacterial activity. In 1959, after two years of attempts to obtain more stable semisynthetic products, a new molecule with a 4-methyl-1-piperazinaminyl side chain exhibiting high efficacy, good tolerability and excellent oral bioavailability was produced, and was named "rifampicin". Rifampicin and other rifamycins are polyketide antibiotics belonging to the family of ansamycins antibiotics, so named because of their basket-like molecular architecture (Latin: ansa = handle) comprising an aromatic moiety bridged at non-adjacent positions by an aliphatic chain. The potent

antibacterial activity of these compounds is due to their specific inhibition of bacterial RNAP (Campbell et al., 2001).

Following its introduction in 1967, rifampicin has become a mainstay of therapy in the treatment of tuberculosis, leprosy and AIDS-associated mycobacterial infections (Shinnick, 1996). In addition to mycobacterial infection, rifampicin is also used in the treatment of infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) in combination with fusidic acid, including difficult-to-treat osteomyelitis and prosthetic joint infections. It is also an excellent prophylactic agent against *Neisseria meningitidis* infections, and is recommended as an alternative treatment for infections with the tick-borne disease pathogens, *Borrelia burgdorferi* and *Anaplasma phagocytophilum*, when treatment with doxycycline is contraindicated. Additional indications are infections sustained by *Listeria* spp., *Neisseria gonorrhoeae*, *Haemophilus influenzae* and *Legionella pneumophila*.

As well as for other antibiotics, resistance to rifampicin was reported shortly after its introduction in the medical practice, particularly in tubercle bacilli (Manten and Van Wijngaarden, 1969). Studies in the model organism *Escherichia coli* helped to define the molecular mechanism most frequently involved in rifampicin-resistance (di Mauro et al., 1969; Ezekiel and Hutchins, 1968; Khesin et al., 1969; Tocchini-Valentini et al., 1968). Importantly, the studies with the *E. coli* rifampicin-resistant (Rif^R) mutants also revealed the amazing potential of the molecular genetics to elucidate the structure–function relationships in bacterial RNAP (Jin and Gross, 1991, 1988; Jin et al., 1988a, 1988b; Korzheva et al., 2000; Landick et al., 1990; Mustaev et al., 1997; Tavormina et al., 1996). The scope of this paper is to review how rifampicin-resistance has been exploited to better understand the regulatory mechanisms that control bacterial cell physiology and virulence, and how this information has been used to maneuver, on a global scale, gene expression in bacteria of industrial interest for strain improvement and drug discovery. To this purpose, we start the next section trying to summarize our current understanding about the structure and function of the bacterial RNAP, the interaction of RNAP with transcription factors and rifampicin, and the so-called "stringent response", the global regulatory system that modulates gene expression in bacteria in response to nutritional stress. Indeed, a functional overlap has been reported between the phenotypes exhibited by certain rifampicin-resistance mutations and the stringent phenotype (Xu et al., 2002; Zhou and Jin, 1998).

2. The bacterial RNA polymerase, the interaction with rifampicin, and the "stringent response"

2.1. The bacterial RNA polymerase

Over the past twenty years, crystallographic structures have been determined for bacterial RNAP complexes with nucleic acids, nucleotides, modulating small molecules, and inhibitors including rifamycins. The structure of *Thermus aquaticus* core RNAP, solved in 1999, revealed that the enzyme with a subunit composition of α2ββ'ω (Murakami et al., 2002a,b; Vassylyev et al., 2002; Zhang et al., 1999) has a shape reminiscent of a "crab claw", which is similar to that of the archaeal RNAP (Hirata et al., 2008) and eukaryotic RNAP (Cramer et al., 2000, 2001). The two largest β and β' subunits form the two pincers of the crab claw, which are separated by a deep cleft about 27 Å wide (Zhang et al., 1999) (Fig. 1A). The two pincers of the claw form a positively charged cleft (known as the active-site or main channel) which contains two catalytically active Mg^{+2} ions, and accommodates the nucleic acids during transcription. A dimer of α subunits (αI and αII) is positioned at the interface of the β and β' pincers, while the small ω subunit wraps around the C-terminal tail of the β' subunit. Each of the two α subunits consists of two domains: an N-terminal domain (α-NTD) and a smaller C-terminal domain (α-CTD) joined by a flexible linker (Blatter et al., 1994). The α-NTD dimerizes and is responsible for the assembly of the large β and β' subunits. The α-CTD plays a different role; it interacts with a diverse range of transcriptional activators and can interact with promoter DNA sequences (Gourse et al., 2000; Haugen et al., 2008).

Based on crystallographic data, biophysical results, biochemical results, models have been proposed for the structures of transcription initiation and elongation complexes. The models propose that nucleic acids completely fill the active-site (or main) channel of RNAP, such that the only route by which incoming nucleoside triphosphate substrates (NTPs) can access the active center is through a narrow funnel-shaped "pore" (also known as the "secondary channel") that leads from the surface of the enzyme to the active site (Gnatt et al., 2001; Ebright, 2000). Crystallographic data show that α-NTD, ω and regions of β and β' subunits form an immobile core surrounded by five domains: the β' clamp, β' jaw, β-flap, β protrusion (or β upstream lobe) and β lobe (or β downstream lobe) (Fig. 1A). These domains are able to move independently from each other as rigid bodies, and their movements enable binding to σ factors and promoter DNA, and opening and closing of the main channel during the transcription cycle, driving loading of the DNA into the active site channel. In particular, the β' clamp folds over the catalytic cleft and holds the nucleic acids (DNA and RNA) more tightly in the active center, stabilizing the elongation complex. Movements of the β upstream and β downstream lobes contribute to open and close the active site channel, while conformational changes of the β flap modulate the elongation behavior of the enzyme at the RNA-exit channel in response to hairpin-dependent pause signals.

In addition to the above-mentioned mobile domains, crystallographic data have also shown other structural motifs (helices or loops) that have been named according to either location or aspect, or presumed role in the transcription process as deduced also by genetic, biochemical and biophysical studies (Fig. 1A). For examples, the β' bridge helix separates the main channel into a downstream DNA entry channel and secondary channel, and is located near the template DNA at the +1 site. Therefore, it has been proposed that this motif is involved in translocation of the nucleic acids during transcription. Just inside the secondary channel lies the β' trigger loop, a structural element that was shown to play a role in elongation by modulating the oscillating properties of the adjacent β' bridge helix. The location of the β' rudder, the β' lid and the β fork loop 1 suggests that these loops are involved in DNA-RNA strand separation, in order to maintain as 8–9 bp long DNA-RNA hybrid of the transcription "bubble" in the active-site cleft. The fork loop 2 and the zipper could be involved in delineating the downstream and upstream boundary of the transcription bubble, respectively. Five loops of β' and β subunits have been termed the switches and could participate in controlling the position of the clamp or, for switches 1–3, in forming a binding site for the DNA–RNA hybrid.

2.2. Interaction of RNA polymerase with sigma factors and regulatory proteins

Although core RNAP is catalytically proficient for transcription, it can only weakly and unselectively bind to the DNA. To begin transcription at a specific promoter, RNAP must first bind a dissociable subunit called the sigma (σ) factor to form a fully functional RNAP holoenzyme (referred to as Eσ). The multiple members of the σ factor family are divided into two classes, the σ^{70} class and the σ^{54} class, with little sequence conservation between the two. The σ^{70} class owes its name to the prototypical *E. coli* "housekeeping" σ^{70} factor. This class is composed of the primary σ factors, which are responsible for transcribing most genes involved in basic cellular metabolism, and many members of alternative σ factors, which

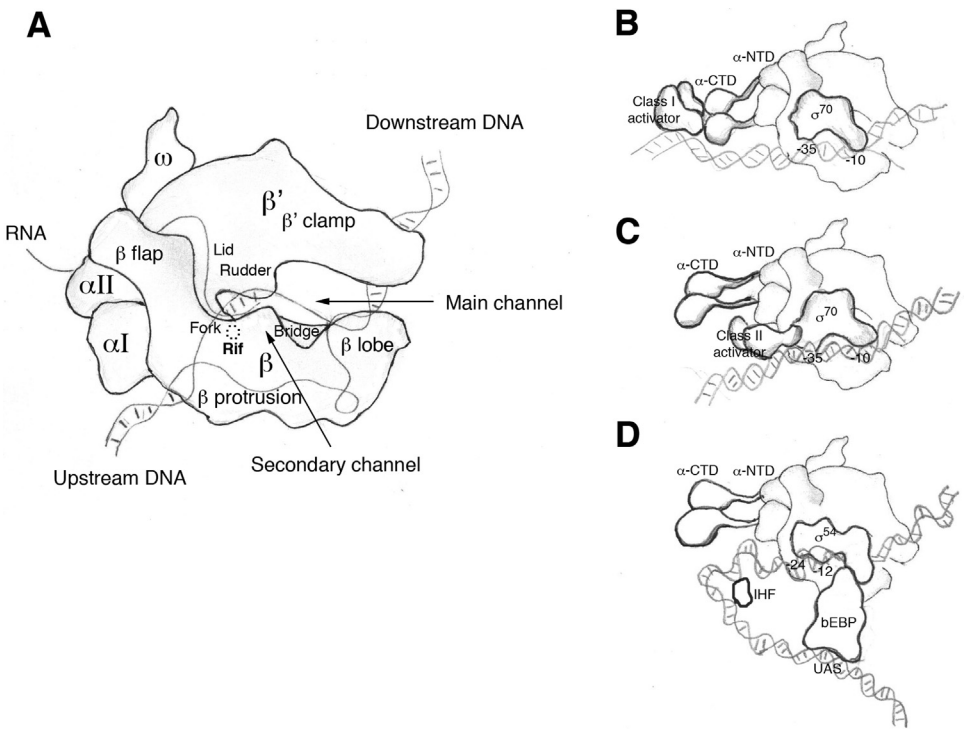

Fig. 1. Schematic representation of bacterial RNA polymerase and its interaction with transcriptional activators. (A) Subunit structure of bacterial core RNA polymerase (RNAP) with location of mobile domains and other structural motifs playing a key role in the transcription process. (B–D) Interaction of bacterial RNAP with transcriptional activators. Eσ^{70} holoenzyme activators are grouped into two classes: Class I and Class II. Class I activators bind well upstream of the promoter −35 element, and establish direct contact with the α-CTD, thereby recruiting RNAP to the promoter (B). Class II activators bind very close to the promoter −35 element, and directly contact domain 4 of σ^{70}, to recruit RNAP to the promoter. At some Class II promoters, the activators make additional contact with the α-NTD of RNAP (C). Eσ^{54} holoenzyme activators (bEBP) hydrolyze ATP and bind upstream activating sequences (UAS) well upstream (80–150 bp) from the transcription site. The interaction of UAS-bound bEBP with Eσ^{54} requires DNA looping, which is often facilitated by DNA bending proteins such as the integrative host factor (IHF) (D).

transcribe subsets of genes required under specific growth conditions, such as heat shock, or specific cellular processes, such as flagella production (Gruber and Gross, 2003; Paget and Helmann, 2003). In contrast, the σ^{54} class has a single member, σ^{54}, is present in most but not all bacteria (about 60% according to EBI-Genome database), and has been implicated in directing the transcription of genes associated with nitrogen metabolism, various stresses and growth-limiting conditions including phage shock, cytotoxicity from nitrosative stress and pathogenicity (Buck et al., 2000; Fisher et al., 2005).

Notably, the two classes of σ factors employ very different modes to activate transcription in the RNAP holoenzyme. The σ^{70} members are composed of four functionally distinct helical domains (σ1 to σ4) connected by flexible linkers, which are involved in promoter recognition, core RNAP binding and DNA melting (Haugen et al., 2008; Murakami and Darst, 2003). The transition from a closed to an open complex (isomerization) occurs spontaneously upon promoter binding. In this system, regulations of transcription initiation is accomplished by a variety of regulatory proteins that recruit (class I and class II activators) or obstruct (repressors) Eσ^{70} binding to the promoter region, or promote the escape of Eσ^{70} from the promoter (Browning and Busby, 2004; Busby and Ebright, 1997; Haugen et al., 2008; Helmann, 2009) (Fig. 1B and C). In contrast to Eσ^{70}, the alternate σ^{54}-RNAP holoenzyme (Eσ^{54}), which recognizes promoters marked by the −24 (GG) and −12 (TGC) consensus sequences upstream of the transcription start site, binds promoter sequences in an energetically favorable closed conformation that rarely isomerizes into an open complex (Buck et al., 2000; Wigneshweraraj et al., 2008). To initiate transcription, Eσ^{54} needs to bind specialized activator proteins (known as bacterial enhancer-binding proteins [bEBP]) that hydrolyze ATP and use the energy released to remodel their substrates (Rappas et al., 2007; Schumacher et al., 2004; Xu and Hoover, 2001; Zhang et al., 2002) (Fig. 1D). Thus, σ54 is structurally different from σ70 members, and is composed of three regions based on function: the N-terminal region I interacts with activator proteins and the −12 promoter element, where DNA melting originates; the central region II is variable and sometimes absent; the C-terminal region III contains a number of functional modules, including determinants that bind core RNAP and promoter DNA (Bordes et al., 2004; Bose et al., 2008a,b; Buck et al., 2000; Chaney et al., 2001; Gallegos et al., 1999).

2.3. Interaction of RNA polymerase with transcription elongation and termination factors

Once the process of transcription begins, sigma factors dissociate, and RNAP makes a stable ternary elongation complex (TEC) with the DNA and nascent RNA (Wilson and von Hippel, 1994; Mooney et al., 1998). During this stage a number of transcription elongation factors interact with RNAP including Nus factors [NusA, NusB, NusG and NusE (ribosomal protein S10)], ribosomal protein S4, RfaH, Gre factors (GreA, GreB), transcription/DNA repair coupling factor Mfd, RapA (HepA) and termination factor Rho (Bailey et al., 2000; Fish and Kane, 2002; Nudler and Gottesman, 2002; Roberts and Park, 2004; Squires and Zaporojets, 2000; Sukhodolets et al., 2001). These factors affect RNAP processivity by modulating transcription pausing, arrest, termination and antitermination.

Two types of mechanisms are known to cause transcription termination: intrinsic (or Rho-independent) termination and factor-mediated (or Rho-dependent) termination. Intrinsic termination occurs in the absence of auxiliary factors at sites where the nascent RNA can form a stable hairpin-like structure immediately preceding a run of uridine residues. Rho-mediated termination

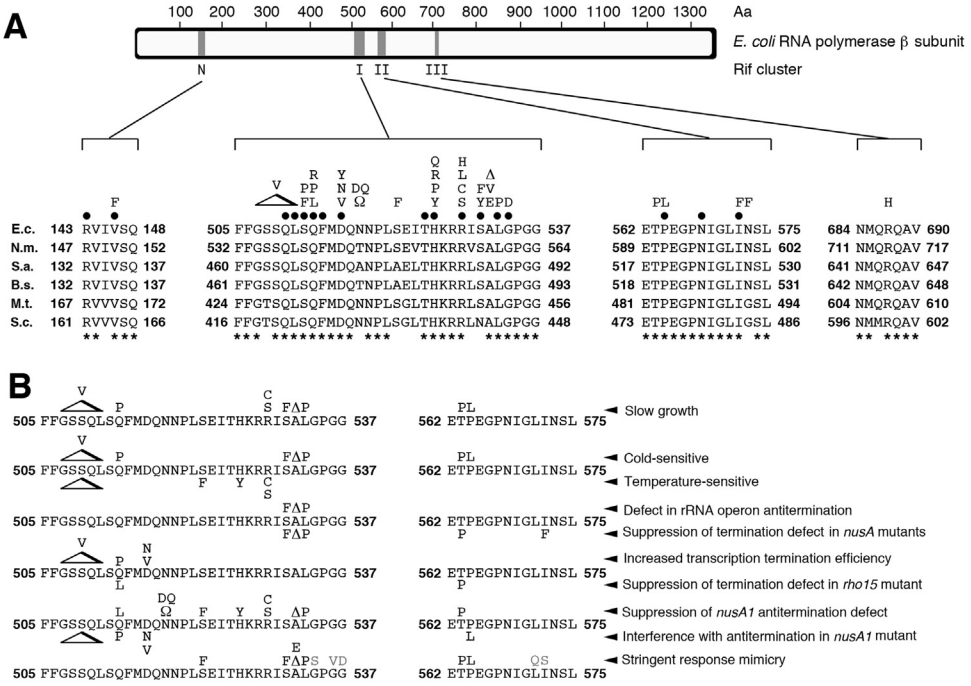

Fig. 2. Interaction between rifampicin and amino acids of RNA polymerase β subunit (RpoB). (A) Map of the *E. coli* RNA polymerase β (RpoB) subunit with location of *rif* clusters N, I, II and III (top), and amino acid sequence alignment of the *rif* clusters from *E. coli* (**E.c.**), *N. meningitidis* (**N.m.**), *S. aureus* (**S.a.**), *B. subtilis* (**B.s.**), *M. tuberculosis* (**M.t.**) and *S. coelicolor* A3(2) (**S.c.**) (bottom). Well-characterized RpoB substitutions causing rifampicin-resistance in *E. coli* are reported above the *E. coli* RpoB sequence. Numbering begins at the first amino acid of the RpoB sequences. Closed circles above the *E. coli* sequence indicate amino acid residues involved in direct binding to rifampicin. Δ indicates amino acid deletion; ▲ indicates amino acid insertion; asterisks indicate evolutionarily conserved RpoB amino acid residues. (B) Phenotypes associated with Rif^R RpoB substitutions in *E. coli*. Amino acid substitutions associated with a stringent-like phenotype but not conferring rifampicin-resistance are shown in gray.

results from the action of the Rho protein that binds to specific sequences called *rut* (Rho utilization) sites in the nascent RNA. After binding Rho acts as a molecular motor translocating along the nascent RNA and promoting dissociation of paused TEC. *rut* sites are generally represented by RNA regions of about 70–80 nucleotides that are rich in cytosine and devoid of secondary structures (Morgan et al., 1985; Bear et al., 1988; Alifano et al., 1991; Rivellini et al., 1991). These sites may be located at the end of transcriptional units contributing to transcriptional punctuation of the bacterial genome, or also intragenically. In this latter case, the *rut* sites become accessible to Rho under conditions that perturb translation leading to premature termination of untranslated or poorly translated transcripts (the so-called transcriptional polarity) (Alifano et al., 1988, 1991; Ciampi et al., 1989; Richardson, 1991). Indeed, under optimal translation conditions, the leading ribosome masks the *rut* sites before Rho can anchor to it. This mechanism contributes to tight coupling between translation and transcription in bacteria (Alifano et al., 1994; Richardson, 1991). The existence of a direct physical link between the leading ribosome and the TEC is further supported by NMR studies showing that NusG, a conserved protein that associates with RNAP, interacts with Rho stimulating transcription termination, and competitively with the ribosomal protein S10 (NusE) (Burmann et al., 2010). It thus appears that Rho competes with the ribosome at two sequential levels, initially for the binding to the *rut* sites, and subsequently for the interaction with NusG. NusE, in turn, may also interact with NusB at the level of specific RNA control sequences (termed "*boxA*"), and the NusB/NusE/*boxA* RNA ternary complex interacts with the TEC promoting N-mediated transcription antitermination in bacteriophage lambda (Das et al., 2008; Stagno et al., 2011). On the other hand, the interaction of NusB with a *boxA*-like element within *S. enterica* sv. Typhimurium *hisG* was reported to stimulate premature Rho-dependent termination at a suboptimal intragenic *rut* site (Carlomagno and Nappo, 2001). Interestingly, Rho protein has been recently involved also in a number of additional mechanisms including the avoidance of potential conflicts between DNA replication and transcription, suppression of pervasive antisense transcription and recruitment in riboswitch and small RNA-dependent regulation (Boudvillain et al., 2013).

