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Essential Biological Processes of an Emerging Pathogen: DNA Replication, Transcription, and Cell Division in *Acinetobacter* spp.

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INTRODUCTION

Species of the bacterial genus *Acinetobacter* are becoming increasingly important as a source of hospital-acquired infections (31, 185, 204). *Acinetobacter* spp. are ubiquitous nonmotile gammaproteobacteria, typified by metabolic versatility and a capacity for natural transformation (172, 204). The species of most clinical relevance is *A. baumannii*; however, pathogenic strains of *A. lwoffi* and *A. baylyi* have also been described (38, 185, 215).

A. baumannii is most commonly associated with pneumonia or bacteremia, although the incidence of soft tissue infections and meningitis appears to be increasing (204). Mortality rates associated with *A. baumannii* infection are a matter of some contention, as there is a tendency for *A. baumannii* to infect patients who are critically ill and already have a poor prognosis (204). It is clear, however, that patients with *A. baumannii* infections stay significantly longer in hospitals than uninfected patients and represent a significant burden on health care systems worldwide (204). Exacerbating this, *A. baumannii* can be highly persistent within the hospital setting, due in part to its resistance to disinfectants (262) and remarkable insensitivity to desiccation (122). Of greatest concern, however, is the rapid emergence of multidrug resistance (65). *A. baumannii* strains that are resistant to every antibiotic compound in clinical use, including recently approved drugs such as tigecyclin, have now been reported (65, 187, 203). In the face of the growing threat posed by these and other intractable Gram-negative pathogens, there is an urgent need for new classes of antibiotic compounds with novel mechanisms of action (245).

Antibiotics act by inhibiting the essential working parts of bacterial cells, with the most common targets being ribosomal protein synthesis, cell wall biosynthesis, and primary metabolic pathways. The processes of chromosomal DNA replication, transcription and cell division are relatively unexplored as drug targets. They could, however, be considered attractive targets for rational design of novel antimicrobial compounds, as they are (i) essential for the propagation of bacterial cells and therefore infection, (ii) conserved across all bacterial species, and (iii) in many ways distinct from the equivalent processes in eukaryotic cells (150). *Acinetobacter* represents an appealing system for studying these processes. In addition to their direct clinical relevance, *Acinetobacter* spp. show some similarities to other problematic pathogens, such as *Pseudomonas aeruginosa*, that are not shared by the more thoroughly studied model bacterium *Escherichia coli* (15). At the same time, the *Acinetobacter* genus has diverged significantly from other gammaproteobacteria, as highlighted by a phylogenetic tree based on the highly conserved sequences of 16S rRNA genes (Fig. 1) (116, 188, 213, 222, 265, 266). As such, *Acinetobacter* sp. proteins generally show considerable sequence divergence from equivalent proteins in other organisms and can therefore be used to highlight the most conserved aspects of essential biological systems. Of course, these are the most attractive targets for the discovery or rational design of broad-spectrum antibiotics.

Currently, complete genome sequences are available for

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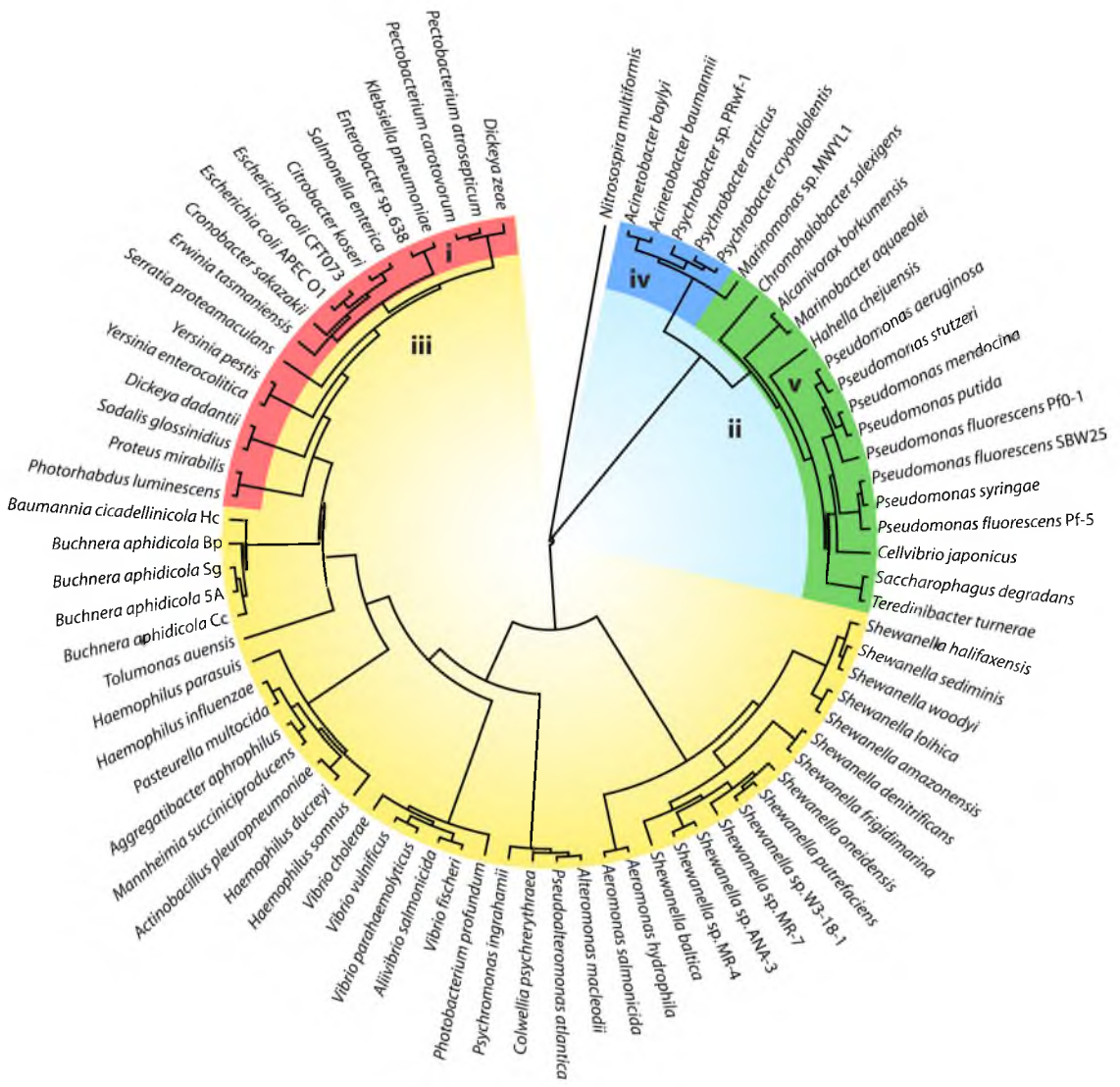

FIG. 1. Subbranch of a phylogenetic tree based on 16S rRNA sequences. 16S rRNA sequences encoded by the *rrsA* gene were from gammaproteobacteria with completely sequenced genomes and were filtered to remove identical sequences prior to tree construction. The tree was constructed using the neighborhood-joining tree method in Geneious (Biomatters, Auckland, New Zealand), using the Jukes-Cantor genetic distance model and employing the bootstrap method with 10,000 replicates. The sequence of *rrsA* from the betaproteobacterium *Nitrosospira multiformis* was included as an outgroup. Colored segments indicate the phylogenetic distribution of particular genetic characteristics: (i) *dnaC*, *holE*, *tus*, *amiC*, and *envC* genes present on chromosome; (ii) long *Pseudomonas*-type ψ subunit of DNA polymerase III; (iii) short *E. coli*-type ψ subunit of DNA polymerase III, *rnhA* and *dnaQ* genes adjacent and transcribed in opposite directions, *yacL* gene present; (iv) *rnhA-dnaQ* fusion gene present and no homologs of *ftsE*, *ftsX*, *sulA*, or *lexA* on chromosome (includes *Acinetobacter* spp.); (v) *rnhA* and *dnaQ* genes adjacent and transcribed in same direction and *amiC* gene present.