Transcription pausing, which is fundamental in transcription termination and antitermination, may occur either by RNAP interaction with nascent RNA secondary structures or by physical barriers to RNAP translocation such as DNA-binding proteins, DNA lesions, misincorporated substrates, and specific DNA sequences (Fish and Kane, 2002). Under certain conditions, both types of pausing can lead to either transcription arrest or termination (Richardson and Greenblatt, 1996). Genetic, biochemical and structural studies have identified four structural determinants in RNAP responsible for stability of TEC: the downstream DNA-binding clamp and the RNA/DNA hybrid binding site (both in the main channel), the single-stranded RNA binding site and the upstream RNA binding site (both in the RNA-exit channel) (Fig. 1A). In addition, when pausing occurs by physical barrier, the secondary channel, which allows NTPs diffusion, is also involved as it binds the RNA 3′-terminus that is extruded from the catalytic center in "backtracked" TEC (Zhang et al., 1999; Vassylyev et al., 2002). The evidence that many mutations affecting transcription elongation and termination map in or in close proximity of these channels, including a number of Rif^R mutations that will be presented below (Fig. 2B), strongly supports this view. Current models also suggest most of transcription elongation factors interact with RNAP at or near these three channels: Mfd near the upstream opening of the main (DNA-binding) channel, NusA in the proximity of both the main channel and the RNA exit channel, and GreA within the secondary (backtracked RNA-binding) channel (Borukhov et al., 2005).

2.4. Interaction of RNA polymerase with rifampicin

In 2001 Campbell and colleagues obtained crystals of RNAP in a complex with rifampicin allowing the identification of the binding

pocket of this antibiotic, and a better understanding of the mode of action of this antibiotic (Campbell et al., 2001). The pocket is centered approximately 20 Å upstream from the active center on the wall of the main channel in a region of the β subunit forming the fork domain. The rifamycin naphthyl moiety (atoms C1–C10) contacts β residues V146, L511, S513, R529, S531, L533, G534, N568, and I572 (*E. coli* RpoB numbering). The rifamycin ansa moiety (atoms C15–C29) contacts β residues R143, Q510, L511, L512, F514, D516, T525, H526, P564, and Q761 (Fig. 2A). Rifampicin positioned in this pocket would block growth of the RNA chain past 2 or 3 nucleotides, explaining the bactericidal effect of the antibiotic. As the synthesis of the first two phosphodiester bonds can occur in the presence of rifampicin, the antibiotic does not interfere with substrate binding, catalytic activity, or the intrinsic translocation mechanism of the RNAP. Moreover, RNAP becomes totally resistant to rifampicin after it has synthesized a transcript of 3 or 4 nucleotides and entered the elongation phase.

Consistently with the crystallographic data, most of the rifampicin-resistant (Rif^R) mutations isolated so far map directly in the fork domain that is proximal to catalytic site, or in adjacent regions including the protrusion and external domains 1 and 2 of the β subunit (Jin and Gross, 1988, 1991; Landick et al., 1990; Lisitsyn et al., 1984; Mustaev et al., 1991; O'Neill et al., 2000; Ovchinnikov et al., 1981, 1983; Severinov et al., 1993; Singer et al., 1993; Tavormina et al., 1996; Vattanaviboon et al., 1995) (Figs. 1A and 2A). Amino acid substitutions at these sites are expected to affect the conformation of the binding pocked, and lower its affinity for rifampicin. In particular, substitutions at three sites, β D516, β H526, and β S531 (*E. coli* numbering), confer high levels of rifamycin-resistance and little or no loss of fitness, and, accordingly, are especially frequently encountered in clinical isolates of Rif^R bacteria. Other mutations conferring lower levels of rifampin resistance are located away from this binding pocket. Many of them are found in the lobe region suggesting that binding of rifampin to the fork region is somehow linked to the lobe's conformation when holding the downstream DNA suggesting that binding of the lobe domain to DNA is necessary for the formation of the rifampin-binding pocket.

2.5. Interaction of RNA polymerase with ppGpp

In *E. coli*, two unusual regulatory nucleotides, the guanosine tetra- and pentaphosphate (collectively referred to here as ppGpp), were identified initially as the effectors of the so-called "stringent response" (Cashel et al., 1996; Potrykus and Cashel, 2008). This response in bacteria may be triggered by nutritional deprivation, such as amino acid starvation, and is accompanied by an extensive reprogramming of gene expression. Binding of ppGpp to *E. coli* RNAP inhibits transcription from many promoters required for ribosome synthesis, activates transcription from a number of promoters for amino acid biosynthesis, and regulates a variety of additional promoters as well (Barker et al., 2001a,b; Haugen et al., 2008). Studies in other microorganisms and genome-wide approaches demonstrate that ppGpp is a key factor in bacterial physiology, and hence is considered a bacterial "alarmone". It regulates the levels of transcripts from several hundred genes involved in macromolecular biosynthetic pathways, a variety of stress response, and a number of adaptive programs including antibiotic biosynthesis in actinomycetes, sporulation, host colonization and virulence in many pathogens (Dalebroux et al., 2010; Durfee et al., 2008; Traxler et al., 2008).

In *E. coli* ppGpp exerts many of its physiological effects by interacting directly with RNAP in cooperation with DnaK suppressor (DksA), a protein that binds in the secondary channel of the enzyme amplifying the effects of the ppGpp on RNAP (Paul et al., 2004; Perederina et al., 2004; Potrykus and Cashel, 2008). ppGpp affects transcription from only a subset of promoters which are characterized by specific structural and kinetic properties. In particular, ppGpp increases decay of the open complex thereby inhibiting transcription initiation from complexes that are intrinsically short-lived (Barker et al., 2001b; Haugen et al., 2008). In *E. coli* short-lived transcription initiation complexes include those formed at the level of rRNA-encoding operons and many tRNA-encoding genes, as well as some promoters for mRNA, for instance *pyrBI* and *fis* promoters (Barker et al., 2001a; Donahue and Turnbough, 1990; Mallik et al., 2006; Potrykus and Cashel, 2008; Murray et al., 2003). As a consequence, ppGpp negatively affects transcription at the level of these promoters. The stimulatory effects of ppGpp at the level of other promoters are probably indirect resulting from the inhibition of rRNA transcription and, thus, liberation of RNAP for binding to other promoters (Barker et al., 2001a; Murray et al., 2003).

The ppGpp binding site on RNAP has remained elusive for a long time. Initial studies using cross-linkable ppGpp derivatives concluded that ppGpp bound to one of the two large subunits of *E. coli* RNAP, i.e., β′ (Toulokhonov et al., 2001) or β (Chatterji et al., 1998), but neither study located the binding site more specifically. A later crystallographic study with a RNAP from *Thermus thermophilus* showed ppGpp to be positioned adjacent to the RNAP catalytic center, partially overlapping with the incoming NTP binding site at the bottom of the secondary channel (Artsimovitch et al., 2004). However, subsequent studies indicated that the *T. thermophilus* RNAP, similar to RNAPs from *Bacillus subtilis*, is not regulated by ppGpp (Kasai et al., 2006; Vrentas et al., 2008; Westover et al., 2004). In fact, at variance with Proteobacteria, in *B. subtilis* and possibly in most other bacteria cessation of rRNA synthesis during the stringent response is not due to a direct action of ppGpp on RNAP, but rather results from the concomitant reduction in the GTP pool because these bacteria rely on GTP as the initiating nucleotide for transcription of rRNA operons (Krásný and Gourse, 2004; Kriel et al., 2012).

More recently, a crystal structure of the *E. coli* RNAP holoenzyme in complex with ppGpp was reported (Zuo et al., 2013). The structure revealed that the alarmone binds at an interface between the core module (containing sections of the β, β′ and α subunits) and the shelf modules (containing sections of the β, β′ and ω subunits) on the surface of RNAP, away from the catalytic center and the nucleic acid binding path. These results were consistent with a parallel study in which the ppGpp binding site on *E. coli* RNA was mapped by crosslinking, protease mapping, and analysis of mutant RNAPs that fail to respond to ppGpp (Ross et al., 2013). The binding site was positioned at the interface between the β′ and ω subunits. Residues implicated in ppGpp binding were β′ K615, β′ I619, β′ D622 and β′ Y626 in the core module, and β′ R362, β′ R417, ω A2, ω R3, ω V4 and ω T5 in the shelf module. These residues are not conserved in RNAPs from distantly related bacterial species, including *B. subtilis*, consistent with the absence of direct effects of ppGpp on *B. subtilis* RNAP (Ross et al., 2013) (Fig. 3).

Identification of the binding site allowed to propose a model for the mechanism of ppGpp action, and to predict in which bacterial species ppGpp exerts its effects by targeting RNAP. The position of ppGpp on the surface of RNAP suggests an allosteric mechanism of action involving restriction of motion between the two mobile core and shelf modules of RNAP. Because ppGpp inhibits transition on the pathway to open complex formation prior to nucleotide addition, the model predicts that by altering the relative orientation of the mobile modules ppGpp weakens or disrupts the interactions between RNAP holoenzyme segments and the DNA template strand in a promoter open complex (Ross et al., 2013). These interactions include σ subunit contacts to several promoter recognition elements, β and β′ subunit contacts to the downstream portion of the transcription bubble, and β and β′ contacts to the downstream DNA duplex (Murakami and Darst, 2003; Zhang et al., 2012; Chakraborty et al., 2012). A "finger" in the σ subunit positions the

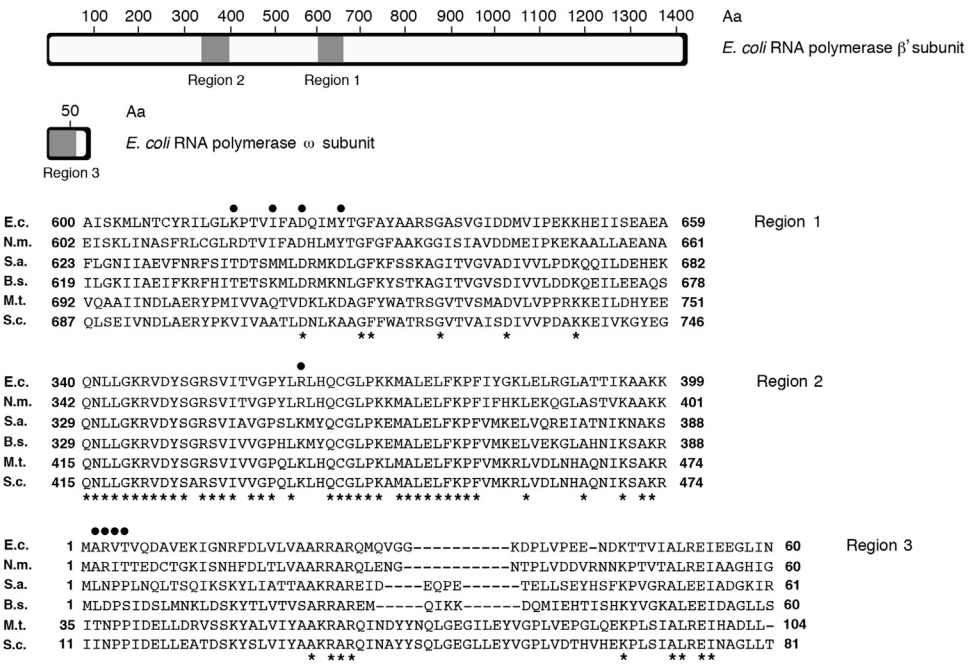

Fig. 3. Interaction between ppGpp and amino acids of *E. coli* RNA polymerase β' (RpoC) and ω (RpoZ) subunits. Map of the *E. coli* RNA polymerase β' (RpoC) and ω (RpoZ) subunits with location of the Regions 1–3 involved in ppGpp binding (top), and amino acid sequence alignment of the Regions 1–3 from *E. coli* (**E.c.**), *N. meningitidis* (**N.m.**), *S. aureus* (**S.a.**), *B. subtilis* (**B.s.**), *M. tuberculosis* (**M.t.**) and *S. coelicolor* A3(2) (**S.c.**) (bottom). Closed circles above the *E. coli* sequence indicate amino acid residues involved in direct binding to ppGpp. Asterisks indicate evolutionarily conserved RpoC amino acid residues.

template strand in the main channel, while the central segment of the β' bridge helix interacts with template strand position +2, and the β "switch 3" region interacts with template strand positions +1 to −3 (Zhang et al., 2012). Residues in two short segments that connect the core and shelf modules establish additional contacts to DNA strands in the downstream portion of the transcription bubble in initiation and elongation complexes (Vassylyev et al., 2007; Tagami et al., 2010; Zhang et al., 2012). Consistently with this model, RNAP substitutions that mimic the effects of ppGpp by reducing open complex stability, thereby suppressing growth defects in strain lacking ppGpp, are located in β, β' and σ subunits along the path of the transcription initiation bubble and downstream duplex. Notably, some of these substitutions map in the fork and fork loop 2 segments of the β subunit and confer rifampicin-resistance (Bartlett et al., 1998; Barker et al., 2001a; Xu et al., 2002; Yang and Ishiguro, 2003; Zhou and Jin, 1998).

2.6. ppGpp targets other than RNAP

Targets other than RNAP mediate additional physiological response of ppGpp. In *E. coli* ppGpp exerts a direct inhibitory effect on the activity of translation factors EF-Tu, EF-G and IF2, on exopolyphosphatase, and on lysine decarboxylase, an enzyme that is involved in acid tolerance (Dalebroux and Swanson, 2012). By indirect mechanisms, ppGpp promotes stability of σ^{38}, a stationary-phase σ-factor that is crucial for stress resistance and the expression of virulence factors by a variety of pathogens, and increases the activity of σ^{24}, a σ-factor that coordinates the cellular response to the presence of misfolded proteins in the periplasm or outer membrane (Bougdour and Gottesman, 2007; Merrikh et al., 2009). ppGpp also controls the activity of transcription factors that are dedicated to regulation of virulence genes including SlyA in *Salmonella enterica* and PigR of *Francisella tularensis* (Charity et al., 2009; Zhao et al., 2008). In *Legionella pneumophila* and *E. coli*, ppGpp induces the expression of non-coding regulatory RNAs that regulate the stability of certain mRNAs indirectly via the carbon storage regulator (CsrA) regulatory pathway (Edwards et al., 2011). Furthermore, in actinomycetes, ppGpp inhibits polynucleotide phosphorylase (PNPase) activity thereby preserving the mRNA during the stationary phase (Gatewood and Jones, 2010; Siculella et al., 2010). In *B. subtilis* ppGpp inhibits the DNA primase, and the activity of the YybT phosphodiesterase leading to an increased pool of cyclic-di-AMP (Rao et al., 2010), a nucleotide that signals DNA damage in this microorganism (Witte et al., 2008). The activity of CodY, a master regulator in Gram-positive Firmicutes, is also indirectly influenced by the alarmone. CodY is a DNA-binding transcriptional repressor that is activated by GTP, and controls antibiotic synthesis, genetic competence, flagellar production, sporulation and virulence. During the stringent response, the pool of GTP is reduced as a consequence of both the ppGpp synthesis that consumes GTP and the inhibition of a key enzyme for GTP synthesis (Gallant et al., 1971; Lopez et al., 1981). The reduced GTP pool releases the CodY repressor from its target promoters permitting their transcriptional activation (Sonenshein, 2005).

3. Rifampicin-resistance in medical practice

3.1. Effects of rpoB mutations on fitness of pathogenic bacteria, and underlying mechanisms

Immediately after the emergence of the first Rif^R mutants in the clinical practice, many epidemiologists and infectious disease specialists were interested to know the relative fitness of the Rif^R strains in order to model the spread of the rifampicin-resistance worldwide (Daddi et al., 1969). Defining the effects of drug resistance on relative fitness can be difficult. Indeed, microbial fitness is by itself a complex trait that encompasses the ability of a give strain to survive and reproduce in a given environment. Furthermore, for commensal, opportunistic or pathogenic microorganisms fitness is also affected by host-to-host transmission capabilities. Reviewing the methods used to estimate this trait is, obviously, outside the scope of this paper. However, it is important to remark that no single method is likely to be sufficient to define it because

fitness is dependent on multiple biological properties, and so multiple approaches including experimental methods and epidemiological studies as well as mathematical models are required.

Rifampicin targets transcription, an essential function. As described above, most (>95%) of the mutations conferring rifampicin-resistance are clustered within three distinct sites, the so-called *rif* cluster I, II and III (Fig. 2A), in the central segment of the β chain of the RNA polymerase. These mutations, which change amino acids directly involved in antibiotic binding to RNA polymerase, affect evolutionarily conserved residues (Campbell et al., 2001) (Fig. 2A). Therefore, it is not surprising that they may compromise transcription efficiency at the level of specific promoters, and hence physiology and fitness of the organism. Indeed, a direct relationship between the fitness cost of *rpoB* mutations and their effects on transcription was demonstrated in *E. coli* (Reynolds, 2000). In contrast, no obvious association between the magnitude of rifampicin-resistance and its allied cost was ever found in *E. coli* (Elena et al., 1998) as well as in other microorganisms (Wichelhaus et al., 2002).

In particular, *E. coli* Rif^R RNA polymerases may confer slow-growth and/or cold- or temperature-sensitive phenotypes (Jin and Gross, 1989), and exhibit altered properties in transcription elongation and/or termination (Jin et al., 1988a,b; Jin and Gross, 1991; Landick et al., 1990). Indeed, several Rif^R mutations are allele-specific suppressors of defective *nusA* and *rho* alleles (Jin et al., 1988a) (Fig. 2B). The H526Y substitution is responsible for a severe defect in transcription termination in both *E. coli* (Jin et al., 1988b; Heisler et al., 1996) (Fig. 2B), and the same phenotype is observed for the corresponding H482Y substitution in *B. subtilis* (Ingham and Furneaux, 2000). In contrast, four Rif^R mutations, i.e. the Q513P, D516V and D516N substitutions, and the 507–511 deletion with valine insertion, lead to increased transcription termination efficiency (Jin et al., 1988b) (Fig. 2B). The well-characterized Q513P mutant exhibits increased Rho-dependent and Rho-independent termination, altered transcription elongation and an increased K_m for purine nucleotides (Jin and Gross, 1991; Heisler et al., 1996). Similarly, in *B. subtilis rpoB* Rif^R mutations that change the equivalent glutamine residue (Q469) to a basic residue (Q469K or Q469R) enhances the action of Rho and sensitivity to the transcription elongation factor NusG (Ingham and Furneaux, 2000). Finally, a number of substitutions affecting the *rpoB* residues 531–533 in *E. coli*, i.e. S531F, Δ532 and L533P, results in a severe defect in rRNA operon antitermination (also known as "cellular antitermination") (Jin et al., 1988a). Interestingly, these mutations along with the S522F, A532E, T563P and P564L substitutions were shown to functionally mimic the modifications induced by the binding of ppGpp to *E. coli* RNAP (Bartlett et al., 1998; Zhou and Jin, 1998; Barker et al., 2001a,b). In fact, similar to ppGpp binding, these mutations weaken the interactions between RNAP and stringently controlled promoters such as rRNA operon promoters (Zhou and Jin, 1998). The defect in rRNA operon antitermination may further contribute to the stringent-like phenotype.

Notwithstanding their effects on transcription, a fitness burden is not always associated with *rpoB* mutations. In general, the genetic nature of the resistant determinant, and the genetic background of the antibiotic-resistant mutant may influence the relative fitness of antibiotic-resistant strains (Alifano, 2014). Hence, for instance, the substitution D516G conferring intermediate resistance to rifampicin was rather associated to a slight fitness advantage in *E. coli* (Reynolds, 2000) (Table 1). Substitution of the conserved histidine residue in the cluster I of the RNA polymerase β chain (H526 in *E. coli* corresponding to H481 in *S. aureus*) is extremely frequent among clinical Rif^R isolates in many bacterial species reflecting the low fitness cost imposed by amino acid substitutions at this position. In fact, the substitution H481N in the *rpoB* gene product of *S. aureus* was not demonstrably associated with a cost of resistance in vitro (Wichelhaus et al., 2002) (Table 1), while the substitution H481Y in *S. aureus*, as well as the corresponding substitution H526Y in *E. coli*, gave only a modest fitness burden (Wichelhaus et al., 2002) (Table 1). Molecular modeling has adequately explained the major cost associated with the substitution H481Y with respect to that of the substitution H481N in *S. aureus* (O'Neill et al., 2006). Substitution of the imidazole ring of histidine 481 with the phenolic moiety of tyrosine leads to the formation of hydrogen bonds between tyrosyl hydroxyl group and the proximal guanidine moiety of the arginine 484. As the arginine 484 lies at the surface of RNA polymerase and is predicted to be in contact with DNA, the hydrogen bonding would move the arginine residue away from its original position thus weakening the electrostatic interaction with the DNA template and decreasing the stability of the transcription complex. The reduced stability in the transcription bubble has consequence not only on transcription initiation by decreasing the stability of the promoter open complex thereby mimicking a stringent phenotype, but also on transcript elongation and termination. In fact, there is evidence that in *E. coli* the equivalent H526Y substitution decreases transcriptional pausing and termination in part through direct effects on overall transcription elongation complex stability (Landick, 2001).

The fitness burden of a given substitution may also vary between different species. In *M. tuberculosis* the RpoB S450L mutation, which is the most frequent rifampicin resistance-conferring mutation in clinical strains worldwide, was associated with the lowest fitness cost in laboratory strains and no fitness defect in clinical strains (Gagneux et al., 2006). However, in *S. aureus* the corresponding substitution S486L significantly affected bacterial growth rates (Wichelhaus et al., 2002) (Table 1). The fitness burden may also vary between different lineages of a given species. By using 3 pairs of linked cases of meningococcal disease by serogroup B, A and C rifampicin-susceptible and rifampicin-resistant isolates harboring the same H552Y substitution (H526Y in *E. coli*), Taha et al. (2006) demonstrated that the virulence of the serogroup B rifampicin-resistant isolate was substantially attenuated when

Table 1
Relative fitness associated with specific Rif^R RpoB mutations.

Bacterial species	RpoB mutation (*S. coelicolor* A3[2] numbering)	Relative fitness	Ref.
Escherichia coli	L511Q (L422Q)	0.86	Reynolds (2000)
	D516G (D427G)	1.03	
	H526Y (H437Y)	0.91	
	H526L (H437L)	0.94	
Staphylococcus aureus	S464P (S420P)	0.93	Wichelhaus et al. (2002)
	Q468R (Q424R)	0.80	
	Q468L (Q424L)	0.95	
	D471Y (D427Y)	0.88	
	D471E (D427E)	0.96	
	D471G (D427G)	0.87	
	N474K (N430K)	0.60	
	A477D (A433D)[a]	0.91	
	A477V (A433V)[a]	0.88	
	H481Y (H437Y)	0.93	
	H481N (H437N)	1	
	R484H (R440H)	0.75	
	S486L (S442L)[b]	0.86	
Mycobacterium tuberculosis	H445R (H437R)	0.38[c]	Billington et al. (1999)
	H445D (H437D)	0.42	
	H445Y (H437Y)	0.79[c]	
	S450Y (S442L)[b]	0.84[d]	

[a] Serine (instead of alanine) is present at position 433 of *S. coelicolor* A3(2) RpoB.
[b] Asparagine (instead of serine) is present at position 440 of *S. coelicolor* A3(2) RpoB.
[c] Calculated as the average of two independent mutants.
[d] Calculated as the average of four independent mutants.

compared to the corresponding susceptible isolate in BALB/c mice infected intraperitoneally. In contrast, attenuation was not significant for the other two pairs of less virulent serogroup C and serogroup A strains.

Microbial cell physiology, metabolism and lifestyle may also influence the relative fitness of antibiotic-resistant strains (Alifano, 2014). Rif^R mutations may confer a conditionally beneficial phenotype depending on the carbon source substrate. For instance, it has been demonstrated that Rif^R *rpoB* mutants of *B. subtilis* can present novel metabolic capabilities with fitness gain when compared with their rifampicin-susceptible parental strain (Perkins and Nicholson, 2008). The resistant mutants make less proficient use of strongly utilized substrates, but increase their capability for degrading weakly utilized substrates (see below). Interestingly, different Rif^R mutations have different effects on the carbon source metabolism likely because their impact on the dynamics of the transcription cycle change depending on the mutation involved (Perkins and Nicholson, 2008) (Fig. 2B).

3.2. Effects of rpoB mutations on bacterial cell physiology and expression of virulence genes

More recently, the studies on microbial fitness have benefited from the application of omics technologies. These technologies are useful not only to explore the connections linking environmental selective pressure, mutagenesis, altered phenotype, and fitness during evolution, but also to better understand the global effects of *rpoB* mutations on bacterial cell physiology and metabolism.

Comparative omics now allows, for instance, to access into the nature and the impact of single nucleotide polymorphisms that accumulate in clinical *S. aureus* isolates under combined antibiotic and host pressures, leading not only to antibiotic resistance but also to altered host-pathogen interactions that favor persistent infection (Garzoni and Kelley, 2011). Using a microarray approach, Gao et al. (2013) investigated at transcriptional levels the host-pathogen consequences of two mutations arising in methicillin-resistant *S. aureus* (MRSA) during persistent infection: the H481Y missense in the RNA polymerase β chain, and the F128Y substitution in RelA protein, which is associated with an active stringent response (Gao et al., 2013). In the *rpoB* and the *relA* mutants significant global transcriptional changes as compared to wild-type were observed including changes in genes encoding mediators of central metabolism, global regulation, and virulence-associated genes with significant overlap in transcriptional patterns associated with the two mutations. Both *rpoB* and *relA* mutations, particularly the RpoB H481Y, led to specific transcriptional effects including up-regulation of capsule and *agr* (accessory gene regulator) gene expression, which in turn reduced virulence, increased resistance to host antimicrobial peptide killing, and resulted in persistent infection (Table 2) (Gao et al., 2013). Up-regulation of the *agr* gene expression is of particular interest because in *S. aureus* the *agr* quorum-sensing system control metabolic operons involved in carbohydrate and amino acid metabolism and utilization, and phenol-soluble modulin (PSM) cytolysin genes, and, via the intracellular effector molecule RNAIII, a wide array of virulence genes (Queck et al., 2008).

As discussed above, the overlapping pattern of the two mutations is consistent with the finding that certain *rpoB* mutations causing rifampicin-resistance exhibit a stringent-like phenotype. In fact, although not reported in *E. coli* (Fig. 2B), the equivalent H437Y substitution in *Streptomycetes* has been shown to confer ppGpp-independent antibiotic production (Lai et al., 2002; Talà et al., 2009; Wang et al., 2010) (Fig. 4A). At this point, it is important to consider that, as discussed above, in Gram-positive Firmicutes and probably also in Actinobacteria most of the effects of ppGpp on RNA polymerase are indirect, and may be caused by perturbed GTP homeostasis in turn affecting transcription initiation at the level of rRNA operons (Krásný and Gourse, 2004; Kriel et al., 2012).

Table 2
Changes in transcriptional patterns associated with RpoB H481Y and RelAF128Y substitutions in *S. aureus*.

Changes in transcriptional patterns[a]		Ref.
Cellular processes and signaling		Gao et al. (2013)
Capsule biosynthesis (*capA, B, C, D, 5E, F, 5G, H, I, J, K, 5L, 5M, N, 50, 5P*)	Up	
Quorum sensing (*agrA, B, C, D*)	Up	
Phenol-soluble modulins (*psm*)	Up	
Amino acid metabolism and ABC transporters		
Branched chain amino acid synthesis (*ilvA, D, B, H, C; leuA, B, C, D*)	Up	
Oligopeptide ABC transporters (*oppB, 3C, 3D, 3F, 3A*)	Up	
Oligopeptide ABC transporters (*opp1F, 1D, 1C, 1B, 1A*)	Down	

[a] Up, up-regulation of transcript levels; Down, down-regulation of transcript levels.

Notably, *rpoB* mutations, irrespective of their ability to confer rifampicin-resistance, promote selection of the vancomycin-intermediate phenotype (Cui et al., 2010; Matsuo et al., 2011; Saito et al., 2014). At least two steps of selection are required to get vancomycin-intermediate *S. aureus* (VISA) when starting from vancomycin-susceptible *S. aureus* (VSSA) strains. Before the VISA phenotype is attained, mutants in the transitional stage of vancomycin resistance, which are called heterogeneously VISA (hVISA), appear. *rpoB* mutations are one of the major contributors to the hVISA-to-VISA phenotype conversion (Matsuo et al., 2011). Indeed, it has been reported that more than 70% of the VISA strains carry *rpoB* mutations (Matsuo et al., 2011; Watanabe et al., 2011). Among the Rif^R *rpoB* mutations, the above-mentioned H481Y was responsible for increased vancomycin resistance, while Q468K and Q468R did not increase resistance to this antibiotic. Promotion of the VISA phenotype by H481Y mutation was associated with prolonged doubling time, activation of the stringent response and cell wall thickening (Matsuo et al., 2011). The different effects of the H481Y and the Q468K/R substitutions could be attributed to the antithetic effects of these mutations on transcription dynamics leading to distinct phenotypes. As mentioned above, in *B. subtilis* the equivalent H482Y and the Q469K/R substitutions result in opposite effects on transcription pausing and termination (Ingham and Furneaux, 2000) (Fig. 4C).

The impact of the *rpoB* mutations on bacterial physiology and metabolism was also explored, by means of proteomic approach, in two *N. meningitidis* invasive clinical isolates carrying, respectively, the RpoB D543V and H553Y substitutions (Neri et al., 2010) (Table 3). A total of twenty-three spots were found to be differentially expressed in both Rif^R strains compared to a susceptible clinical isolate. Enzymes involved in carbohydrate metabolism and in the reactions of the tricarboxylic acid (TCA) cycle resulted up-expressed. The NADP-dependent glutamate dehydrogenase that in *N. meningitidis* supplies the TCA cycle with 2-oxoglutarate (Monaco et al., 2006; Pagliarulo et al., 2004; Talà et al., 2011) and is essential for virulence in the infant rat model (Sun et al., 2000) was also up-regulated (Table 3). By assuming that these RpoB substitutions may mimic a stringent phenotype, up-regulation of these proteins is unexpected because TCA enzyme-encoding genes are generally down-regulated under stringent conditions in *E. coli* (Durfee et al., 2008). In contrast, similar to *E. coli* under stringent conditions, a number of proteins involved in ATP production, lipid metabolism and cell division were down-expressed in the *N. meningitidis rpoB* mutants (Table 3). In addition to metabolic enzymes, proteins involved in transcription, translation and protein turnover were