seven *Acinetobacter* strains: the highly transformable laboratory strain *A. baylyi* ADP1 (15); a human body louse symbiont, *A. baumannii* SDF (248); and the human pathogens *A. baumannii* strains ATCC 17978 (233), AYE (248), ACICU (115), AB0057 (4), and AB307-0294 (4). At least eight additional genome sequence projects are currently in progress or at draft assembly stage at the time of writing of this review. In addition, the Genoscope group has produced a complete single-gene deletion library for *A. baylyi* ADP1, allowing the essentiality of individual genes to be assessed (61). These resources provide a firm starting point for the investigation of essential biological processes in these organisms. The goal of this review is to examine this recently available information, primarily by extrapolating knowledge from well-studied model organisms, in the context of DNA replication, transcription, and cell division, with a view toward highlighting unusual aspects and identification of candidate enzymes and complexes most suitable for future drug discovery and design.

DNA REPLICATION

Our knowledge of bacterial DNA replication draws extensively from studies of the model organism *E. coli* (16, 209, 226,

242). Over the last 5 decades, each of the individual DNA replication proteins have been identified, for most their three-dimensional structures (or those of their domains) have been determined, and many protein-protein and protein-DNA interactions have been mapped (Table 1).

In *E. coli* and many other bacteria, DNA replication is initiated at a single site on the circular chromosome, the origin of replication or *oriC*, which contains a series of 9-bp sequence repeats known as DnaA boxes (180). Binding of multiple DnaA molecules to *oriC* leads to separation of the two template DNA strands at a nearby AT-rich region. One molecule of the replicative helicase DnaB is loaded onto each of the resulting single DNA strands, and each proceeds to unwind the parental DNA duplex to create replication forks that move away from the origin in opposite directions. DNA primase associates with DnaB and constructs short RNA primers, onto which the multisubunit DNA polymerase III holoenzyme (Pol III HE) builds each new DNA strand. DNA synthesis by Pol III is carried out only in the 5′-to-3′ direction; thus, synthesis of one strand (the leading strand) is continuous while synthesis of the other strand (the lagging strand) is discontinuous. The DnaB helicase translocates on the lagging strand at each fork. DNA replication continues bidirectionally around the circular chromosome until the two replication forks meet in the terminus region, located approximately opposite the origin (182, 189). This eventually yields two copies of the bacterial chromosome, each containing one strand from the parental chromosome and one nascent strand.

With the availability of complete genome sequences for more than 900 bacterial strains, we can now extrapolate our solid understanding of *E. coli* DNA replication into genera outside the *Enterobacteriaceae*, such as *Acinetobacter*. While both are members of the gammaproteobacteria, the *Acinetobacter* genus is relatively remote from *E. coli* (see the phylogenetic tree in Fig. 1). The sequences of *Acinetobacter* DNA replication proteins have diverged significantly from those of other organisms, in some cases beyond the detection level of standard homology searches. While this in some ways restricts the level of information that can be extrapolated from the *E. coli* system, it also provides strong indications of which aspects of DNA replication are conserved and which are not. *Acinetobacter* spp. display several peculiarities among their replication components, some of which are discussed in detail below. Some of these, such as the fusion of *rnhA* and *dnaQ* genes in a single cistron as previously noted by Barbe and colleagues (15), are unique to members of *Acinetobacter* and the related genus *Psychrobacter*. Others highlight the previously identified relationship between the *Acinetobacter* and *Pseudomonas* genera (15), with the two groups often displaying common protein features despite significant divergence at the DNA sequence level.

Initiation of Replication in *Acinetobacter*

All *Acinetobacter* spp. for which genome sequences have been completed contain a single circular chromosome, with the origin of replication (*oriC*) located between the *rpmH* and *dnaA* loci (15). This arrangement is also seen in *Pseudomonas* spp. (270) and contrasts with the case for *E. coli*, whose origin maps between the *gidA* (*mnmG*) and *mioC* genes, some 40 kb from *rpmH* and *dnaA*. For each *Acinetobacter* sp., the replication initiator protein DnaA is relatively well conserved (46% identity with *E. coli* DnaA [Table 1]), particularly within the N-terminal DnaB-interacting domain (domain I), the C-terminal AAA+ ATPase domain (domain III), and the DNA-binding domain (domain IV) (134). Domain II, identified as a flexible linker in the *E. coli* protein, has diverged beyond recognition in *Acinetobacter* DnaA proteins.

Initiation of DNA replication in *E. coli* is regulated by a paralog of DnaA known as Hda (138, 180). When bound to a DNA-associated β sliding clamp, Hda causes the active ATP-bound form of DnaA to hydrolyze its substrate and thus become inactive, preventing unscheduled reinitiation of replication (142). Genes encoding proteins with moderate homology to *E. coli* Hda (30% sequence identity) are found within the *Acinetobacter* sp. genomes. Although they are frequently annotated as "putative chromosomal replication initiator, DnaA type," these proteins likely perform a role analogous to that of Hda, i.e., suppression of untimely initiation of DNA replication.

Historically, the study of DNA replication has focused on two model organisms: the Gram-negative *E. coli* (a gammaproteobacterium) and the Gram-positive *Bacillus subtilis* (a firmicute). Both these organisms utilize AAA+ ATPase family helicase loader proteins to load the replicative DNA helicase (DnaB in *E. coli* and DnaC in *B. subtilis*) onto the template DNA at origins of replication or during replication restart after fork blockage (59). In *E. coli*, helicase loading is carried out by DnaC, which complexes and thereby inactivates DnaB until its activity is required and dissociates upon helicase loading (226). In *B. subtilis*, three proteins, DnaB, DnaD, and DnaI, are used; the last is a homolog of *E. coli* DnaC (26, 117). No homologs of *E. coli* DnaC or *B. subtilis* DnaB, -D, or -I are identifiable within the *Acinetobacter* sp. genomes, with the exception of prophage-associated DnaC homologs in *A. baumannii* strains AYE and ACICU. Homology searches across those bacteria with completed genome sequences show the phylogenetic distribution of DnaC to be restricted to the *Enterobacteriaceae* (Fig. 1). The DnaB, -D, and -I helicase-loading proteins are similarly restricted to the *Firmicutes*, which include the genera *Bacillus*, *Staphylococcus*, and *Mycoplasma*. This implies that *Acinetobacter* spp., and for that matter all other bacteria outside the *Enterobacteriaceae* and the *Firmicutes*, either are capable of loading the replicative helicase without additional loader proteins or have helicase loaders whose sequences are not related to the *E. coli* and *B. subtilis* proteins. In support of the former scenario, the *dnaB* (helicase) gene from the epsilonproteobacterium *Helicobacter pylori* has been found to successfully complement temperature-sensitive *dnaC* mutations in *E. coli* (236). This suggests that *H. pylori* DnaB not only can be loaded without the activity of DnaC *in vivo* but also can be assembled successfully into an *E. coli* replisome. Further evidence comes from the observation that DnaB proteins from *Pseudomonas* spp. can be loaded onto the origin of a broad-host-range plasmid *in vitro*, using only DnaA, i.e., without assistance from a loader protein (35). Since the *E. coli* DnaA and DnaC proteins are structurally related (58, 77, 181, 226), it is possible that DnaA (or a DnaA homolog) loads DnaB in other organisms, not just at *oriC* but also during replication restart. Further work is clearly required to better understand

TABLE 1. Conservation of chromosomal DNA replication proteins in *Acinetobacter* spp.