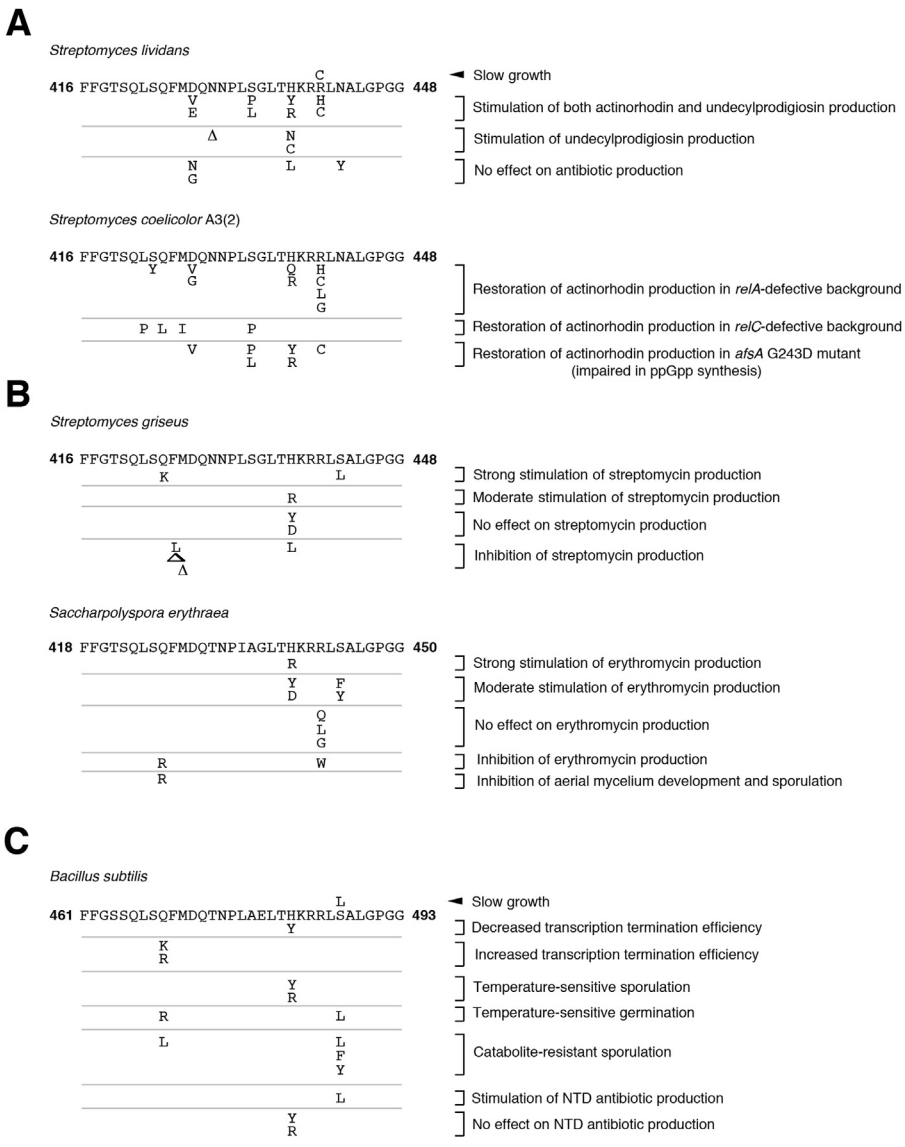

Fig. 4. Phenotypes associated with Rif^R RpoB substitutions in *S. coelicolor* A3(2) and *S. lividans* (A) *S. griseus* and *S. erythraea* (B) and *B. subtilis* (C). NTD, aminosugar antibiotic 3,3′-neotrehalosadiamine biosynthesis pathway.

differentially expressed. In particular, the DNA-directed RNA polymerase subunit α, the transcription elongation factor NusA, and the translation elongation factor G were up-expressed (Table 3). These changes are somewhat expected by considering the possible effects of the RpoB D543V and H553Y substitutions on transcription dynamics as inferred from studies addressing the effects on transcription elongation and termination of the equivalent RpoB substitutions in *E. coli* (Heisler et al., 1996; Jin et al., 1988a,b; Jin and Gross, 1991; Landick et al., 1990) (Fig. 2B). In fact, the mechanisms controlling transcription elongation and termination appear to be highly conserved in Neisseriaceae and Enterobacteriaceae (Alifano et al., 1988, 1991; Lavitola et al., 1999; Miloso et al., 1993).

Although resolution of proteomic analysis is limited by the relative abundance of the proteins, these results demonstrate a wide effect of *rpoB* mutations on carbon (and energy) metabolism and on basic cell functions and processes in *N. meningitidis*.

In *M. tuberculosis*, omic approaches have been mainly focused on global changes in gene expression profile upon exposure of rifampicin-resistant and rifampicin-susceptible strains to different rifampicin concentrations in the attempt to better define the mechanisms of rifampicin-resistance (de Knegt et al., 2013; Gupta et al., 2010; Louw et al., 2011). These studies are outside the scope of the present review. In contrast, the study of Bisson et al. (2012) investigated the impacts of two RpoB substitutions, H445D and S450L, on the proteome and metabolome in the absence of rifampicin in order to determine protein and metabolite abundance changes that are associated with the two *rpoB* mutations independent of drug exposure (Fig. 2B and Table 4). Noteworthy, both *rpoB* mutants showed significant up-regulation of the polyketide synthase (PKS) genes *ppsA-ppsE* and *drrA*, which constitute an operon encoding multifunctional enzymes involved in the biosynthesis of phthiocerol dimycocerosate and other lipids in *M. tuberculosis* (Table 4). In addition, metabolomics identified precursors of phthiocerol dimycocerosate (PDIM), but not the intact molecule itself, in greater abundance in both *rpoB* mutant isolates. PDIM is a long-chain ß-diol (phthiocerol) esterified with two branched-chain mycocerosic acids located in the outer mycobacterial cell wall that has been implicated in *M. tuberculosis* virulence (Cox et al., 1999; Reed et al., 2004). Indeed, *M. tuberculosis* strains with defects in this pathway have been shown to possess increased cell envelope

Table 3
Changes in protein expression patterns associated with RpoB substitutions D542V and H552Y in *N. meningitidis*.

Changes in protein expression patterns[a]		Ref.
Intermediary and energy metabolism		Neri et al. (2010)
Phosphoenolpyruvate synthase (PpsA)	Up	
Pyruvate dehydrogenase E1	Up	
Isocitrate dehydrogenase	Up	
Succinyl-CoA synthetase subunit β (SucC)	Up	
Aconitate hydratase (AcnB)	Up	
Malate quinone oxidoreductase (Mqo)	Down	
Enolase	Down	
Zinc-binding alcohol dehydrogenase	Down	
Carboxyphosphonoenol pyruvate phosphonomutase (PrpB)	Down	
NADP-dependent glutamate dehydrogenase	Up	
F_0F_1 ATP synthase subunit α (AtpA)	Down	
Lipid metabolism		
Malonyl-CoA-acyl carrier protein transacylase (FabD)	Down	
Transcription		
DNA-directed RNA polymerase subunit α (RpoA)	Up	
Transcription elongation factor (NusA)	Up	
Translation		
Elongation factor G (FusA)	Up	
Folding, sorting and degradation		
Trigger factor	Down	
60 kDa chaperonin (GroEL)	Down	
Peptidyl-prolyl cis-trans isomerase (PpiB)	Down	
Cell division		
Cell division protein (FtsA)	Down	
Septum site-determining protein (MinD)	Down	

[a] Up, up-regulation of protein levels; Down, down-regulation of protein levels.

Table 4
Changes in protein expression patterns associated with RpoB H445D and RpoB S450L substitutions in *M. tuberculosis*.

Changes in protein expression patterns[a]		Ref.
Lipid metabolism		Bisson et al. (2012)
Modular type I PKS system PpsA-E (involved in PDIM biosynthetis)	Up	
Acyl-CoA dehydrogenase FadE31	Up	
Cell wall and cell processes		
ABC transporter DrrA (involved in PDIM biosynthetis)	Up	
Intermediary and energy metabolism		
Succinic semialdehyde dehydrogenase (GabD1)	Up	
Information pathways		
Bifunctional polyribonucleotide nucleotidyltransferase (GpsI)	Up	
Putative integration host factor (MIHF)	Up	
Conserved hypothetical proteins		
Rv1056	Up	
Rv3038c	Up	
Rv3661b	Up	

[a] Up, up-regulation of protein levels; Down, down-regulation of protein levels.

permeability (Camacho et al., 2001), and are more susceptible to IFN-γ-mediated and IFN-γ-independent immunity (Kirksey et al., 2011; Murry et al., 2009).

The genes *ppsA-ppsE* encode a type I modular PKS responsible for biosynthesis of the phtiocerol backbone of PDIM, with PpsA-PpsC sequentially loading ketide units onto long-chain fatty acids and PpsD and PpsE subsequently extending the phthiocerol further by adding a 4-methyl branch and malonyl- or methylmalonyl-CoA, respectively (Azad et al., 1997; Trivedi et al., 2005). The finding of up-regulation of several genes encoding PKS in both *rpoB* mutants of *M. tuberculosis* is of interest because in related Actinobacteria PKS are involved in the biosynthesis of various secondary metabolites such as erythromycin and rifamycin, and, as we will discuss in more detail below, several *rpoB* mutations lead to up-regulation of otherwise dormant gene clusters in Actinobacteria, resulting in increased abundance of specific secondary metabolites that are absent or minimally present in wild-type strains. These data suggest that *rpoB* mutations in *M. tuberculosis* may trigger compensatory transcriptional changes in secondary metabolism genes analogous to those observed in related Actinobacteria.

4. Rifampicin-resistance in biotechnology: RNA polymerase genetic engineering

4.1. The rifampicin-resistance selection method as a tool to improve antibiotic production and activate silent antibiotic gene clusters in Actinomycetes

The reviewed literature provides many examples of activation of silent or weakly expressed gene clusters coding for secondary metabolites by Rif^R *rpoB* mutations in Actinomycetes and *B. subtilis* (Ochi and Hosaka, 2013; Ochi et al., 2014). For instance, gene clusters coding for enzymes involved in actinorhodin (Act) and undecylprodigiosin (Red) biosynthesis are found in *Streptomyces lividans* but normally are not expressed. In contrast, these clusters are expressed in the phylogenetically related bacterium *Streptomyces coelicolor* A3(2). However, certain rifampicin-resistant *rpoB* mutants of *S. lividans* produce both Act and Red in abundance by activating transcription of the key regulatory genes *act*II-ORF4 and *redD* (Hu et al., 2002). The RpoB substitutions D427V, S433P, S433L, H437Y, H437R, R440H, R440C, R599C were among the most effective in activating both Act and Red production (Fig. 4A). Some of these *rpoB* mutations result in ppGpp-independent antibiotic production in both *S. coelicolor* A3(2) and *S. lividans* (Lai et al., 2002; Xu et al., 2002; Wang et al., 2010). This result, together with the evidence that the guanine nucleotide ppGpp is a pivotal signal molecule for initiating the onset of antibiotic production in *Streptomycetes* (Artsimovitch et al., 2004; Bibb, 2005; Martínez-Costa et al., 1996; Ochi, 2007, 1987; Saito et al., 2006; Takano and Bibb, 1994), is consistent with the hypothesis that the *rpoB* mutations activating antibiotic production in *S. lividans* can functionally mimic the physiological modifications induced by the ppGpp (Hu et al., 2002; Lai et al., 2002; Talà et al., 2009; Xu et al., 2002; Wang et al., 2010, 2008). However, it is also important to remember that a number of specific effects of these mutations on global gene expression may due to their impact on transcription elongation/termination, and that there is not a straightforward correlation between the effects of these mutations on RNAP promoter binding/isomerization, and their effects on transcription elongation/termination as clearly demonstrated in *E. coli* and *B. subtilis* (Heisler et al., 1996; Ingham and Furneaux, 2000).

The broad applicability of the Rif^R selection method to strain improvement was recently demonstrated by Tanaka et al. (2013). The introduction of certain *rpoB* mutations effectively increased antibiotic production by *Streptomyces griseus* (streptomycin producer), *S. coelicolor* (actinorhodin producer), *Streptomyces antibioticus* (actinomycin producer), *Streptomyces lavendulae* (formycin producer), *Saccharopolyspora erythraea* (erythromycin producer), and *Amycolatopsis orientalis* (vancomycin producer). Importantly, this method worked successfully regardless of the chemical class of the antibiotics (polyketides, polyethers, glycopeptides, macrolides,

polypeptides, nucleotides, and aminoglycosides). In contrast, it was restricted by strain biology and environmental conditions (e.g., growth media). For instance, although actinomycin production by *S. antibioticus* was markedly enhanced by introducing certain *rpoB* mutations, production of the same antibiotic by *Streptomyces parvulus* could not be enhanced using this method (Tanaka et al., 2013). Moreover, the activation of the gene clusters for secondary metabolites was medium dependent, with each *rpoB* mutation exerting differential effects on activation of each gene cluster. For instance, although Q424K and S442L mutations were quite effective at enhancing streptomycin production (Fig. 4B), these mutations, at variance with the H437Y (and, partially, H437R) mutations, were not effective in enhancing the activity of cryptic gene clusters for other secondary metabolites in *S. griseus*. In contrast, H439Y and H439R mutations (corresponding to H437Y/R in *S. coelicolor* A3[2]), which were effective in enhancing erythromycin production (Fig. 4B), were widely effective in enhancing the activity of cryptic genes of *S. erythraea*. Similarly, S433L and H437Y mutations, which were effective in enhancing actinorhodin production, were also effective in enhancing the activity of the cryptic genes of *S. coelicolor*.

Overall, this study demonstrates that H437Y and H437R mutations are among the most effective in a wide variety of Actinomycetes and on a wide variety secondary metabolites. The mutations affecting the histidine residue at position 437 have been shown to circumvent the detrimental effects of the *relA* and *asfB* mutations (in *S. coelicolor*) and the *relC* mutations (in *S. lividans*), perhaps by mimicking the stringent phenotype (Hu et al., 2002; Lai et al., 2002; Talà et al., 2009; Xu et al., 2002; Wang et al., 2010, 2008). In many cases, enhancement of antibiotic production by these mutations may be mediated by up-regulation of poorly transcribed genes as represented by pathway-specific regulatory genes *actII*-ORF4 in actinorhodin production or pleiotropic regulatory genes as represented by *bldD* in biosynthesis of erythromycin (Tanaka et al., 2013). A similar mechanism is involved in the enhancement of streptomycin production in the *S. griseus* Q424K mutant, in which the expression of the pathway-specific regulatory gene *strR* was markedly up-regulated (Tanaka et al., 2013).

StrR is member of a regulatory cascade starting with binding of quorum sensing A-factor to ArpA repressor dissociating it from *adpA* promoter thereby inducing transcription of this gene. The transcriptional factor AdpA, in turn, activates a number of genes required for secondary metabolism and morphological differentiation (Ohnishi et al., 2005). Members of the AdpA regulon include StrR (Retzlaff and Distler, 1995). Notably, in the Q424K mutant, in spite of *strR* transcription stimulation, *adpA* transcription was rather impaired. However, since translation of *adpA* is modulated by *bldA*, coding for the only tRNA species able to read the rare leucine codon UUA efficiently in the high G+C Actinomycetes, it would be interesting to analyze in this mutant both *bldA* RNA and AdpA protein levels. Indeed, there is evidence that *bldA* is apparently subject to positive stringent control (Chater and Chandra, 2008). Analogously, it would be intriguing to check ActII-ORF4 protein levels in the H437R/Y mutants of *S. coelicolor* since translation of *actII*-ORF4 is also subject to *bldA* regulation (Fernández-Moreno et al., 1991).

In addition to H437Y and H437R mutations, substitutions of Ser at position 433 (S433P/L) or 442 (S442F/Y/L) were also often effective in boosting antibiotic production, although position 442 (S444 in *S. erythraea*) is variable among species (e.g., asparagine in *S. coelicolor*). For instance, the S444F/Y (position 442 in *S. coelicolor* A3[2] RpoB) mutations markedly potentiated the erythromycin production by *S. erythraea* (Carata et al., 2009; Tanaka et al., 2013) (Fig. 4B). The mechanisms of activation of secondary metabolism by these mutations, however, might be different from that of the H439Y/R mutations in *S. erythraea* (H437Y/R in *S. coelicolor* A3[2]). The S444F mutation in *S. erythraea* markedly altered the transcriptional profile of this microorganism (Table 5). The expression of genes coding for key enzymes of carbon (and energy) and nitrogen central metabolism was dramatically altered in turn affecting the flux of metabolites through erythromycin feeder pathways. In particular, the valine catabolic pathway that supplies propionyl-CoA for biosynthesis of the erythromycin precursor 6-deoxyerythronolide B was strongly up-regulated in this mutant, while, at variance with the H439Y/R mutants, the expression of the biosynthetic gene cluster of erythromycin and regulatory genes including *bldD* was not significantly affected (Carata et al., 2009).

Altogether these data indicate that distinct mechanisms are responsible for the stimulatory effects on antibiotic production caused by *rpoB* mutations because each mutation may exert a distinct effect on gene expression depending on its impact on transcription dynamics and the genome context. For instance, it may be noteworthy the circumstance that the regulatory gene *bldD* and the gene coding for the transcription elongation factor *nusB* are located adjacent, in a convergent orientation on the *S. erythraea* chromosome. In the H439R/Y mutants it is reasonable that the termination deficiency that is associated with the corresponding substitutions in model organisms (Heisler et al., 1996; Ingham and Furneaux, 2000) may perturb the expression of the two convergently transcribed genes leading to *bldD* up-regulation. This hypothesis is strongly supported by the finding of a nonsense mutation in *nusB* in an industrial erythromycin-producing strain with possible consequence on *bldD* expression (Peano et al., 2012).

Table 5
Changes in transcriptional patterns associated with RpoB S444F or RpoB Q426R substitutions in *S. erythraea*.

Changes in transcriptional patterns[a]		Ref.
S. erythraea RpoB S444F vs. parent strain		Carata et al. (2009)
Metabolism		
Energy-generating NADH dehydrogenase complex I (*nuoC*, *D*, *E*, *F*)	Up	
Valine catabolic pathway (*mmsA2*-SACE_1456, SACE_1457)	Up	
Information storage and processing		
Replication, recombination and repair (*gyrA*, *gyrB*, *deaD*)	Down	
Ribosome biogenesis (*rpsB*, *rpsD*, *rplD*, *rplO*, *rplW*, *rpmC*, *rpmD*)	Down	
S. erythraea RpoB Q426R vs. parent strain		
Metabolism		
Nitrogen regulon (*glnB*, *amt*, *glnA1*, *gudB*, *ureA*, *ureC*, *nirB*, *nirD*, *narK*)	Up	
Valine catabolic pathway (*mmsA2*-SACE_1456, SACE_1457)	Down	
Amino acid biosynthesis (*metH*, *hisC2*, *lat*)	Up	
Nucleotide metabolism (*purF*, *pyre*, *adk*)	Up	
Nucleotide metabolism (*purE*, *pyrH*)	Down	
Vitamin metabolism (*pdx1*, *folK*)	Up	
Carbon metabolism (*eno*, SACE_5675, SACE_7048)	Up	
Energy-generating NADH dehydrogenase complex I (*nuoA*, *C*, *D*, *E*, *F*, *L*)	Down	
Information storage and processing		
Ribosome biogenesis (*rpsA*)	Up	
Ribosome biogenesis (*rpsB*, *rpsD*, *rplD*, *rplO*, *rplW*, *rpmC*, *rpmD*)	Down	
Global transcriptional regulators (*SACE_3299*, *SACE_4349*, *SACE_6128*)	Up	
Putative stress proteins (*smpB*, *uspA3*)	Up	
Replication, recombination and repair (*gyrA*, *gyrB*, *deaD*)	Down	

[a] Up, up-regulation of transcript levels; Down, down-regulation of transcript levels.

4.2. The rifampicin-resistance selection method as a tool to manipulate the cell cycle, activate dormant genes, and expand the metabolic capabilities of Bacillus

The efficacy of *rpoB* mutations in activating the production of a dormant antibiotic has been also demonstrated in *B. subtilis*. In particular, RpoB substitution S487L in *B. subtilis* led to dramatic autoinduction of the amino sugar antibiotic 3,3'-neotrehalosadiamine (NTD), which wild-type strains do not produce (Inaoka et al., 2004) (Fig. 4C). In contrast, H482Y and H482R substitutions that effectively activated antibiotic production in *S. lividans* (Hu et al., 2002) were ineffective in *B. subtilis* NTD production that is not controlled by ppGpp (Inaoka et al., 2004). This finding is reminiscent of the mutational pattern that was associated with enhanced production of streptomycin (an amino sugar antibiotic, just like NTD) by *S. griseus* (Fig. 4B).

Mechanistically, it has been proposed that the mutant RNA polymerase harboring the RpoB S487L missense, with respect to the wild-type enzyme, acquired a superior ability to efficiently transcribe σ^A-dependent promoters including *ntdABC* operon promoter resulting in the dramatic activation of the NTD biosynthesis pathway by an autoinduction mechanism (Inaoka et al., 2004). The same substitution was proven to be effective for overproduction of extracellular enzymes such as amylase and protease by several *Bacillus* species (Inaoka et al., 2004).

Interestingly, Maughan et al. (2004) demonstrated that four RpoB substitutions, Q469R, H482R, H482Y and the above-described S487L caused global changes in *B. subtilis* growth, sporulation, germination and competence for DNA-mediated transformation (Fig. 4C). Compared to the growth rate of the wild-type strain, lowered growth rates were observed in certain mutants (e.g., in the S487L mutant) but not in others (e.g., in the Q469R and H482R/Y mutants) in rich Luria Bertani (LB) medium. Furthermore, temperature sensitive germination was observed in Q469R and S487L mutants, while the H482R/Y mutants exhibited temperature sensitive sporulation.

This study further supports the concept that the *rpoB* mutations activating secondary metabolism can be functionally classified into three major groups: (i) group I mutations affecting the H482 residue (H437 in *S. coelicolor* A3[2] numbering) including the H482R/Y (H437R/Y in *S. coelicolor* A3[2]); (ii) group II mutations affecting the Q469 residue (Q424 in *S. coelicolor* A3[2]) including the Q469R/K (Q469R/K in *S. coelicolor* A3[2]); group III mutations (with some phenotypic overlaps with group II mutations) affecting the S487 residue (only present in several *Streptomycetes*; N442 in *S. coelicolor* A3[2]) including S487L/F/Y. This classification is consistent with the results of a more recent study, in which different levels of glucose catabolite repression during sporulation and spore resistance to heat and chemicals were observed in Rif^R *rpoB* mutants (Moeller et al., 2012) (Fig. 4C). In particular, strains carrying the Q469L, S487F, S487L or S487Y exhibited a strong catabolite-resistant sporulation (Crs) phenotype. Interestingly, the Crs phenotype also results from missense mutation P290F in the *sigA* (*rpoD*) gene encoding the major vegetative RNA polymerase σ^A factor (Kawamura et al., 1985). This observation, together with the evidence that *rpoB* mutation may suppress temperature-sensitive sporulation initiation caused by *sigA* missense mutation E314K (Nanamiya et al., 2000), implicates that the effects of several *rpoB* substitutions including S487L on NTD biosynthesis, and activation ad catabolite repression of sporulation initiation involve the activity of the σ^A factor.

As above mentioned, Rif^R selection also led to discover new metabolic capabilities of *B. subtilis* (Perkins and Nicholson, 2008). By using global metabolic profiling approach, a number of alteration of substrate utilization patterns were observed in the Rif^R *rpoB* mutants including the utilization of novel substrates previously unknown in *B. subtilis*, such as gentiobiose, ß-methyl-d-glucoside, and D-psicose. In general, compared to the wild-type, the Rif^R mutation led to a decrease in strongly utilized substrates and an increase in weakly utilized substrates. However, specific *rpoB* mutations were associated with specific stimulatory or inhibitory effects on substrate utilization (Perkins and Nicholson, 2008). This study demonstrates that combining Rif^R with metabolic profiling is system-wide approach for uncovering previously unknown metabolic capabilities and further understanding global transcriptional control circuitry. The biotechnological implications are obvious, since extending the range of substrate utilization and/or altering substrate preference may represent major goals in microbial strain improvement processes.

4.3. Strain improvement and drug discovery methods based on natural "mutant-type" or duplicated RNA polymerases

As already mentioned, under standard laboratory conditions (standardized media and growth conditions) and in different experimental setups (pure cultures, co-cultures, cellular or animal infection models), a fitness burden is often associated with the Rif^R *rpoB* mutations. However, laboratory conditions cannot replicate the entire complexity of a natural environment. Soil microorganisms such as Bacillus and Actinomycetes have to thrive in a hostile environment. They are not bathed in rich sources of nutrients such as in laboratory conditions, and need to rely on the ability to utilize ephemeral, rare, or less readily metabolized substrates, including ß-glucosides released through breakdown of cellulose and hemicellulose. They have to face the competition with other organisms and microorganisms for food and space, and sometime have to colonize hosts as diverse as nematodes, insects, plants or mammals. As reported above, the ability (i) to utilize less readily metabolized substrates, (ii) to modulate sporulation, germination and spore resistance to chemical and physical agents, (iii) to activate cryptic clusters for antibiotics and siderophores, and (iv) to tune the expression of host colonization factors may be conferred by particular *rpoB* mutations. Thus, it is entirely possible that under more realistic environmental conditions, *rpoB* mutants may indeed exhibit greater fitness than the wild-type strain.

The discovery of natural "mutant-type" or duplicated RNA polymerase genes in certain "rare" Actinomycetes is consistent with this view. In contrast to the widely accepted consensus of the existence of a single RNA polymerase in bacteria, Actinomycetes with two or multiple *rpoB* paralogs were recently discovered (Vigliotta et al., 2005; Ishikawa et al., 2006; Talà et al., 2009). In *Nonomuraea* sp. ATCC 39727, the producer of the glycopeptide antibiotic A40926, the presence of duplicated *rpoB* genes, *rpoB*(S) (rifampicin-sensitive wild-type *rpoB*) and *rpoB*(R) (rifampicin-resistant mutant-type *rpoB*), provides the microorganism with two functionally distinct RNA polymerase (Vigliotta et al., 2005) (Fig. 5). With respect to the *rpoB*(S) gene product, the product of *rpoB*(R) is characterized by an 18-bp in-frame deletion in a hyper-variable region of the lobe domain, and mutations causing five amino acid substitutions located within or close to the *rif* clusters I and II (Fig. 5). Of these substitutions, the histidine to asparagine substitution (H437N in *S. coelicolor* A3[2]) in the *rif* cluster I is of particular interest because it corresponds to one of the rifampicin resistance *rpoB* mutations that activate antibiotic production in *S. lividans* by mimicking the stringent phenotype (Hu et al., 2002). Notably, the corresponding histidine to asparagine substitution (H481N) in *S. aureus* RpoB is not associated to fitness cost (Table 1). The serine to tyrosine substitution (S485Y in *S. coelicolor* A3[2]) in the *rif* cluster II (Fig. 5) is also of interest because the equivalent substitution in *E. coli* is responsible for moderate-level resistance to rifampicin and high-level resistance to sorangicin (Campbell et al., 2005; Rommele et al., 1990; Xu et al., 2005). Sorangicin is a polyketide-derived

Fig. 5. *rpoB* duplication in Actinomycetes. Amino acid sequences of the region spanning the *rif* clusters I and II of RpoB proteins encoded by *Nonomuraea* sp. ATCC 39727 *rpoB*(S) and *rpoB*(R), *Nocardia farcinica* IFM 10152 *rpoB* and *rpoB2* paralogous genes, and *S. coelicolor* A3(2) *rpoB* are shown. Closed triangle, amino acid substitution common to *rpoB*(R)- and *rpoB2*-encoded proteins; closed circle, amino acid substitution specific to *rpoB*(R)- or *rpoB2*-encoded proteins.

macrocyclic-polyether produced by the Myxobacterium *Sorangium cellulosum* (Jansen et al., 1985). Similarly to rifampicin it inhibits transcription initiation but does not inhibit transcription elongation, and exhibits partial cross-resistance with rifampicin (Irschik et al., 1987; Campbell et al., 2005; Rommele et al., 1990; Xu et al., 2005). It is also worth noting the presence of both histidine-to-asparagine and serine-to-tyrosine substitutions in the corresponding regions of *rpoB2* from *Nocardia farcinica* (strain IFM 10152), an Actinomycete that shares with *Nonomuraea* sp. strain ATCC 39727 the distinction of having two *rpoB* paralogs (*rpoB* and *rpoB2*) (Ishikawa et al., 2006) (Fig. 5).

The presence of both wild-type *rpoB*(S) and mutant-type *rpoB*(R) genes in the same genome may represent an elaborate strategy enabling certain Actinomycetes to cohabit with microorganisms that produce antibiotic targeting the bacterial RNAP, minimizing, at the same time, the disadvantage associated with rifampicin-resistance (Ishikawa et al., 2006). However, the more intriguing possibility is that *rpoB* duplication may contribute to the developmental strategy of these bacteria. This hypothesis is supported by the observation that *rpoB*(R) transcription is tightly regulated during *Nonomuraea* growth, and that the constitutive expression of this gene increases the production of the glycopeptide antibiotic A40926 in this organism (Alduina et al., 2007; Vigliotta et al., 2005). Moreover, *rpoB*(R) markedly activated antibiotic biosynthesis in the wild-type *S. lividans* strain 1326, which is considered a good host for genetic analysis of *Nonomuraea* (Alduina et al., 2005), and also in strain KO-421, a relaxed (*rel*) mutant unable to produce ppGpp. Site-directed mutagenesis demonstrated that the RpoB(R)-specific histidine-to-asparagine substitution in the *rif* cluster I (Fig. 5) is essential for the activation of secondary metabolism (Talà et al., 2009).

This physiological role of *rpoB* duplication is also consistent with results of studies with *Nonomuraea terrinata* strains with single *rpoB* [*rpoB*(R)] or duplicated *rpoB* [*rpoB*(S) + *rpoB*(R)] demonstrating that the strain with duplicated *rpoB* shows much greater capability than the single *rpoB* strain for growth (representing primary metabolism) and sporulation and antibiotic production (representing the developmental strategy), especially under stressful conditions (Talà et al., 2009). There is also evidence that mutant-type, or duplicated, *rpoB* often exists in nature, with *rpoB* gene polymorphisms detected in five of 75 inherently rifampicin-resistant Actinomycetes isolated from nature, although these polymorphisms are preferentially distributed in the rare Actinomycetes, not in *Streptomyces* spp. Notably, most of these rifampicin-resistant rare Actinomycete isolates obtained to date are able to produce antibiotics (Talà et al., 2009).

From a practical viewpoint, these findings suggest the intriguing possibility of using *rpoB*(R)-based technology to improve strains and to search for novel bioactive molecules by activating dormant genes. This technology should have greater potential than simple Rif^R selection method currently used to improve the production of secondary metabolites (Hu and Ochi, 2001; Ochi and Hosaka, 2013; Ochi et al., 2014, 2004; Tamehiro et al., 2003; Tanaka et al., 2013), as the introduction of *rpoB*(R) was much more effective in boosting antibiotic production in *S. lividans* than the introduction, for instance, of simply the H426Y mutation (Talà et al., 2009). This may be due to the additional *rif* cluster-associated RpoB(R)-specific substitutions that likely interact functionally with the histidine-to-asparagine substitutions, leading to the marked effect of *rpoB*(R). In the future, it will be of interest to examine whether various *rpoB*(R) forms found in nature in other Actinomycetes are more capable of activating silent bacterial genes than *Nonomuraea rpoB*(R).

5. Concluding remarks

Rifampicin-resistance, with the underlying mechanisms and biological effects, is an area of wide interest to scientists operating in many different fields, from epidemiology to infectious disease, from molecular biology to cell physiology and biochemistry, from industrial microbiology to biotechnology, from microbial ecology to evolution.

The above-reviewed literature shows that specific *rpoB* mutations conferring rifampicin-resistance may exert variable effects on bacterial fitness and on a variety of biological processes including nutrition, primary, intermediary and secondary metabolism, sporulation, germination, host colonization and virulence depending on the microorganisms and their genetic backgrounds, the model systems, and the experimental conditions. However, in spite of such a wide range of biological effects associated with a given mutation, mutation-specific mechanisms mediating the biological effects have to be better understood. In this review, a general pattern is proposed to explain the differential and complex effects on gene expression that are caused by the *rpoB* mutations. The available evidence suggests that a number of specific effects of *rpoB* Rif^R mutations may be due to their specific impact on transcription dynamics including promoter binding/isomerization and transcription elongation/termination that are not directly correlated with each other. In fact, while most analyzed *rpoB* Rif^R mutations appear to reduce the open complex stability thereby mimicking the direct or indirect effects of ppGpp on RNAP, their effect on transcription elongation/termination appear to be specific and antithetical in some cases.

Based on these traits and their effects on global gene expression, a functional classification is presented for most studied RpoB substitutions in both clinical and industrially-important bacteria:

(i) group I substitutions affecting the histidine residue in *rif* cluster I (H526 in *E. coli*; H437 in *S. coelicolor* A3[2]) that decrease transcription termination in *E. coli* and *B. subtilis*; (ii) group II substitutions affecting a glutamine residue in *rif* cluster I (Q513 in *E. coli*; H424 in *S. coelicolor* A3[2]) that increase transcription termination in *E. coli* and *B. subtilis*; (iii) group III substitutions affecting a serine residue in *rif* cluster I (S531 in *E. coli*; in *S. coelicolor* A3[2] the serine is replaced by asparagine 442) that decrease cellular antitermination in *E. coli.*

Noteworthy, substitution of the histidine residue in *rif* cluster I (H526 in *E. coli*; H437 in *S. coelicolor* A3[2]), the most frequent genetic alteration in Rif^R clinically isolated, is also the most effective in promoting adaptive responses (alternative substrates utilization, secondary metabolism, sporulation/germination, genetic exchange, antibiotic hetero-resistance) in soil bacteria by mimicking the effects of alarmone ppGpp and altering the transcription dynamics. This means that, in certain ecological niches and/or environmental conditions, the disadvantage of a reduced growth potential, which is often associated with Rif^R *rpoB* mutations, may be compensated by the advantage of having induced adaptive strategies. This hypothesis is supported by the recent discovery of natural "mutant-type" or duplicated *rpoB* genes with the histidine-to-asparagine substitution (H437N in *S. coelicolor* A3[2]) in the so-called rare Actinomycetes. By a theoretical point of view, the *rpoB* polymorphism may, therefore, act as a modulator of microbial evolution.

By a practical point of view, the rifampicin-resistance selection method is routinely used to maneuver, on a global scale, gene expression in bacteria of industrial interest for strain improvement and drug discovery. The natural polymorphisms associated with the *rpoB* gene may be also exploited to this purpose by transferring natural "mutant-type" or duplicated *rpoB* genes into target bacteria. The advantage of this procedure, as compared to the classical rifampicin-resistance selection method, relies on the introduction of multiple, functionally interacting *rpoB* mutations. Importantly, the RNA polymerase-based methods can be successfully combined with other methods including ribosome engineering, metabolism remodeling, co-cultivation and utilization of rare earth elements (REE) (Ochi and Hosaka, 2013; Ochi et al., 2014), and/or rational genome-guided approaches (Chaudhary et al., 2013; Peano et al., 2012; Stephanopoulos et al., 2004; Xu et al., 2013). In particular, the utilization of REE, whose effectiveness in activating cryptic gene expression and antibiotic production in bacteria has been recently demonstrated (Inaoka and Ochi, 2011; Kawai et al., 2007; Ochi et al., 2014; Tanaka et al., 2010), is of great interest. In fact, REE may represent environmental stress signals that trigger adaptive microbial responses, and therefore may be useful to understand the mechanisms underlying the silencing of cryptic genes, and the ecological role of the "mutant-type" and duplicated RNA polymerases in rare Actinomycetes. Indeed, "mutant-type" and duplicated RNA polymerases are involved in cryptic gene activation, and, such as hypothesized by Ochi et al. (2014), REE may either induce their expression or modulate their activity.