Protein	Function	Protein length (amino acids)[a]	% Sequence identity (residue range)			Essentiality[e]	Homologs with known structures[b] (residue range of sequence match; % sequence identity)	Known protein-protein interactions in *E. coli*	References
			A. baylyi vs *E. coli*[b]	*A. baumannii* vs *E. coli*[d]	*A. baylyi* vs *A. baumannii*[b]				
DnaA	Initiator	465	46 (2–465)	47 (2–465)	91 (1–465)	A, P, E	*E. coli* (2–108; 32), *Thermatoga maritima* (109–432; 36)	DnaB, Hda, DiaA, Dps, HU	1, 14, 40, 41, 142, 145, 196
DnaB	DNA helicase	481	51 (24–480)	51 (24–480)	92 (1–481)	A, P, E	*Geobacillus stearothermophilus* (29–473; 42)	DnaA, DnaC, DnaG, τ, Rep	1, 12, 82, 83, 97, 191
DnaC	Helicase loader	—[f]	—	—	—	E	*Aquifex aoelicus* (no homolog)	DnaB	82, 181
DnaE	α subunit, polymerase III core	1,187	48 (2–1145)	48 (2–1145)	87 (1–1187)	A, P, E	*E. coli* (2–901; 53), *Thermus aquaticus* (1–1010; 38)	ε, β, τ	13, 55, 125, 149, 157, 192, 197, 206, 241
DnaG	DNA primase	629	39 (3–472)	40 (3–464)	81 (1–629)	A, E	*G. stearothermophilus* (3–95; 51), *E. coli* (165–472; 44)	DnaB, SSB	12, 51, 191, 275
DnaN	β sliding clamp	382	44 (1–382)	45 (1–382)	91 (1–382)	A, P, E	*E. coli* (1–382; 44)	α, δ, Hda, UmuC, UmuD, DinB1, MutS, MutL, DNA ligase, PolA, PolB	55, 70, 87, 127, 142, 158, 159
DnaQ (fusion with RnhA)	ε subunit, polymerase III core	450	46 (223–447)	46 (232–456)	78 (1–450)	A, P	*E. coli* (1–136 RNase HI; 49), *E. coli* (223–393; 52)	α, θ	99, 137, 197, 206
DnaX	τ and γ subunits, clamp loader complex	711	56 (2–360)	55 (2–363)	66 (1–711)	A, P, E	*E. coli* (2–360; 56)	δ, δ′, α, γ, ψ	83, 88, 125, 126, 231, 240
HolA	δ subunit, clamp loader complex	331	26 (34–303)	29 (46–308)	83 (1–327)	A, P, E	*E. coli* (34–303; 26)	τ, γ, δ′, β	88, 126, 127, 231
HolB	δ′ subunit, clamp loader complex	331	28 (15–189)	31 (26–170)	72 (11–328)	A, P, E	*E. coli* (15–189; 30)	τ, γ, δ	88, 126, 231
HolC	χ subunit, clamp loader complex	135	23 (1–127)	21 (1–127)	68 (1–135)	D	*E. coli* (1–127; 23)	ψ, SSB	96, 263, 275
HolD	ψ subunit, clamp loader complex	204	7 (1–204)[c]	7 (1–204)[c]	39 (1–204)[c]	A, P	*E. coli* (not detected)	χ, γ/τ	96, 231
HolE	θ subunit, polymerase III core	—	—	—	—	D	*E. coli* (no homolog)	ε	144, 208
SSB	Single-stranded-DNA binding	192	49 (2–192)	64 (2–114)	76 (1–192)	A, P, E	*E. coli* (2–192; 46)	DnaG, χ, RecQ, TopB, UmuC, RecJ, PriA, RecO, exonuclease I, GroEL	9, 30, 86, 100, 135, 148, 162, 229, 243, 263, 275
Tus	Terminator	—	—	—	—	D	*E. coli* (no homolog)	DnaB	182, 183, 189
Hda	Initiation repressor	235	30 (29–229)	29 (29–226)	88 (1–235)	P	*Shewanella amazonensis* (3–157; 28)	β, DnaA	137, 142
PolA	DNA polymerase I	920	49 (3–920)	48 (3–923)	83 (1–920)	A	*T. aquaticus* (2–920; 39), *E. coli* (324–920; 55)	β	17, 154, 159
LigA	DNA ligase	676	50 (10–673)	51 (16–684)	86 (1–674)	A, P, E	*E. coli* (10–673; 50)	β	159, 186

[a] *Acinetobacter baylyi* protein (15).
[b] Determined from BLAST output (6); residue range is for *A. baylyi* protein (15).
[c] Determined from multiple-sequence alignment created with MAFFT (139–141).
[d] Determined from BLAST output (6); residue range is for *A. baumannii* AYE protein (248).
[e] A, essential in *Acinetobacter baylyi* (61); P, essential in *Pseudomonas aeruginosa* (118); E, essential in *Escherichia coli* (11); D, dispensable gene.
[f] —, not present in *Acinetobacter* spp.

the process of helicase loading in organisms outside the *Enterobacteriaceae* and *Firmicutes*.

A Highly Diverged *Pseudomonas*-Type ψ Subunit

Most of the 10 components of the Pol III HE complex are readily identifiable in *Acinetobacter* spp. based on homology searches with the *E. coli* proteins (Table 1). Nevertheless, such searches could not locate a homolog of the *holD* gene, which encodes the ψ subunit of the clamp loader complex in *E. coli* (7). A heterodimer formed between the χ and ψ subunits binds to the clamp loader complex [$(\tau/\gamma)_3\delta\delta'$] of DNA polymerase III through a region at the N terminus of ψ (96, 231). The ψχ complex mediates the handoff of the primed template molecule from the DnaG primase via single-stranded DNA-binding protein (SSB) to the clamp loader complex ($\tau_3\delta\delta'$), thus representing a functional link between the primosome complex (DnaB-DnaG) and Pol III HE within the replisome (275). Originally thought to represent a simple "bridge" between the clamp loader complex and the χ subunit, the ψ subunit has now been shown to play a significant role in loading of the β clamp onto its DNA template in *E. coli* (7).

During their work on the *Pseudomonas* replisome, McHenry's group noted that *Pseudomonas* spp. also lacked an identifiable homolog of the *E. coli holD* gene (120). This group found, however, that addition of purified ψχ complex from *E. coli* dramatically stimulated the activity of a minimal *Pseudomonas* replication complex (αε-τδδ′-β) *in vitro*. As this stimulation was not observed following the addition of *Pseudomonas* χ subunit alone, this strongly suggested the existence of a cryptic ψ subunit in *Pseudomonas*, which the group later identified and characterized (121). The *Pseudomonas*-type ψ subunit was found to be considerably longer than its *E. coli* counterpart (282 versus 137 residues). Despite this, multiple-sequence alignments revealed three distinct conserved regions corresponding to helices α1 and α4 of the *E. coli* ψ and a region at the N terminus known to interact with domain III of τ/γ (7, 96).