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References

Alduina, R., Lo Piccolo, L., D'Alia, D., Ferraro, C., Gunnarsson, N., Donadio, S., Puglia, A.M., 2007. Phosphate-controlled regulator for the biosynthesis of the dalbavancin precursor A40926. J. Bacteriol. 189, 8120–8129.

Alduina, R., Gierdina, A., Gallo, G., Renzone, G., Ferraro, C., Contino, A., Scaloni, A., Donadio, S., Puglia, A.M., 2005. Expression in *Streptomyces lividans* of *Nonomuraea* genes cloned in an artificial chromosome. Appl. Microbiol. Biotechnol. 68, 656–662.

Alifano, P., 2014. Fitness costs of antibiotic resistance. In: Gualerzi, C.O., Brandi, L., Fabbretti, A., Pon, C.L. (Eds.), Antibiotics: Targets, Mechanisms and Resistance. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, pp. 109–132.

Alifano, P., Ciampi, M.S., Nappo, A.G., Bruni, C.B., Carlomagno, M.S., 1988. In vivo analysis of the mechanisms responsible for strong transcriptional polarity in a "sense" mutant within an intercistronic region. Cell 55, 351–360.

Alifano, P., Rivellini, F., Limauro, D., Bruni, C.B., Carlomagno, M.S., 1991. A consensus motif common to all Rho-dependent prokaryotic transcription terminators. Cell 64, 553–563.

Alifano, P., Rivellini, F., Nappo, A.G., Bruni, C.B., Carlomagno, M.S., 1994. Alternative patterns of *his* operon transcription and mRNA processing generated by metabolic perturbation. Gene 146, 15–21.

Aronson, J., 1999. When I use a word . . . That's show business. Br. Med. J. 319, 972.

Artsimovitch, I., Patlan, V., Sekine, S., Vassylyeva, M.N., Hosaka, T., Ochi, K., Yokoyama, S., Vassylyev, D.G., 2004. Structural basis for transcription regulation by alarmone ppGpp. Cell 117, 299–310.

Azad, A.K., Sirakova, T.D., Fernandes, N.D., Kolattukudy, P.E., 1997. Gene knockout reveals a novel gene cluster for the synthesis of a class of cell wall lipids unique to pathogenic mycobacteria. J. Biol. Chem. 272, 16741–16745.

Bailey, M.J., Hughes, C., Koronakis, V., 2000. *In vitro* recruitment of the RfaH regulatory protein into a specialised transcription complex, directed by the nucleic acid *ops* element. Mol. Gen. Genet. 262, 1052–1059.

Barker, M.M., Gaal, T., Gourse, R.L., 2001a. Mechanism of regulation of transcription initiation by ppGpp. II. Models for positive control based on properties of RNAP mutants and competition for RNAP. J. Mol. Biol. 305, 689–702.

Barker, M.M., Gaal, T., Josaitis, C.A., Gourse, R.L., 2001b. Mechanism of regulation of transcription initiation by ppGpp. I. Effects of ppGpp on transcription initiation in vivo and in vitro. J. Mol. Biol. 305, 673–688.

Bartlett, M.S., Gaal, T., Ross, W., Gourse, R.L., 1998. RNA polymerase mutants that destabilize RNA polymerase-promoter complexes alter NTP-sensing by *rrn* P1 promoters. J. Mol. Biol. 279, 331–345.

Bear, D.G., Hicks, P.S., Escudero, K.W., Andrews, C.L., McSwiggen, J.A., von Hippel, P.H., 1988. Interactions of *Escherichia coli* transcription termination factor Rho with RNA. II. Electron microscopy and nuclease protection experiments. J. Mol. Biol. 199, 623–635.

Billington, O.J., McHugh, T.D., Gillespie, S.H., 1999. Physiological cost of rifampin resistance induced *in vitro* in *Mycobacterium tuberculosis*. Antimicrob. Agents Chemother. 43, 1866–1869.

Bibb, M.J., 2005. Regulation of secondary metabolism in *Streptomycetes*. Curr. Opin. Microbiol. 8, 208–215.

Bisson, G.P., Mehaffy, C., Broeckling, C., Prenni, J., Rifat, D., Lun, D.S., Burgos, M., Weissman, D., Karakousis, P.C., Dobos, K., 2012. Upregulation of the phthiocerol dimycocerosate biosynthetic pathway by rifampin-resistant, *rpoB* mutant *Mycobacterium tuberculosis*. J. Bacteriol. 194, 6441–6452.

Blatter, E.E., Ross, W., Tang, H., Gourse, R.L., Ebright, R.H., 1994. Domain organization of RNA polymerase alpha subunit: C-terminal 85 amino acids constitute a domain capable of dimerization and DNA binding. Cell 78, 889–896.

Bordes, P., Wigneshweraraj, S., Chaney, M., Dago, A., Morett, E., Buck, M., 2004. Communication between Esigma(54), promoter DNA and the conserved threonine residue in the GAFTGA motif of the PspF sigma-dependent activator during transcription activation. Mol. Microbiol. 54, 489–506.

Borukhov, S., Lee, J., Laptenko, O., 2005. Bacterial transcription elongation factors: new insights into molecular mechanism of action. Mol. Microbiol. 55, 1315–1324.

Bose, D., Joly, N., Pape, T., Rappas, M., Schumacher, J., Buck, M., Zhang, X., 2008a. Dissecting the ATP hydrolysis pathway of bacterial enhancer-binding proteins. Biochem. Soc. Trans. 36, 83–88.

Bose, D., Pape, T., Burrows, P.C., Rappas, M., Wigneshweraraj, S.R., Buck, M., Zhang, X., 2008b. Organization of an activator-bound RNA polymerase holoenzyme. Mol. Cell 32, 337–346.

Boudvillain, M., Figueroa-Bossi, N., Bossi, L., 2013. Terminator still moving forward: expanding roles for Rho factor. Curr. Opin. Microbiol. 16, 118–124.

Bougdour, A., Gottesman, S., 2007. ppGpp regulation of RpoS degradation via anti-adaptor protein IraP. Proc. Natl. Acad. Sci. U.S.A. 104, 12896–12901.

Browning, D.F., Busby, S.J., 2004. The regulation of bacterial transcription initiation. Nat. Rev. Microbiol. 2, 57–65.

Buck, M., Gallegos, M., Studholme, D., Guo, Y., Gralla, J., 2000. The bacterial enhancer-dependent sigma(54) (sigma(N)) transcription factor. J. Bacteriol. 182, 4129–4136.

Burmann, B.M., Schweimer, K., Luo, X., Wahl, M.C., Stitt, B.L., Gottesman, M.E., Rosch, P., 2010. A NusE:NusG complex links transcription and translation. Science 328, 501–504.

Busby, S., Ebright, R.H., 1997. Transcription activation at class II CAP-dependent promoters. Mol. Microbiol. 23, 853–859.

Camacho, L.R., Constant, P., Raynaud, C., Laneelle, M.A., Triccas, J.A., Gicquel, B., Daffe, M., Guilhot, C., 2001. Analysis of the phthiocerol dimycocerosate locus of *Mycobacterium tuberculosis.* Evidence that this lipid is involved in the cell wall permeability barrier. J. Biol. Chem. 276, 19845–19854.

Campbell, E.A., Korzheva, N., Mustaev, A., Murakami, K., Nair, S., Goldfarb, A., Darst, S.A., 2001. Structural mechanism for rifampicin inhibition of bacterial RNA polymerase. Cell 104, 901–912.

Campbell, E.A., Pavlova, O., Zenkin, N., Leon, F., Irschik, H., Jansen, R., Severinov, K., Darst, S.A., 2005. Structural, functional, and genetic analysis of sorangicin inhibition of bacterial RNA polymerase. EMBO J. 24, 674–682.

Carata, E., Peano, C., Tredici, S.M., Ferrari, F., Talà, A., Corti, G., Bicciato, S., De Bellis, G., Alifano, P., 2009. Phenotypes and gene expression profiles of *Saccharopolyspora erythraea* rifampicin-resistant (*rif*) mutants affected in erythromycin production. Microb. Cell Fact. 8, 18–32.

Carlomagno, M.S., Nappo, A., 2001. The antiterminator NusB enhances termination at a sub-optimal Rho site. J. Mol. Biol. 309, 19–28.

Cashel, M., Gentry, D.R., Hernandez, V.H., Vinella, D., 1996. The stringent response. In: Neidhardt, F.C. (Ed.), *Escherichia coli* and *Salmonella*: Cellular and Molecular Biology. ASM Press, Washington, DC, pp. 1458–1496.

Chakraborty, A., Wang, D., Ebright, Y.W., Korlann, Y., Kortkhonjia, E., Kim, T., Chowdhury, S., Wigneshweraraj, S., Irschik, H., Jansen, R., Nixon, B.T., Knight, J., Weiss, S., Ebright, R.H., 2012. Opening and closing of the bacterial RNA polymerase clamp. Science 337, 591–595.

Chaney, M., Grande, R., Wigneshweraraj, S.R., Cannon, W., Casaz, P., Gallegos, M.T., Schumacher, J., Jones, S., Elderkin, S., Dago, A.E., Morett, E., Buck, M., 2001. Binding of transcriptional activators to sigma 54 in the presence of the transition state analog ADP-aluminum fluoride: insights into activator mechanochemical action. Genes Dev. 15, 2282–2294.

Charity, J.C., Blalock, L.T., Costante-Hamm, M.M., Kasper, D.L., Dove, S.L., 2009. Small molecule control of virulence gene expression in *Francisella tularensis*. PLoS Pathog. 5, e1000641.

Chater, K.F., Chandra, G., 2008. The use of the rare UUA codon to define expression "space" for genes involved in secondary metabolism, development and environmental adaptation in streptomyces. J. Microbiol. 46, 1–11.

Chatterji, D., Fujita, N., Ishihama, A., 1998. The mediator for stringent control, ppGpp, binds to the beta-subunit of *Escherichia coli* RNA polymerase. Genes Cells 3, 279–287.

Chaudhary, A.K., Dhakal, D., Sohng, J.K., 2013. An insight into the "-omics" based engineering of *Streptomycetes* for secondary metabolite overproduction. Biomed. Res. Int. 2013, 968518.

Chopra, I., 2007. Bacterial RNA polymerase: a promising target for the discovery of new antimicrobial agents. Curr. Opin. Investig. Drugs 8, 600–607.

Ciampi, M.S., Alifano, P., Nappo, A.G., Bruni, C.B., Carlomagno, M.S., 1989. Features of the *rho*-dependent transcription termination polar element within the *hisG* cistron of *Salmonella typhimurium*. J. Bacteriol. 171, 4472–4478.

Cox, J.S., Chen, B., McNeil, M., Jacobs Jr., W.R., 1999. Complex lipid determines tissue-specific replication of *Mycobacterium tuberculosis* in mice. Nature 402, 79–83.

Cramer, P., Bushnell, D.A., Fu, J., Gnatt, A.L., Maier-Davis, B., Thompson, N.E., Burgess, R.R., Edwards, A.M., David, P.R., Kornberg, R.D., 2000. Architecture of RNA polymerase II and implications for the transcription mechanism. Science 288, 640–649.

Cramer, P., Bushnell, D.A., Kornberg, R.D., 2001. Structural basis of transcription: RNA polymerase II at 2.8 angstrom resolution. Science 292, 1863–1876.

Cui, L., Isii, T., Fukuda, M., Ochiai, T., Neoh, H.M., Camargo, I.L., Watanabe, Y., Shoji, M., Hishinuma, T., Hiramatsu, K., 2010. An RpoB mutation confers dual heteroresistance to daptomycin and vancomycin in *Staphylococcus aureus*. Antimicrob. Agents Chemother. 54, 5222–5233.

Daddi, G., Lucchesi, M., Mancini, P., Termine, A., 1969. The virulence of *Mycobacterium tuberculosis* strains resistant to rifampicin (Preliminary note). Ann. Ist. Carlo Forlanini 29, 29–33.

Dalebroux, Z.D., Svensson, S.L., Gaynor, E.C., Swanson, M.S., 2010. ppGpp conjures bacterial virulence. Microbiol. Mol. Biol. Rev. 74, 171–199.

Dalebroux, Z.D., Swanson, M.S., 2012. ppGpp: magic beyond RNA polymerase. Nat. Rev. Microbiol. 10, 203–212.

Darst, S.A., 2004. New inhibitors targeting bacterial RNA polymerase. Trends Biochem. Sci. 29, 159–160.

Das, R., Loss, S., Li, J., Waugh, D.S., Tarasov, S., Wingfield, P.T., Byrd, R.A., Altieri, A.S., 2008. Structural biophysics of the NusB:NusE antitermination complex. J. Mol. Biol. 376, 705–720.

de Knegt, G.J., Bruning, O., ten Kate, M.T., de Jong, M., van Belkum, A., Endtz, H.P., Breit, T.M., Bakker-Woudenberg, I.A., de Steenwinkel, J.E., 2013. Rifampicin-induced transcriptome response in rifampicin-resistant *Mycobacterium tuberculosis*. Tuberculosis 93, 96–101.

di Mauro, E., Synder, L., Marino, P., Lamberti, A., Coppo, A., Tocchini-Valentini, G.P., 1969. Rifampicin sensitivity of the components of DNA-dependent RNA polymerase. Nature 222, 533–537.

Donahue, J.P., Turnbough Jr., C.L., 1990. Characterization of transcriptional initiation from promoters P1 and P2 of the *pyrBI* operon of *Escherichia coli* K12. J. Biol. Chem. 265, 19091–19099.

Durfee, T., Hansen, A.-M., Zhi, H., Blattner, F.R., Jin, D.J., 2008. Transcription profiling of the stringent response in *Escherichia coli*. J. Bacteriol. 190, 1084–1096.

Ebright, R.H., 2000. RNA polymerase: structural similarities between bacterial RNA polymerase and eukaryotic RNA polymerase II. J. Mol. Biol. 304,687–698.

Edwards, A.N., Patterson-Fortin, L.M., Vakulskas, C.A., Mercante, J.W., Potrykus, K., Vinella, D., Camacho, M.I., Fields, J.A., Thompson, S.A., Georgellis, D., Cashel, M., Babitzke, P., Romeo, T., 2011. Circuitry linking the Csr and stringent response global regulatory systems. Mol. Microbiol. 80, 1561–1580.

Elena, S.F., Ekunwe, L., Hajela, N., Oden, S.A., Lenski, R.E., 1998. Distribution of fitness effects caused by random insertion mutations in *Escherichia coli*. Genetica 102/103, 349–358.

Ezekiel, D.H., Hutchins, J.E., 1968. Mutations affecting RNA polymerase associated with rifampicin resistance in *Escherichia coli*. Nature 220, 276–277.

Fernández-Moreno, M.A., Caballero, J.L., Hopwood, D.A., Malpartida, F., 1991. The *act* cluster contains regulatory and antibiotic export genes, direct targets for translational control by the *bldA* tRNA gene of *Streptomyces*. Cell 66, 769–780.

Fish, R.N., Kane, C.M., 2002. Promoting elongation with transcript cleavage stimulatory factors. Biochim. Biophys. Acta 1577, 287–307.

Fisher, M.A., Grimm, D., Henion, A.K., Elias, A.F., Stewart, P.E., Rosa, P.A., Gherardini, F.C., 2005. *Borrelia burgdorferi* sigma54 is required for mammalian infection and vector transmission but not for tick colonization. Proc. Natl. Acad. Sci. U.S.A. 102, 5162–5167.

Gagneux, S., Long, C.D., Small, P.M., Van, T., Schoolnik, G.K., Bohannan, B.J., 2006. The competitive cost of antibiotic resistance in *Mycobacterium tuberculosis*. Science 312, 1944–1946.

Gallant, J., Irr, J., Cashel, M., 1971. The mechanism of amino acid control of guanylate and adenylate biosynthesis. J. Biol. Chem. 246, 5812–5816.

Gallegos, M., Cannon, W., Buck, M., 1999. Functions of the sigma(54) region I in trans and implications for transcription activation. J. Biol. Chem. 274, 25285–25290.

Gao, W., Cameron, D.R., Davies, J.K., Kostoulias, X., Stepnell, J., Tuck, K.L., Yeaman, M.R., Peleg, A.Y., Stinear, T.P., Howden, B.P., 2013. The RpoB H481Y rifampicin resistance mutation and an active stringent response reduce virulence and increase resistance to innate immune responses in *Staphylococcus aureus*. J. Infect. Dis. 207, 929–939.

Garzoni, C., Kelley, W.L., 2011. Return of the Trojan horse: intracellular phenotype switching and immune evasion by *Staphylococcus aureus*. EMBO Mol. Med. 3, 115–117.

Gatewood, M.L., Jones, G.H., 2010. (p)ppGpp inhibits polynucleotide phosphorylase from streptomyces but not from *Escherichia coli* and increases the stability of bulk mRNA in *Streptomyces coelicolor*. J. Bacteriol. 192, 4275–4280.

Gnatt, A., Cramer, P., Fu, J., Bushnell, D., Kornberg, R., 2001. Structural basis of transcription: an RNA polymerase II elongation complex at 3.3 Å resolution. Science 292, 1876–1882.

Gourse, R.L., Ross, W., Gaal, T., 2000. Ups and downs in bacterial transcription initiation: the role of the alpha subunit of RNA polymerase in promoter recognition. Mol. Microbiol. 37, 687–695.

Gruber, T.M., Gross, C.A., 2003. Multiple sigma subunits and the partitioning of bacterial transcription space. Annu. Rev. Microbiol. 57, 441–466.

Gupta, A.K., Katoch, V.M., Chauhan, D.S., Sharma, R., Singh, M., Venkatesan, K., Sharma, V.D., 2010. Microarray analysis of efflux pump genes in multidrug-resistant *Mycobacterium tuberculosis* during stress induced by common anti-tuberculous drugs. Microb. Drug Resist. 16, 21–28.

Haugen, S.P., Ross, W., Gourse, R.L., 2008. Advances in bacterial promoter recognition and its control by factors that do not bind DNA. Nat. Rev. Microbiol. 6, 507–519.

Heisler, L.M., Feng, G., Jin, D.J., Gross, C.A., Landick, R., 1996. Amino acid substitutions in the two largest subunits of *Escherichia coli* RNA polymerase that suppress a defective Rho termination factor affect different parts of the transcription complex. J. Biol. Chem. 271, 14572–14583.

Helmann, J.D., 2009. RNA polymerase: a nexus of gene regulation. Methods 47, 1–5.

Hirata, A., Klein, B.J., Murakami, K.S., 2008. The X-ray crystal structure of RNA polymerase from Archaea. Nature 451, 851–854.

Hu, H., Ochi, K., 2001. Novel approach for improving the productivity of antibiotic-producing strains by inducing combined resistant mutations. Appl. Environ. Microbiol. 67, 1885–1892.

Hu, H., Zhang, Q., Ochi, K., 2002. Activation of antibiotic biosynthesis by specified mutations in the *rpoB* gene (encoding the RNA polymerase beta subunit) of *Streptomyces lividans*. J. Bacteriol. 184, 3984–3991.

Inaoka, T., Ochi, K., 2011. Scandium stimulates the production of amylase and bacilysin in *Bacillus subtilis*. Appl. Environ. Microbiol. 77, 8181–8183.

Inaoka, T., Takahashi, K., Yada, H., Yoshida, M., Ochi, K., 2004. RNA polymerase mutation activates the production of a dormant antibiotic 3,3′-neotrehalosadiamine via an autoinduction mechanism in *Bacillus subtilis*. J. Biol. Chem. 279, 3885–3892.

Ingham, C.J., Furneaux, P.A., 2000. Mutations in the β subunit of the *Bacillus subtilis* RNA polymerase that confer both rifampicin resistance and hypersensitivity to NusG. Microbiology 146, 3041–3049.

Irschik, H., Jansen, R., Gerth, K., Hofle, G., Reichenbach, H., 1987. The sorangicins: novel and powerful inhibitors of eubacterial RNA polymerase isolated from myxobacteria. J. Antibiot. (Tokyo) 40, 7–13.

Ishikawa, J., Chiba, K., Kurita, H., Satoh, H., 2006. Contribution of *rpoB2* RNA polymerase beta subunit gene to rifampin resistance in *Nocardia* species. Antimicrob. Agents Chemother. 50, 1342–1346.

Jansen, R., Wray, V., Irschik, H., Reichenbach, H., Hofle, G., 1985. Isolation and spectroscopic structure elucidation of sorangicin A, a new type of macrolide-polyether antibiotic from gliding bacteria. Tetrahedron Lett. 26, 6031–6034.

Jin, D.J., Gross, C.A., 1988. Mapping and sequencing of mutations in the *Escherichia coli rpoB* gene that lead to rifampicin resistance. J. Mol. Biol. 202, 45–58.

Jin, D.J., Gross, C.A., 1989. Characterization of the pleiotropic phenotypes of rifampicin-resistant *rpoB* mutants of *Escherichia coli*. J. Bacteriol. 171, 5229–5231.

Jin, D.J., Gross, C.A., 1991. RpoB8, a rifampicin-resistant termination-proficient RNA polymerase, has an increased Km for purine nucleotides during transcription elongation. J. Biol. Chem. 266, 14478–14485.

Jin, D.J., Cashel, M., Friedman, D.I., Nakamaru, Y., Walter, W.A., Gross, C.A., 1988a. Effects of rifampicin resistant *rpoB* mutations on antitermination and interaction with *nusA* in *Escherichia coli*. J. Mol. Biol. 204, 247–261.

Jin, D.J., Walter, W., Gross, C.A., 1988b. Characterization of the termination phenotypes of rifampicin resistant *rpoB* mutants in *Escherichia coli*. J. Mol. Biol. 202, 245–263.

Kasai, K., Nishizawa, T., Takahashi, K., Hosaka, T., Aoki, H., Ochi, K., 2006. Physiological analysis of the stringent response elicited in an extreme thermophilic bacterium, *Thermus thermophilus*. J. Bacteriol. 188, 7111–7122.

Kawai, K., Wang, G., Okamoto, S., Ochi, K., 2007. The rare earth, scandium, causes antibiotic overproduction in *Streptomyces* spp. FEMS Microbiol. Lett. 274, 311–315.

Kawamura, F., Wang, F.L., Doi, R.H., 1985. Catabolite-resistant sporulation (*crsA*) mutations in the *Bacillus subtilis* RNA polymerase 43 gene (*rpoD*) can suppress and be suppressed by mutations in the *spoO* genes. Proc. Natl. Acad. Sci. U.S.A. 82, 8124–8128.

Khesin, R.B., Gorlenko, Z.M., Shemyakin, M.F., Stvolinsky, S.L., Mindlin, S.Z., Ilyina, T.S., 1969. Studies on the functions of the RNA polymerase components by means of mutations. Mol. Gen. Genet. 105, 243–261.

Kirksey, M.A., Tischler, A.D., Siméone, R., Hisert, K.B., Uplekar, S., Guilhot, C., McKinney, J.D., 2011. Spontaneous phthiocerol dimycocerosate-deficient variants of *Mycobacterium tuberculosis* are susceptible to gamma interferon-mediated immunity. Infect. Immun. 79, 2829–2838.

Korzheva, N., Mustaev, A., Kozlov, M., Malhotra, A., Nikiforov, V., Goldfarb, A., Darst, S.A., 2000. A structural model of transcription elongation. Science 289, 619–625.

Krásný, L., Gourse, R.L., 2004. An alternative strategy for bacterial ribosome synthesis: *Bacillus subtilis* rRNA transcription regulation. EMBO J. 23, 4473–4483.

Kriel, A., Bittner, A.N., Kim, S.H., Liu, K., Tehranchi, A.K., Zou, W.Y., Rendon, S., Chen, R., Tu, B.P., Wang, J.D., 2012. Direct regulation of GTP homeostasis by (p)ppGpp: a critical component of viability and stress resistance. Mol. Cell 48, 231–241.

Lai, C., Xu, J., Tozawa, Y., Okamoto-Hosoya, Y., Yao, X., Ochi, K., 2002. Genetic and physiological characterization of *rpoB* mutations that activate antibiotic production in *Streptomyces lividans*. Microbiology 148, 3365–3373.

Landick, R., 2001. RNA polymerase clamps down. Cell 105, 567–570.

Landick, R., Stewart, J., Lee, D.N., 1990. Amino acid changes in conserved regions of the beta-subunit of *Escherichia coli* RNA polymerase alter transcription pausing and termination. Genes Dev. 4, 1623–1636.

Lavitola, A., Bucci, C., Salvatore, P., Maresca, G., Bruni, C.B., Alifano, P., 1999. Intracistronic transcription termination in polysialyltransferase gene (*siaD*) affects phase variation in *Neisseria meningitidis*. Mol. Microbiol. 33, 119–127.

Lechevalier, M.P., Prauser, H., Labeda, D.P., Ruan, J.S., 1986. Two genera of nocardioform actinomycetes: *Amycolata* gen. nov. and *Amycolatopsis* gen. nov. Int. J. Syst. Bacteriol. 36, 29–37.

Lisitsyn, N.A., Sverdlov, E.D., Moiseyeva, E.P., Danilevskaya, O.N., Nikiforov, V.G., 1984. Mutation to rifampicin resistance at the beginning of the RNA polymerase beta subunit gene in *Escherichia coli*. Mol. Gen. Genet. 196, 173–174.

Lopez, J.M., Dromerick, A., Freese, E., 1981. Response of guanosine 5′-triphosphate concentration to nutritional changes and its significance for *Bacillus subtilis* sporulation. J. Bacteriol. 146, 605–613.

Louw, G.E., Warren, R.M., Gey van Pittius, N.C., Leon, R., Jimenez, A., Hernandez-Pando, R., McEvoy, C.R., Grobbelaar, M., Murray, M., van Helden, P.D., Victor, T.C., 2011. Rifampicin reduces susceptibility to ofloxacin in rifampicin-resistant *Mycobacterium tuberculosis* through efflux. Am. J. Respir. Crit. Care Med. 184, 269–276.

Mallik, P., Paul, B.J., Rutherford, S.T., Gourse, R.L., Osuna, R., 2006. DksA is required for growth phase-dependent regulation, growth rate-dependent control, and stringent control of *fis* expression in *Escherichia coli*. J. Bacteriol. 188, 5775–5782.

Manten, A., Van Wijngaarden, L.J., 1969. Development of drug resistance to rifampicin. Chemotherapy 14, 93–100.

Martínez-Costa, O.H., Arias, P., Romero, N.M., Parro, V., Mellado, R.P., Malpartida, F., 1996. A *relA/spoT* homologous gene from *Streptomyces coelicolor* A3(2) controls antibiotic biosynthetic genes. J. Biol. Chem. 271, 10627–10634.

Matsuo, M., Hishinuma, T., Katayama, Y., Cui, L., Kapi, M., Hiramatsu, K., 2011. Mutation of RNA polymerase beta subunit (*rpoB*) promotes hVISA-to-VISA phenotypic conversion of strain Mu3. Antimicrob. Agents Chemother. 55, 4188–4195.

Maughan, H., Galeano, B., Nicholson, W.L., 2004. Novel *rpoB* mutations conferring rifampin resistance on *Bacillus subtilis*: global effects on growth, competence, sporulation, and germination. J. Bacteriol. 186, 2481–2486.

Merrikh, H., Ferrazzoli, A.E., Bougdour, A., Olivier-Mason, A., Lovett, S.T., 2009. A DNA damage response in *Escherichia coli* involving the alternative sigma factor, RpoS. Proc. Natl. Acad. Sci. U.S.A. 106, 611–616.

Miloso, M., Limauro, D., Alifano, P., Rivellini, F., Lavitola, A., Gulletta, E., Bruni, C.B., 1993. Characterization of the *rho* genes of *Neisseria gonorrhoeae* and *Salmonella typhimurium*. J. Bacteriol. 175, 8030–8037.

Moeller, R., Vlašić, I., Reitz, G., Nicholson, W.L., 2012. Role of altered *rpoB* alleles in *Bacillus subtilis* sporulation and spore resistance to heat, hydrogen peroxide, formaldehyde, and glutaraldehyde. Arch. Microbiol. 194, 759–767.

Monaco, C., Talà, A., Spinosa, M.R., Progida, C., De Nitto, E., Gaballo, A., Bruni, C.B., Bucci, C., Alifano, P., 2006. Identification of a meningococcal L-glutamate ABC transporter operon essential for growth in low-sodium environments. Infect. Immun. 74, 1725–1740.

Mooney, R.A., Artsimovitch, I., Landick, R., 1998. Information processing by RNA polymerase: recognition of regulatory signals during RNA chain elongation. J. Bacteriol. 180, 3265–3275.

Morgan, W.D., Bear, D.G., Litchman, B.L., von Hippel, P.H., 1985. RNA sequence and secondary structure requirements for Rho-dependent transcription termination. Nucleic Acids Res. 13, 3739–3754.

Murakami, K.S., Darst, S.A., 2003. Bacterial RNA polymerases: the whole story. Curr. Opin. Struct. Biol. 13, 31–39.

Murakami, K., Masuda, S., Darst, S., 2002a. Structural basis of transcription initiation: RNA polymerase holoenzyme at 4 Å resolution. Science 296, 1280–1284.

Murakami, K., Masuda, S., Campbell, E., Muzzin, O., Darst, S., 2002b. Structural basis of transcription initiation: an RNA polymerase holoenzyme–DNA complex. Science 296, 1285–1290.

Murray, H.D., Schneider, D.A., Gourse, R.L., 2003. Control of rRNA expression by small molecules is dynamic and nonredundant. Mol. Cell 12, 125–134.

Murry, J.P., Pandey, A.K., Sassetti, C.M., Rubin, E.J., 2009. Phthiocerol dimycocerosate transport is required for resisting interferon-gamma-independent immunity. J. Infect. Dis. 200, 774–782.

Mustaev, A., Kashlev, M., Lee, J.Y., Polyakov, A., Lebedev, A., Zalenskaya, K., Grachev, M., Goldfarb, M., Nikiforov, A.V., 1991. Mapping of the priming substrate contacts in the active center of *Escherichia coli* RNA polymerase. J. Biol. Chem. 266, 23927–23931.

Mustaev, A., Kozlov, M., Markovtsov, V., Zaychikov, E., Denissova, L., Goldfarb, A., 1997. Modular organization of the catalytic center of RNA polymerase. Proc. Natl. Acad. Sci. U.S.A. 94, 6641–6645.

Nanamiya, H., Fugono, N., Asai, K., Doi, R.H., Kawamura, F., 2000. Suppression of temperature-sensitive sporulation mutation in the *Bacillus subtilis sigA* gene by *rpoB* mutation. FEMS Microbiol. Lett. 192, 237–241.

Neri, A., Mignogna, G., Fazio, C., Giorgi, A., Schininà, M.E., Stefanelli, P., 2010. *Neisseria meningitidis* rifampicin resistant strains: analysis of protein differentially expressed. BMC Microbiol. 10, 246.

Nudler, E., Gottesman, M., 2002. Transcription termination and anti-termination in *E. coli*. Genes Cells 7, 755–768.

Ochi, K., 2007. From microbial differentiation to ribosome engineering. Biosci. Biotechnol. Biochem. 71, 1373–1386.

Ochi, K., 1987. Metabolic initiation of differentiation and secondary metabolism by *Streptomyces griseus*: significance of the stringent response (ppGpp) and GTP content in relation to A factor. J. Bacteriol. 169, 3608–3616.

Ochi, K., Hosaka, T., 2013. New strategies for drug discovery: activation of silent or weakly expressed microbial gene clusters. Appl. Microbiol. Biotechnol. 97, 87–98.

Ochi, K., Okamoto, S., Tozawa, S., Inaoka, Y., Hosaka, T., Xu, T., Kurosawa, J.K., 2004. Ribosome engineering and secondary metabolite production. Adv. Appl. Microbiol. 56, 155–184.

Ochi, K., Tanaka, Y., Tojo, S., 2014. Activating the expression of bacterial cryptic genes by *rpoB* mutations in RNA polymerase or by rare earth elements. J. Ind. Microbiol. Biotechnol. 41, 403–414.

Ohnishi, Y., Yamazaki, H., Kato, J.Y., Tomono, A., Horinouchi, S., 2005. AdpA, a central transcriptional regulator in the A-factor regulatory cascade that leads to morphological development and secondary metabolism in *Streptomyces griseus*. Biosci. Biotechnol. Biochem. 69, 431–439.

O'Neill, A., Oliva, B., Storey, C., Hoyle, A., Fishwick, C., Chopra, I., 2000. RNA polymerase inhibitors with activity against rifampin-resistant mutants of *Staphylococcus aureus*. Antimicrob. Agents Chemother. 44, 3163–3166.

O'Neill, A.J., Huovinen, T., Fishwick, C.W., Chopra, I., 2006. Molecular genetic and structural modeling studies of *Staphylococcus aureus* RNA polymerase and the fitness of rifampin resistance genotypes in relation to clinical prevalence. Antimicrob. Agents Chemother. 50, 298–309.

Ovchinnikov, Y., Monastyrskaya, G.S., Gubanov, V.V., Lipkin, V.M., Sverdlov, E.D., Kiver, I.F., Bass, I.A., Mindlin, S.Z., Danilevskaya, O.N., Khesin, R.B., 1981. Primary structure of *Escherichia coli* RNA polymerase nucleotide substitution in the beta subunit gene of the rifampicin resistant *rpoB255* mutant. Mol. Gen. Genet. 184, 536–538.

Ovchinnikov, Y.A., Monastyrskaya, G.S., Guriev, S.O., Kalinina, N.F., Sverdlov, E.D., Gragerov, A.I., Bass, I.A., Kiver, I.F., Moiseyeva, E.P., Igumnov, V.N., Mindlin, S.Z., Nikiforov, V.G., Khesin, R.B., 1983. RNA polymerase rifampicin resistance mutations in *Escherichia coli*: sequence changes and dominance. Mol. Gen. Genet. 190, 344–348.

Paget, M.S.B., Helmann, J.D., 2003. The sigma70 family of sigma factors. Genome Biol. 4 (1), 203.