We have used hidden Markov model (HMM)-based homology searches of the *A. baylyi* ADP1 genome with the program HHsearch (234, 235) to reveal a poorly conserved homolog of *P. aeruginosa* ψ (hypothetical protein ACIAD1326, 17% sequence identity). Homologs of this putative ψ protein are found within each of the available *A. baumannii* genome sequences (62% sequence identity with *A. baylyi* ψ [Table 1]). From multiple-sequence alignments, it appears that the open reading frame within the *A. baylyi* gene should begin 11 residues earlier than in the published genome annotation. Using these newly identified homologs as input for HHsearch, it was further possible to detect extremely weak, but significant, homology between these proteins and the *E. coli* ψ subunit (for the *A. baylyi* protein, E value = 1.8×10^{-4}; 13% overall sequence identity). These newly identified *Acinetobacter* ψ proteins maintain the three conserved regions (Fig. 2A) that were previously identified within the *E. coli* and *Pseudomonas* ψ subunits (121). When the conserved residues are mapped onto the *E. coli* clamp loader ($\gamma_3\delta\delta'\psi$) and ψχ structures (96, 231), they fall within the N-terminal clamp loader-interacting region and the χ-contacting helices α1 and α4 (Fig. 2B). When these ψ types are mapped to a phylogenetic tree for the gammaproteobacteria (Fig. 1), a decisive split is seen, in which *Pseudomonas*, *Acinetobacter*, and their relatives use the longer *Pseudomonas*-type ψ subunit, while members of the *Enterobacteriaceae*, *Vibrio*, *Haemophilus*, and *Shewanella* have the shorter *E. coli*-type protein. Interestingly, gene knockout studies show that the *holD* gene is dispensable in *E. coli* (11) but is essential in both *A. baylyi* ADP1 (61) and *P. aeruginosa* PA01 (118) (Table 1). This might suggest that the longer, *Pseudomonas*-type ψ subunits have an additional, as-yet-unidentified function *in vivo*.

An Unusual Primosome Complex: Unique Features of *Acinetobacter* DNA Primase

DNA polymerase III cannot synthesize new DNA strands *de novo* and first requires the construction of a short RNA primer by the DnaG primase (226); this occurs every second or two during Okazaki fragment synthesis on the lagging strands (67). During this step, DnaG binds to the replicative helicase DnaB, forming a complex known as the primosome. This interaction formed by DnaB and DnaG has been shown to modulate the activities of both enzymes (39).

DnaB consists of two domains: a primase-binding domain at the N terminus and an ATP-utilizing DNA helicase domain at the C terminus (12). Bacterial primases contain three distinct domains: a zinc-binding domain at the N terminus, a central RNA polymerase (RNAP) domain, and a C-terminal helicase-binding domain that is structurally related to the N-terminal domain of DnaB (51, 78, 191, 237, 241, 258). The structural basis for the primosome interaction was revealed recently in crystal structures of the complex between DnaB and the C-terminal domain of DnaG from *Geobacillus stearothermophilus* (12). DnaB was seen to form a 3-fold symmetric hexamer, mediated by the formation of a ring of N-terminal primase-binding domains. The C-terminal helicase-binding domains of three DnaG molecules were found to associate with this ring, with DnaG residues forming a network of hydrogen bonds, which were later shown to play a role in modulating DnaB activity (12, 39).

In *Acinetobacter* spp., the enzymatic sites of DnaB and DnaG are well conserved. The domains responsible for forming the primosome complex, however, have diverged significantly. In fact, the DnaG helicase-binding domain has diverged so greatly that it bears no resemblance to any other protein in the current sequence databases, including DnaG proteins from members of the closely related genus *Psychrobacter*. Therefore, it is not currently possible to model the *Acinetobacter* primosome complex based on the *G. stearothermophilus* structure. In contrast, the DnaG helicase-binding domains of other Gram-negative organisms, namely, *E. coli* and *P. aeruginosa*, do show detectable sequence homology with the *G. stearothermophilus* domain. As such, primosome complexes could conceivably be modeled for these organisms using the *G. stearothermophilus* structure as a template.

The DnaG proteins of *Acinetobacter* spp. and *Psychrobacter* spp. are further complicated by a large insertion (50 to 100 residues) between the N-terminal zinc-binding domain and the central RNA polymerase domain. Based on secondary structure and disorder predictions (155, 221), this insertion appears to be disordered and thus probably acts as a linker region.

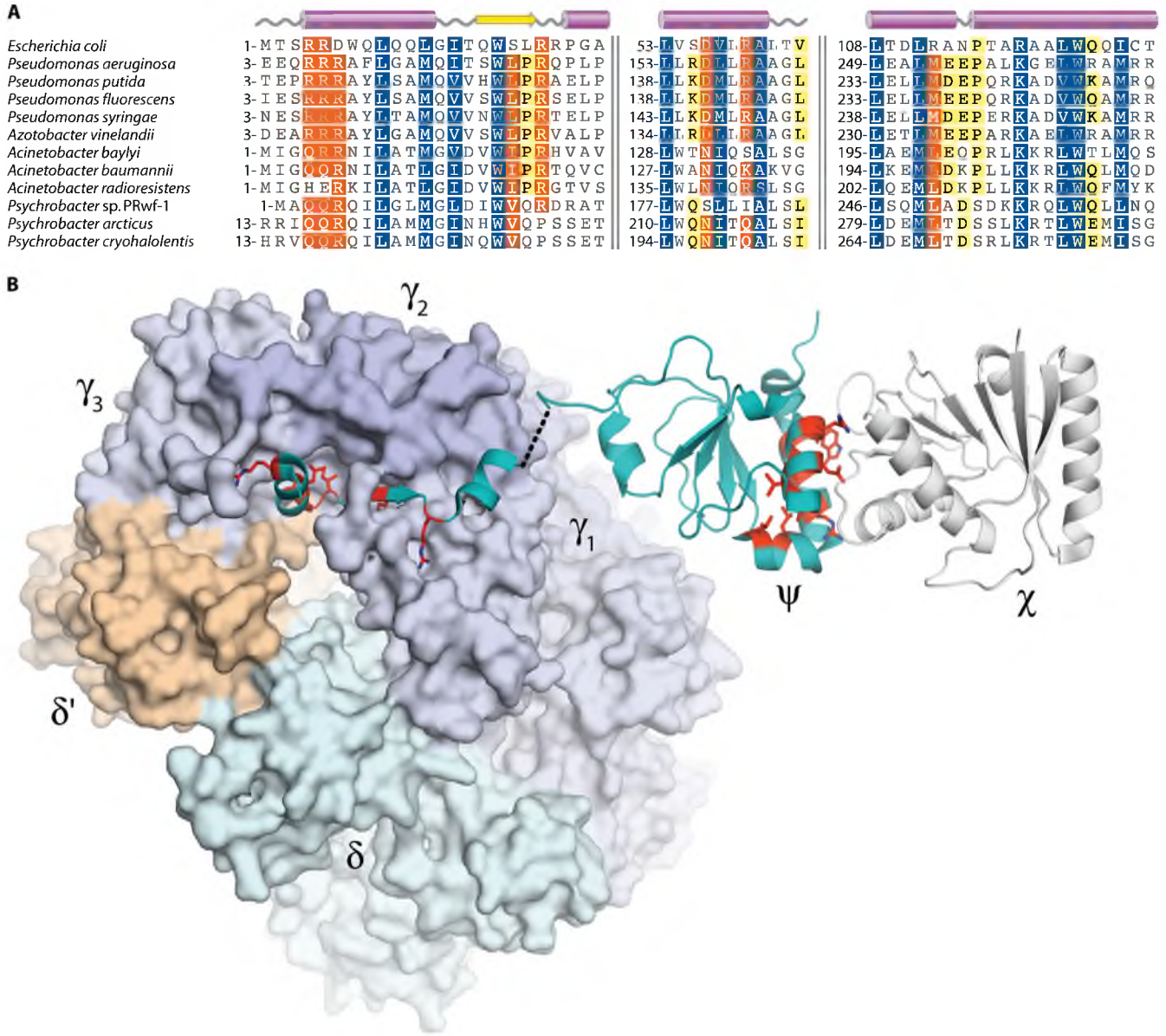

FIG. 2. Sequence conservation within the ψ subunit of DNA polymerase III. (A) Multiple alignment of ψ amino acid sequences. Numbers indicate the sequence position of the first residue in each alignment block; pairs of vertical lines denote discontinuities in the sequences. White characters with blue shading indicate positions with homology across all sequences. White characters with orange shading indicate residues conserved in 80% of sequences, while black characters with yellow shading indicate 60% conservation. The sequence alignment was produced using the MUSCLE algorithm (72) within the Geneious software package (Biomatters). (B) Crystal structures of the *E. coli* $\gamma_3\delta\delta'\psi$ and $\chi\psi$ complexes. Residues conserved in the *A. baylyi* ψ subunit are colored red and have side chains shown. (Produced with PyMOL [64] using data from Protein Data Bank entries 3GLI [231] and 1EM8 [96].)