Pagliarulo, C., Salvatore, P., De Vitis, L.R., Colicchio, R., Monaco, C., Tredici, M., Talà, A., Bardaro, M., Lavitola, A., Bruni, C.B., Alifano, P., 2004. Regulation and differential expression of *gdhA* encoding NADP-specific glutamate dehydrogenase in *Neisseria meningitidis* clinical isolates. Mol. Microbiol. 51, 1757–1772.

Paul, B.J., Barker, M.M., Ross, W., Schneider, D.A., Webb, C., Foster, J.W., Gourse, R.L., 2004. DksA: a critical component of the transcription initiation machinery that potentiates the regulation of rRNA promoters by ppGpp and the initiating NTP. Cell 118, 311–322.

Peano, C., Talà, A., Corti, G., Pasanisi, D., Durante, M., Mita, G., Bicciato, S., De Bellis, G., Alifano, P., 2012. Comparative genomics and transcriptional profiles of *Saccharopolyspora erythraea* NRRL 2338 and a classically improved erythromycin over-producing strain. Microb. Cell Fact. 11, 32.

Perederina, A., Svetlov, V., Vassylyeva, M.N., Tahirov, T.H., Yokoyama, S., Artsimovitch, I., Vassylyev, D.G., 2004. Regulation through the secondary channel—structural framework for ppGpp-DksA synergism during transcription. Cell 118, 297–309.

Perkins, A.E., Nicholson, W.L., 2008. Uncovering new metabolic capabilities of *Bacillus subtilis* using phenotype profiling of rifampin-resistant *rpoB* mutants. J. Bacteriol. 190, 807–814.

Potrykus, K., Cashel, M., 2008. (p)ppGpp: still magical? Annu. Rev. Microbiol. 62, 35–51.

Queck, S.Y., Jameson-Lee, M., Villaruz, A.E., Bach, T.H., Khan, B.A., Sturdevant, D.E., Ricklefs, S., Li, M., Otto, M.M., 2008. RNAIII-independent target gene control by the *agr* quorum-sensing system: insight into the evolution of virulence regulation in *Staphylococcus aureus*. Mol. Cell 32, 150–158.

Rao, F., See, R.Y., Zhang, D., Toh, D.C., Ji, Q., Liang, Z.X., 2010. YybT is a signaling protein that contains a cyclic dinucleotide phosphodiesterase domain and a GGDEF domain with ATPase activity. J. Biol. Chem. 285, 473–482.

Rappas, M., Bose, D., Zhang, X., 2007. Bacterial enhancer-binding proteins: unlocking sigma54-dependent gene transcription. Curr. Opin. Struct. Biol. 17, 110–116.

Reed, M.B., Domenech, P., Manca, C., Su, H., Barczak, A.K., Kreiswirth, B.N., Kaplan, G., Barry 3rd., C.E., 2004. A glycolipid of hypervirulent tuberculosis strains that inhibits the innate immune response. Nature 431, 84–87.

Retzlaff, L., Distler, J., 1995. The regulator of streptomycin gene expression, StrR, of *Streptomyces griseus* is a DNA binding activator protein with multiple recognition sites. Mol. Microbiol. 18, 151–162.

Reynolds, M.G., 2000. Compensatory evolution in rifampin-resistant *Escherichia coli*. Genetics 156, 1471–1481.

Richardson, J.P., 1991. Preventing the synthesis of unused transcripts by Rho factor. Cell 64, 1047–1049.

Richardson, J.P., Greenblatt, J.L., 1996. Control of RNA chain elongation and termination. In: Neidhardt, F.C. (Ed.), *Escherichia coli* and *Salmonella*: Cellular and Molecular Biology. , 2nd ed. ASM Press, Washington, DC, pp. 822–848.

Rivellini, F., Alifano, P., Piscitelli, C., Blasi, V., Bruni, C.B., Carlomagno, M.S., 1991. A cytosine-over guanosine-rich sequence in RNA activates *rho*-dependent transcription termination. Mol. Microbiol. 5, 3049–3054.

Roberts, J., Park, J.S., 2004. Mfd, the bacterial transcription repair coupling factor: translocation, repair and termination. Curr. Opin. Microbiol. 7, 120–125.

Rommele, G., Wirz, G., Solf, R., Vosbeck, K., Gruner, J., Wehrli, W., 1990. Resistance of *Escherichia coli* to rifampicin and sorangicin A—a comparison. J. Antibiot. (Tokyo) 43, 88–91.

Ross, W., Verntas, C.E., Sanchez-Vazquez, P., Gaal, T., Gourse, R.L., 2013. The magic spot: a ppGpp binding site on *E. coli* RNA polymerase responsible for regulation of transcription initiation. Mol. Cell 50, 420–429.

Saito, M., Katayama, Y., Hishinuma, T., Iwamoto, A., Aiba, Y., Kuwahara-Arai, K., Cui, L., Matsuo, M., Aritaka, N., Hiramatsu, K., 2014. Slow VISA' (sVISA), a novel phenotype of vancomycin resistance, obtained in vitro from hVISA. Antimicrob. Agents Chemother. 58, 5024–5035.

Saito, N., Xu, J., Hosaka, T., Okamoto, S., Aoki, H., Bibb, M.J., Ochi, K., 2006. EshA accentuates ppGpp accumulation and is conditionally required for antibiotic production in *Streptomyces coelicolor* A3(2). J. Bacteriol. 188, 4952–4961.

Schumacher, J., Zhang, X., Jones, S., Bordes, P., Buck, M., 2004. ATP-dependent transcriptional activation by bacterial PspF AAA1 protein. J. Mol. Biol. 338, 863–875.

Severinov, K., Soushko, M., Goldfarb, A., Nikiforov, V., 1993. Rifampicin region revisited. New rifampicin-resistant and streptolydigin-resistant mutants in the beta subunit of *Escherichia coli* RNA polymerase. J. Biol. Chem. 268, 14820–14825.

Shinnick, T. (Ed.), 1996. Current Topics in Microbiology and Immunology. Academic Press, New York.

Siculella, L., Damiano, F., di Summa, R., Tredici, S.M., Alduina, R., Gnoni, G.V., Alifano, P., 2010. Guanosine 5′-diphosphate 3′-diphosphate (ppGpp) as a negative modulator of polynucleotide phosphorylase activity in a 'rare' actinomycete. Mol. Microbiol. 77, 716–729.

Singer, M., Jin, D.J., Walter, W.A., Gross, C.A., 1993. Genetic evidence for the interaction between cluster I and cluster III rifampicin resistant mutations. J. Mol. Biol. 231, 1–5.

Sonenshein, A.L., 2005. CodY, a global regulator of stationary phase and virulence in Gram-positive bacteria. Curr. Opin. Microbiol. 8, 203–207.

Squires, C.L., Zaporojets, D., 2000. Proteins shared by the transcription and translation machines. Annu. Rev. Microbiol. 54, 775–798.

Stagno, J.R., Altieri, A.S., Bubunenko, M., Tarasov, S.G., Li, J., Court, D.L., Byrd, R.A., Ji, X., 2011. Structural basis for RNA recognition by NusB and NusE in the initiation of transcription antitermination. Nucleic Acids Res. 39, 7803–7815.

Stephanopoulos, G., Alper, H., Moxley, J., 2004. Exploiting biological complexity for strain improvement through systems biology. Nat. Biotechnol. 22, 1261–1267.

Sukhodolets, M.V., Cabrera, J.E., Zhi, H., Jin, D.J., 2001. RapA, a bacterial homolog of SWI2/SNF2, stimulates RNA polymerase recycling in transcription. Genes Dev. 15, 3330–3341.

Sun, Y.H., Bakshi, S., Chalmers, R., Tang, C.M., 2000. Functional genomics of *Neisseria meningitidis* pathogenesis. Nat. Med. 6, 1269–1273.

Tagami, S., Sekine, S., Kumarevel, T., Hino, N., Murayama, Y., Kamegamori, S., Yamamoto, M., Sakamoto, K., Yokoyama, S., 2010. Crystal structure of bacterial RNA polymerase bound with a transcription inhibitor protein. Nature 468, 978–982.

Taha, M.K., Zarantonelli, M.L., Ruckly, C., Giorgini, D., Alonso, J.M., 2006. Rifampin-resistant *Neisseria meningitidis*. Emerg. Infect. Dis. 12, 859–860.

Takano, E., Bibb, M.J., 1994. The stringent response, ppGpp and antibiotic production in *Streptomyces coelicolor* A3(2). Actinomycetologica 8, 1–10.

Talà, A., Monaco, C., Nagorska, K., Exley, R.M., Corbett, A., Zychlinsky, A., Alifano, P., Tang, C.M., 2011. Glutamate utilization promotes meningococcal survival *in vivo* through avoidance of the neutrophil oxidative burst. Mol. Microbiol. 81, 1330–1342.

Talà, A., Wang, G., Zemanova, M., Okamoto, S., Ochi, K., Alifano, P., 2009. Activation of dormant bacterial genes by *Nonomuraea* sp. strain ATCC 39727 mutant-type RNA polymerase. J. Bacteriol. 191, 805–814.

Tamehiro, N., Hosaka, T., Xu, J., Hu, H., Otake, N., Ochi, K., 2003. Innovative approach for improvement of an antibiotic-overproducing industrial strain of *Streptomyces albus*. Appl. Environ. Microbiol. 69, 6412–6417.

Tanaka, Y., Hosaka, T., Ochi, K., 2010. Rare earth elements activate the secondary metabolite-biosynthetic gene clusters in *Streptomyces coelicolor* A3(2). J. Antibiot. (Tokyo) 63, 477–481.

Tanaka, Y., Kasahara, K., Hirose, Y., Murakami, K., Kugimiya, R., Ochi, K., 2013. Activation and products of the cryptic secondary metabolite biosynthetic gene clusters by rifampin resistance (*rpoB*) mutations in actinomycetes. J. Bacteriol. 195, 2959–2970.

Tavormina, P.L., Landick, R., Gross, C.A., 1996. Isolation, purification and *in vitro* characterization of recessive-lethal-mutant RNA polymerases from *Escherichia coli*. J. Bacteriol. 178, 5263–5271.

Tocchini-Valentini, G.P., Marino, P., Colvill, A.J., 1968. Mutant of *E. coli* containing an altered DNA-dependent RNA polymerase. Nature 220, 275–276.

Toulokhonov, I.I., Shulgina, I., Hernandez, V.J., 2001. Binding of the transcription effector ppGpp to *Escherichia coli* RNA polymerase is allosteric, modular, and occurs near the N terminus of the beta'-subunit. J. Biol. Chem. 276, 1220–1225.

Trivedi, O.A., Arora, P., Vats, A., Ansari, M.Z., Tickoo, R., Sridharan, V., Mohanty, D., Gokhale, R.S., 2005. Dissecting the mechanism and assembly of a complex virulence mycobacterial lipid. Mol. Cell 17, 631–643.

Traxler, M.F., Summers, S.M., Nguyen, H.-T., Zacharia, V.M., Hightower, G.A., Smith, J.T., Conway, T., 2008. The global, ppGpp-mediated stringent response to amino acid starvation in *Escherichia coli*. Mol. Microbiol. 68, 1128–1148.

Vassylyev, D.G., Sekine, S., Laptenko, O., Lee, J., Vassylyeva, M.N., Borukhov, S., Yokoyama, S., 2002. Crystal structure of a bacterial RNA polymerase holoenzyme at 2.6 Å resolution. Nature 417, 712–719.

Vassylyev, D.G., Vassylyeva, M.N., Perederina, A., Tahirov, T.H., Artsimovitch, I., 2007. Structural basis for transcription elongation by bacterial RNA polymerase. Nature 448, 157–162.

Vattanaviboon, P., Sukchawalit, R., Jearanaikoon, P., Chuchottaworn, C., Ponglikitmongkol, M., 1995. Analysis of RNA polymerase gene mutation in three isolates of rifampicin resistant *Mycobacterium tuberculosis*. Southeast Asian J. Trop. Med. Public Health 26 (Suppl. 1), 333–336.

Vigliotta, G., Tredici, S.M., Damiano, F., Montinaro, M.R., Pulimeno, R., di Summa, R., Massardo, D.R., Gnoni, G.V., Alifano, P., 2005. Natural merodiploidy involving duplicated *rpoB* alleles affects secondary metabolism in a producer actinomycete. Mol. Microbiol. 55, 396–412.

Villain-Guillot, P., Bastide, L., Gualtieri, M., Leonetti, J., 2007. Progress in targeting bacterial transcription. Drug Discov. Today 12, 200–208.

Vrentas, C.E., Gaal, T., Berkmen, M.B., Rutherford, S.T., Haugen, S.P., Vassylyev, D.G., Ross, W., Gourse, R.L., 2008. Still looking for the magic spot: the crystallographically defined binding site for ppGpp on RNA polymerase is unlikely to be responsible for rRNA transcription regulation. J. Mol. Biol. 377, 551–564.

Wang, G., Hosaka, T., Ochi, K., 2008. Dramatic activation of antibiotic production in *Streptomyces coelicolor* by cumulative drug resistance mutations. Appl. Environ. Microbiol. 74, 2834–2840.

Wang, G., Tanaka, Y., Ochi, K., 2010. The G243D mutation (*afsB* mutation) in the principal sigma factor sigma HrdB alters intracellular ppGpp level and antibiotic production in *Streptomyces coelicolor* A3(2). Microbiology 156, 2384–2392.

Watanabe, Y., Cui, L., Katayama, Y., Kozue, K., Hiramatsu, K., 2011. Impact of *rpoB* mutations on reduced vancomycin susceptibility in *Staphylococcus aureus*. J. Clin. Microbiol. 49, 2680–2684.

Westover, K.D., Bushnell, D.A., Kornberg, R.D., 2004. Structural basis of transcription: nucleotide selection by rotation in the RNA polymerase II active center. Cell 119, 481–489.

Wichelhaus, T.A., Böddinghaus, B., Besier, S., Schäfer, V., Brade, V., Ludwig, A., 2002. Biological cost of rifampin resistance from the perspective of *Staphylococcus aureus*. Antimicrob. Agents Chemother. 46, 3381–3385.

Wigneshweraraj, S., Bose, D., Burrows, P.C., Joly, N., Schumacher, J., Rappas, M., Pape, T., Zhang, X., Stockley, P., Severinov, K., Buck, M., 2008. Modus operandi of the bacterial RNA polymerase containing the sigma(54) promoter-specificity factor. Mol. Microbiol. 68, 538–546.

Wilson, K.S., von Hippel, P.H., 1994. Stability of *Escherichia coli* transcription complexes near an intrinsic terminator. J. Mol. Biol. 244, 36–51.

Witte, G., Hartung, S., Buttner, K., Hopfner, K.P., 2008. Structural biochemistry of a bacterial checkpoint protein reveals diadenylate cyclase activity regulated by DNA recombination intermediates. Mol. Cell 30, 167–178.

Xu, H., Hoover, T.R., 2001. Transcriptional regulation at a distance in bacteria. Curr. Opin. Microbiol. 4, 138–144.

Xu, C., Liu, L., Zhang, Z., Jin, D., Qiu, J., Chen, M., 2013. Genome-scale metabolic model in guiding metabolic engineering of microbial improvement. Appl. Microbiol. Biotechnol. 97, 519–539.

Xu, J., Tozawa, Y., Lai, C., Hayashi, H., Ochi, K., 2002. A rifampicin resistance mutation in the *rpoB* gene confers ppGpp-independent antibiotic production in *Streptomyces coelicolor* A3 (2). Mol. Genet. Genomics 268, 179–189.

Xu, M., Zhou, Y., Goldstein, B., Jin, D., 2005. Cross-resistance of *Escherichia coli* RNA polymerases conferring rifampin resistance to different antibiotics. J. Bacteriol. 187, 2783–2792.

Yang, X., Ishiguro, E.E., 2003. Temperature-sensitive growth and decreased thermotolerance associated with *relA* mutations in *Escherichia coli*. J. Bacteriol. 185, 5765–5771.

Zhang, G., Campbell, E.A., Minakhin, L., Richter, C., Severinov, K., Darst, S.A., 1999. Crystal structure of *Thermus aquaticus* core RNA polymerase at 3.3 Å resolution. Cell 98, 811–824.

Zhang, X., Chaney, M., Wigneshweraraj, S., Schumacher, J., Bordes, P., Cannon, W., Buck, M., 2002. Mechanochemical ATPases and transcriptional activation. Mol. Microbiol. 45, 895–903.

Zhang, Y., Feng, Y., Chatterjee, S., Tuske, S., Ho, M.X., Arnold, E., Ebright, R.H., 2012. Structural basis of transcription initiation. Science 338, 1076–1080.

Zhao, G., Weatherspoon, N., Kong, W., Curtiss, R., Shi, Y., 2008. A dual-signal regulatory circuit activates transcription of a set of divergent operons in *Salmonella typhimurium*. Proc. Natl. Acad. Sci. U.S.A. 105, 20924–20929.

Zhou, Y.N., Jin, D.J., 1998. The *rpoB* mutants destabilizing initiation complexes at stringently controlled promoters behave like stringent RNA polymerases in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. 95, 2908–2913.

Zuo, Y., Wang, Y., Steitz, T.A., 2013. The mechanism of *E. coli* RNA polymerase regulation by ppGpp is suggested by the structure of their complex. Mol. Cell 50, 430–436.