Increased flexibility between the zinc-binding and RNA polymerase domains may have significant implications for primase activity, as (i) the zinc-binding domain is believed to act as an anchor that recognizes priming sites in DNA and regulates RNA primer length and (ii) primase zinc-binding domains have been demonstrated to function in *trans* with other primase molecules in *E. coli* (50). A thorough biochemical and structural investigation of these unusual DnaG proteins is required to fully elucidate the similarities to and differences from the better-studied *E. coli* enzyme.

Highly Conserved Protein Interaction Modules within the β Sliding Clamp and SSB

In addition to binding the Pol III δ subunit during clamp loading/unloading (127) and the α polymerase subunit (261), greatly increasing its processivity, the *E. coli* β sliding clamp also binds to DNA polymerases I, II, IV, and V; DNA ligase; Hda; and the mismatch repair proteins MutS and MutL (55, 159, 260). Each of these proteins binds to a hydrophobic groove (127) on the β sliding clamp by way of a pentameric or

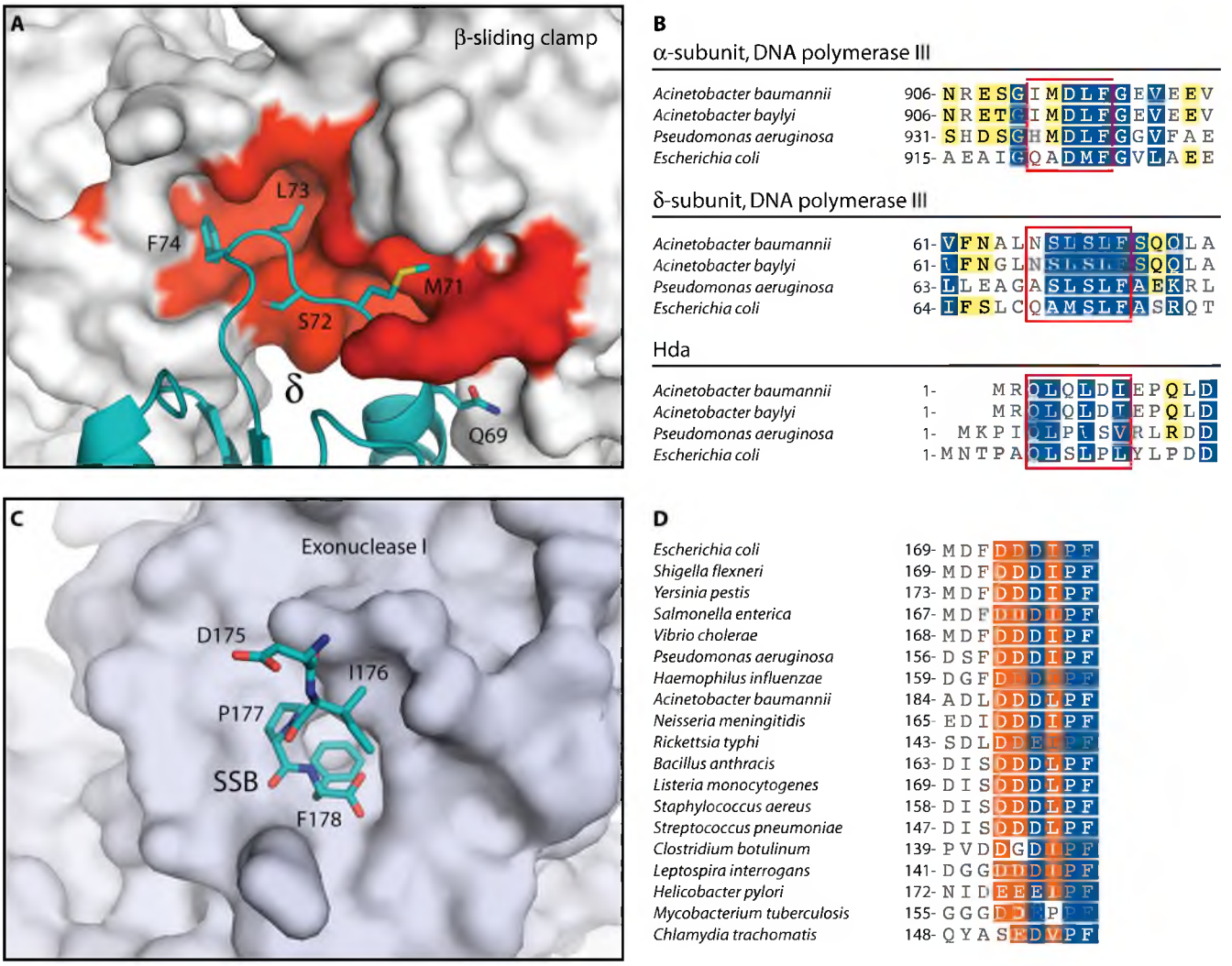

FIG. 3. Highly conserved protein interaction modules in the *Acinetobacter* replisome. (A) Structure of the *E. coli* βδ complex (127). The solvent-accessible surface of β is shown in gray and red. Red indicates residues that are conserved in *A. baylyi*. The δ subunit is colored teal, and side chains are shown for the β-binding consensus residues. (B) Portions of sequence alignments for DNA replication proteins containing β-binding motifs. Boxes indicate β-binding motifs. Residues are highlighted as in Fig. 2. (C) Structure of *E. coli* exonuclease I bound to the C-terminal peptide of SSB (162). The solvent-accessible surface of exonuclease I is shown in gray. The C-terminal peptide of SSB is shown in teal. (D) Portion of a sequence alignment for SSB proteins. In panels B and D, white characters with blue shading indicate positions with homology across all sequences, white characters with orange shading indicate residues conserved in 80% of sequences, and black characters with yellow shading indicate 60% conservation. Sequence alignments were produced using the MUSCLE algorithm (72) within the Geneious software package (Biomatters). (Panels A and C were produced with PyMOL [64] using data from Protein Data Bank entries IJQL [127] and 3C94 [162], respectively.)

hexameric peptide motif, which is often located close to the N or C terminus (55). In *Acinetobacter* spp., the residues comprising the protein-binding groove of the β sliding clamp are highly conserved (Fig. 3A). Variants of the β-binding motifs ($Qx^{D}/_{S}LF$ or QLxLxL) are also conserved within several *Acinetobacter* proteins, including the α subunits of Pol III (IMDLF) and Hda (QLQLDI) (Fig. 3B). In *E. coli*, a β-binding motif within the Pol III δ subunit (QAMSLF) is utilized in the loading of the β sliding clamp by the clamp loader complex (Fig. 3B) (127). Despite sharing only weak overall sequence homology with the *E. coli* δ (26% identity for the *A. baylyi* protein), this motif is also maintained in *Acinetobacter* sp. δ subunits (NSLSLF). This suggests that despite significant sequence divergence in δ and other components of the clamp loader complex in *Acinetobacter* spp. (Table 1), loading of the β sliding clamp onto DNA is likely to utilize a mechanism similar to that of the *E. coli* system.

The hydrophobic groove on the β sliding clamp and its binding motifs in other proteins are strongly conserved across bacterial species (27, 260). An analogous mechanism is also shared by the PCNA sliding clamp of archaea and eukaryotes (209), although the consensus sequence recognized by PCNA (QxxLxxFF) is different from that of the bacterial β-binding motifs (55). The strong conservation of this protein-binding mechanism across bacteria, together with the essentiality of the interactions formed, makes the protein-binding groove of the β sliding clamp (Fig. 3B) an attractive target for the rational design of novel antibiotic compounds. Promisingly,

the O'Donnell group has recently described a small molecule that binds within the β clamp binding groove and is capable of disrupting key protein-protein interactions in diverse bacteria without inhibiting analogous interactions in the eukaryotic PCNA system (87).

A second replication protein, the single-stranded DNA-binding protein (SSB), also interacts with many other proteins by way of a conserved peptide motif (229). In addition to interacting with the DnaG primase and the χ subunit of Pol III HE during DNA replication, SSB also binds to a range of DNA repair enzymes (9, 30, 86, 100, 135, 148, 162, 225, 229, 243, 263, 275). These interactions are mediated by the final six to nine residues at the SSB C terminus, which in *E. coli* has the sequence MDFDDDIPF (229, 275). The crystal structure of the terminal SSB peptide in complex with exonuclease I (Fig. 3C) was recently determined by Keck's group (162). The structure and additional biochemical studies (163) revealed a key role for the final two residues of SSB in binding to a hydrophobic pocket in exonuclease I. While no high-resolution structures have been reported for any other proteins in complex with the SSB peptide (230), it is anticipated that its binding to other proteins occurs in a similar fashion; i.e., the terminal residues of SSB will be inserted into specific pockets on the protein surfaces.

The SSB C-terminal peptide represents a universal system for tying together processes transacted on single-stranded DNA (229). Even in genetically remote organisms, such as *Acinetobacter* spp., the final six residues of SSB are strongly conserved (Fig. 3D). This conservation extends, in fact, to SSB proteins from a wide range of bacterial species, indicating that this system is probably used universally across all of the bacteria (Fig. 3D) (229).

The β clamp and SSB constitute major protein interaction hubs in bacteria. Each is known to interact with at least 10 other proteins, forming interactions with different partners at different times. In doing so, the β clamp and SSB tie together functional processes required for genome maintenance by recruiting other proteins to their sites of action on double- and single-stranded DNA, respectively. Many of these interacting partners are essential across a wide range of bacteria (277). At the heart of these functional linkages are short, highly conserved peptide motifs that could be exploited as the basis for the design of novel antibiotics. At the individual level, inhibitors of β clamp and SSB interactions should be capable of simultaneously disrupting several functions critical to the survival of a bacterial cell. Furthermore, as these modules show strong sequence conservation across diverse bacterial genera, inhibitors should also have broad-spectrum activity. Finally, and perhaps most crucially, the fact that numerous proteins interact at a single site on each of these hubs might help to preclude the opportunity for resistance to such inhibitors. The accumulation of point mutations that decrease the affinity of inhibitory compounds for their target is a common theme in antibiotic resistance (168). Presumably, in the case of protein-protein interaction sites, any mutation occurring on one interacting partner would need to be compensated for with a complementary mutation on the other partner to maintain the integrity of the interaction site. For this to occur in the β-clamp and SSB protein-binding sites, it would necessitate the unlikely acquisition of simultaneous compensatory mutations in the binding sites of every one of the many proteins that bind at these sites. These protein interaction hubs thus represent an unutilized and relatively unexplored resource for the future discovery of novel antibiotic drugs.

CELL DIVISION

Cell division is an appealing target for the design of antibacterial drugs, as it is also essential for the viability of bacterial populations and, as many of the proteins involved are external to the cytoplasm, it is a target relatively accessible to attack with small-molecule inhibitors. In addition, the majority of proteins have little or no homology to human proteins. In contrast to the case for the processes of DNA replication and transcription, the majority of our knowledge on cell division has emerged only over the past 10 to 15 years. Most studies so far have focused on two rod-shaped organisms, *B. subtilis* and *E. coli*, both of which divide precisely at the cell center. Currently, at least 12 proteins are known to be involved in the cell division process, and these are distributed between the cytoplasm, cell membranes, and periplasmic space (76, 89, 103, 156, 259). For the majority of these proteins, their exact biochemical functions remain unknown. Three-dimensional structures of the entire proteins or parts of them have been solved for many, however, and protein-protein interactions are now being determined and characterized (Table 2).

The process of cell division involves the ingrowth of the cell envelope layers to form a septum at the cell center between two newly replicated chromosomes. This septum then splits to form two new daughter cells. The first step in cell division is the assembly of the tubulin-like protein FtsZ into a structure known as the Z ring, precisely at midcell (103). This assembly involves ZipA in *E. coli* (98) and the membrane-attaching protein FtsA in both *E. coli* and *B. subtilis* (54, 103, 255). The Z ring marks the future site of cell division and acts as a "landing pad" for the other proteins involved in the division process. Once in place, the remaining cell division proteins are recruited to the Z ring, forming a complex collectively known as the divisome (103). In *E. coli* these later-assembling proteins include FtsE, -X, -K, -Q, -L, -B, -W, -I, and -N; AmiC; and EnvC (255, 259). Homologs of all of these proteins except FtsN are found in *B. subtilis*.

Homologs of most of these proteins can also be found within the genomes of *Acinetobacter* spp., although in most cases their sequences have diverged substantially from those of their *E. coli* counterparts (Table 2). Interestingly however, no homologs could be found for FtsE, FtsX, or SulA, which are conserved in almost all other gammaproteobacteria. FtsE and -X together form a membrane-associated ABC transporter of unknown cellular function that is essential for the survival of *E. coli* only under low-ionic-strength conditions (218). In *B. subtilis* this protein complex has recently been linked to the initiation of sporulation (85). SulA is an inhibitor of FtsZ polymerization that is induced as part of the SOS response in *E. coli*, where it serves to temporarily halt cell division in the event of DNA damage (49, 112). As discussed in a later section, the lack of SulA in *Acinetobacter* spp. has strong implications for the mechanisms underlying global stress responses in these organisms.

TABLE 2. Conservation of cell division proteins in *Acinetobacter* spp.

Protein	Function	Protein location[a]	Protein length (amino acids)[b]	% Sequence identity (residue range)			Essentiality[e]	Homologs with known structures[b] (residue range of sequence match; % sequence identity)	Known protein-protein interactions in *E. coli*	Reference(s)
				A. baylyi vs *E. coli*[c]	*A. baumannii* vs *E. coli*[d]	*A. baylyi* vs *A. baumannii*[c]				
FtsA	Tethers FtsZ to membrane, stabilizes Z rings at membrane	c	420	29 (8–409)	30 (8–409)	88 (1–420)	A, E, P	*Thermotoga maritima* (10–351; 23)	FtsZ	101, 207, 249, 257
FtsB (DivIC)	Unknown; role in septal peptidoglycan synthesis?	m	110	41 (6–77)	38 (25–96)	91 (6–110)	A, E, P	—[f]	FtsQ, FtsL	28, 92
FtsE	Unknown; similar to ABC transporter	c	—	—	—	—	E	—	FtsZ, FtsX	46, 218, 227
FtsI/PBP3 (PBPB)	Cross-links septal peptidoglycan	m	621	40 (15–607)	39 (7–598)	89 (10–617)	A, E, P	—	FtsQ, FtsN	66, 69
FtsK	Role in chromosome segregation and septum formation	m	1018	60 (528–1017)	59 (516–1009)	77 (1–1018)	A, E, P	*Pseudomonas aeruginosa* (527–942; 59), *E. coli* (528–1017; 59)	FtsZ, FtsL, FtsQ	66, 169
FtsL	Unknown; role in septal peptidoglycan synthesis?	m	110	24 (26–100)	26 (28–100)	88 (29–109)	A, E, P	—	FtsQ, FtsB, FtsW, FtsK	28, 66, 69
FtsN	Unknown	m	200	23 (44–197)	16 (3–206)	87 (1–200)	A, E, P	*E. coli* (120–200; 19)	FtsQ, FtsI, FtsW	52, 53, 66, 69, 268
FtsQ (DivIB)	Unknown; role in septal peptidoglycan synthesis?	m	284	25 (61–261)	26 (90–259)	81 (1–284)	A, E, P	*Yersinia enterocolitica* (59–266; 24), *E. coli* (61–267; 23)	FtsB, FtsL, FtsI, FtsN, FtsW, FtsK	28, 34, 69, 220, 250
FtsW	Septal peptidoglycan synthesis	m	399	42 (32–388)	42 (31–387)	85 (2–399)	A, E, P	—	FtsI, FtsL, FtsN, FtsQ	66, 69
FtsX	Unknown; similar to ABC transporter	m	—	—	—	—	E	—	FtsE	218, 227
FtsZ	First protein to form midcell ring in cell division	c	389	47 (31–386)	48 (31–388)	90 (1–389)	A, E, P	*P. aeruginosa* (31–322; 55), *B. subtilis* (35–389; 46), *Mycobacterium tuberculosis* (31–322; 53), *Methanococcus jannaschii* (5–322; 42), *A. aeolicus* (31–320; 43)	FtsA, ZapA, ZapB, ZipA, FtsE, FtsK, MinC, SulA, ClpX, SlmA	22, 46, 66, 71, 79, 95, 98, 101, 108, 110, 151, 160, 161, 165, 179, 193, 194, 207, 228, 257
ZapA	Promotes FtsZ polymerization and protofilament bundling	c	94	21 (7–94)	26 (1–88)	80 (1–92)	D	*Pseudomonas aeruginosa* (1–93; 16)	FtsZ	95, 160
ZapB	Promotes FtsZ polymerization	c	—	—	—	—	D	—	FtsZ	71
ZipA	Stabilizes Z rings at membrane	m	325	28 (185–310)	27 (205–330)	62 (1–324)	E	*E. coli* (183–323; 26)	FtsZ	98, 101, 179
MinC	cell division inhibitor	c	240	40 (111–230)	41 (122–229)	78 (1–240)	D	*T. maritima* (111–213; 28)	MinD, FtsZ	47, 108–110, 193, 228
MinD	Membrane ATPase of the MinC-MinD-MinE system	m	278	64 (9–278)	65 (9–278)	95 (1–278)	E	*Archaeglobus fulgidis* (9–277; 30), *Pyrococcus furiosus* (9–256; 30)	MinC, MinE	48, 104, 108–110, 224
MinE	Cell division topological specificity factor	c	90	33 (9–88)	32 (9–88)	92 (1–90)	E	*E. coli* (36–90; 28)	MinD	62, 110, 147, 214, 278
SlmA	Spatial regulation of Z-ring formation (nucleoid occlusion)	c	—	—	—	—	D	—	FtsZ	22
SulA	FtsZ assembly inhibitor	c	—	—	—	—	D	*P. aeruginosa* (no homolog)	FtsZ	49, 110
ClpX	FtsZ assembly inhibitor	m	436	70 (10–408)	71 (10–408)	91 (1–436)	D	*Helicobacter pylori* (54–417; 58)	FtsZ	32, 79, 146
AmiC	Amidase; required for septal wall degradation and cell separation	p	—	—	—	—	D	—	—	20
EnvC	Murein hydrolase; required for septal wall degradation and cell separation	p	—	—	—	—	D	—	—	21

[a] c, cytoplasmic; p, periplasmic; m, membrane associated.
[b] *Acinetobacter baylyi* protein (15).
[c] Determined from BLAST output (6); residue range is for *A. baylyi* protein (15).
[d] Determined from BLAST output (6); residue range is for *A. baumannii* AYE protein (248).
[e] A, essential in *Acinetobacter baylyi* (61); P, essential in *Pseudomonas aeruginosa* (118); E, essential in *Escherichia coli* (11); D, dispensable gene.
[f] —, not applicable, no structure determined, or not present in *Acinetobacter* spp.

Assembly of FtsZ at Midcell

FtsZ is the most conserved bacterial cell division protein and is an essential player in the cell division process (103). Homologs of FtsZ have been identified in almost all prokaryotes as well as in plant chloroplasts and the mitochondria of lower eukaryotes (165, 253). In *E. coli*, the *ftsZ* gene resides in the *dcw* cluster, from which *ftsQ*, *-A*, and *-Z* are cotranscribed (63, 84, 271). Analysis of the available *Acinetobacter* sp. genomes suggests the same to be true for this genus, with *ftsQ*, *-A*, and *-Z* being adjacent to each other in the chromosome. The sequences of FtsZ from *Acinetobacter* spp. are relatively well conserved (Table 2), particularly within the core domain, which contains the GTP-binding motif and is important for the polymerization of FtsZ into the Z ring. Amino acid residues involved in GTP hydrolysis and formation of the active site (190, 257) are also conserved.

Assembly of FtsZ into the Z ring at precisely midcell requires a balance among several proteins that interact directly with FtsZ to regulate its assembly (3, 54, 156). These proteins either promote or inhibit FtsZ polymerization and are thought to act together to support Z-ring assembly at the right place and time. In *E. coli*, FtsZ assembly is promoted by FtsA, ZipA, and ZapA and -B and is inhibited by MinC, ClpX, SulA, and SlmA (3, 54, 156).

At the C terminus of FtsZ is a nine-amino-acid consensus sequence, often referred to as the C-terminal core domain, which has been shown to be involved in interactions with both ZipA and FtsA (101, 165, 179, 267). In *E. coli* and most other gammaproteobacteria, the sequence of this region is DIPAFLRKQ. The Somers group has determined the crystal structure of the C-terminal core domain in complex with ZipA (Fig. 4A). The nonapeptide protein-binding motif of FtsZ was shown to form an amphipathic helical structure in which residues I374, F377, and L378 form the primary contacts with ZipA. Earlier studies using alanine-scanning mutagenesis showed that these same three residues provide nearly all of the binding strength in the interaction with ZipA in *E. coli* (179). Residues D373 and P375 of *E. coli* FtsZ are also important in the interaction with ZipA, however, presumably setting the C-terminal region in the correct conformation for binding (101).

By projecting the nonapeptide motifs from the FtsZ proteins of diverse bacteria onto a helical wheel, it can be seen that most maintain an amphipathic helix structure with hydrophobic residues at positions 2, 5, and 6; Asp at position 1; and Pro at position 3 (Fig. 4B). The fact that this motif is conserved in Gram-positive organisms (which lack ZipA) supports the idea that the interaction of FtsZ with other proteins, most notably FtsA, also involves the C-terminal amphipathic helix. Interestingly, while hydrophobic residues are maintained at positions 2, 5, and 6 of the FtsZ C-terminal peptides in *Acinetobacter* spp. (SIQDYLKNQ), the conserved Asp and Pro residues found in other organisms at positions 1 and 3 have been replaced with Ser and Gln. It has previously been shown that site-specific mutation of these positions (D373 and P375) in *E. coli* FtsZ results in disrupted binding to ZipA (101). Importantly, P375 mutants also show reduced binding to FtsA (165). The unusual replacement of this residue with Gln in *Acinetobacter* spp. might suggest a somewhat different binding mode for FtsZ to both ZipA and FtsA in these organisms.

Complexation of FtsZ with FtsA, ZipA, ZapA, and ZapB represents the starting point of the cell division pathway in *E. coli*. As such, these interactions mediated by the FtsZ C-terminal helix have been viewed as attractive targets for the design of novel antibiotics. Wyeth, in particular, has invested considerable effort in the development of antagonists of the FtsZ-ZipA interaction and has discovered several compounds that bind to ZipA at the FtsZ interaction site (123, 124, 179, 223, 244, 247). Importantly however, none of these have led to a useful antagonist of the FtsZ-ZipA interaction, despite the opportunities for structure-guided design afforded by crystal structures of ZipA in complex with these ligands (123, 124, 223, 244, 247). The difficulty in expanding these lead compounds lies with the fact that FtsZ binds into a shallow, and relatively featureless, hydrophobic groove on the ZipA surface (179). The broad interacting surface provides few opportunities to incorporate polar contacts between ZipA and the designed ligands. The few attempts to do so have yielded little or no increase in binding strength (247). Wyeth has since abandoned its efforts to develop an FtsZ-ZipA antagonist.

Wyeth's inability to successfully extend apparently promising lead compounds into useful inhibitors of this interaction highlights the difficulty associated with targeting protein-protein interactions using structure-aided design. The broad and relatively shallow interaction surface of ZipA ultimately decreases the utility of this interaction as a target. Furthermore, analysis of the C-terminal helices of FtsZ proteins from diverse bacteria suggests only a vague requirement for hydrophobic residues on the interacting face of the helix (Fig. 4B). This implies that there is significant variation in the shape of FtsZ-binding sites in proteins from different bacteria, further complicating drug design efforts. Importantly, as the binding site for FtsA within FtsZ overlaps significantly with the binding site for ZipA, this might imply that the FtsZ-FtsA interaction, also viewed as a potential drug target, could be equally difficult to target.

Recruitment of Membrane-Associated Proteins

Following formation of the Z ring, the later-assembling membrane-associated cell division proteins are recruited to the midcell site and drive the process of septum formation (89, 254). These proteins represent appealing targets for the development of novel antibacterials, as regions essential for their function are largely located outside the (inner) cell membrane, making them more accessible to inhibitors. Their potential as antibacterial targets is also increased because most of these proteins are essential and the majority lack homologs in eukaryotes (156). The core set of essential membrane-associated cell division proteins have now been identified (103). However, few biochemical data are yet available for these proteins, and many are still to be structurally defined owing to the inherent difficulties associated with *in vitro* analyses of membrane proteins. All of the core membrane cell division proteins are identifiable in *Acinetobacter* spp. on the basis of HMM-based homology searches with *E. coli* proteins (Table 2).

Of the membrane-associated cell division proteins in *E. coli*, FtsQ interacts with the greatest number of other proteins, with two-hybrid studies and coimmunoprecipitation experiments revealing interactions with FtsA, -K, -X, -I, -W, -B, -L, and -N (28, 66, 69, 136). Separate regions within the periplasmic por-

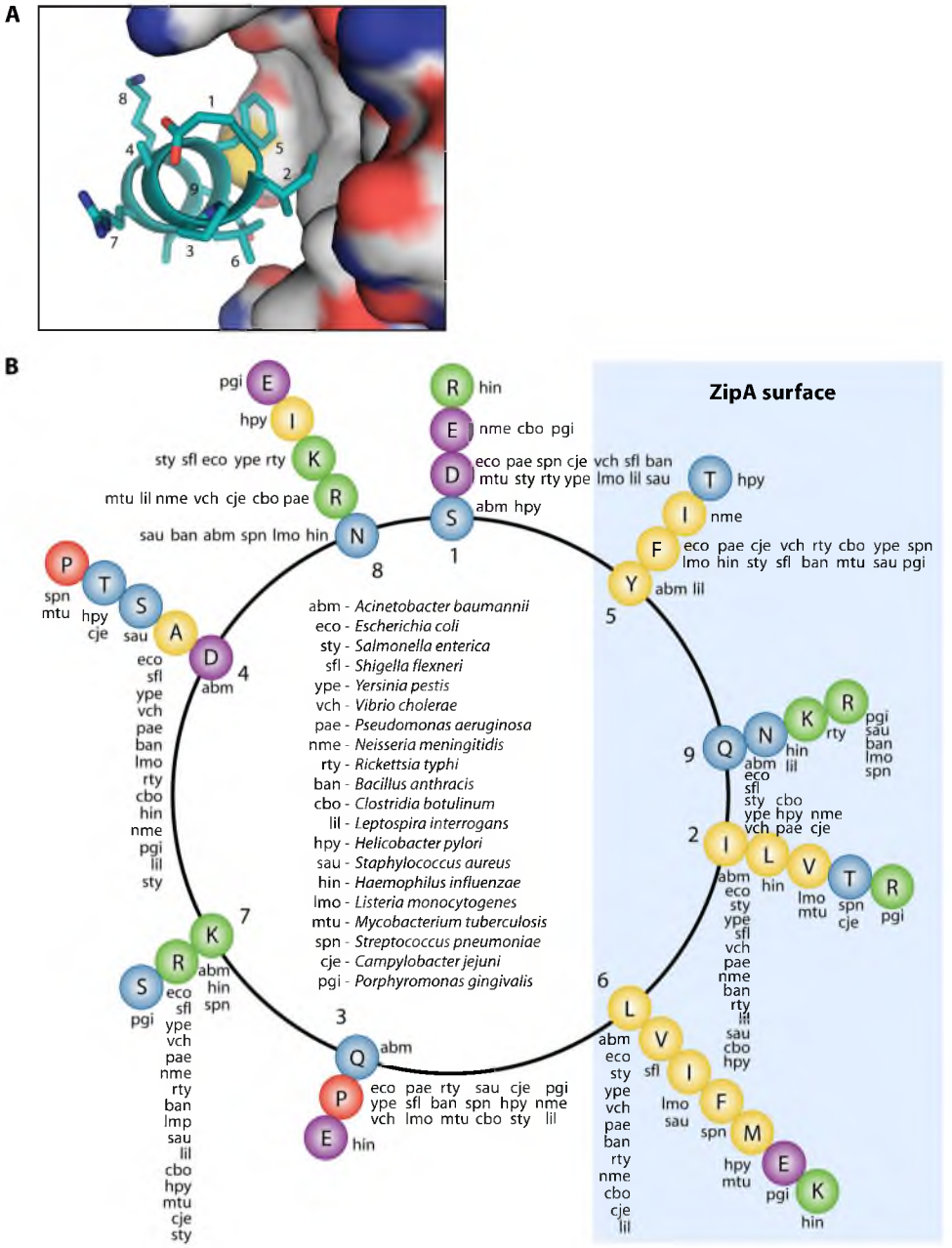

FIG. 4. The FtsZ-ZipA interaction. (A) Diagram of the FtsZ C-terminal peptide in contact with the ZipA surface (179). The solvent-accessible surface of ZipA is colored according to atom type: C, white; N, blue; O, red; S, yellow. Numbering indicates positions within the conserved nine-residue motif. (Produced with PyMOL [64] using data from Protein Data Bank entry 1F47 [179].) (B) Helical projection of the FtsZ C-terminal peptide. Letters at each position indicate the residue occupying that position in different bacterial species.

tion of FtsQ are required for localization of the protein to the division site and recruitment of downstream proteins (37, 250). The periplasmic region forms a modular two-domain structure that crystallizes as a dimer yet is monomeric in solution (250). Localization to the Z ring requires interaction with upstream proteins such as FtsK and is mediated largely by the α domain (residues 58 to 125) (250). *Acinetobacter* FtsQ proteins are only moderately conserved, sharing 26% overall sequence identity with *E. coli* FtsQ (Table 2). Residues previously shown to be required for localization of *E. coli* FtsQ (V92, Q108, V111, and K113) (37, 90, 250) map to the outermost tip of the α domain and are conserved within *Acinetobacter* FtsQ proteins (equivalent positions are L95, R110, V113, and R115).

Immunoprecipitation experiments have demonstrated that in *E. coli* the three proteins FtsL, -B, and -Q assemble away from the midcell site and arrive as a complex (28). Recruitment of these proteins by FtsQ involves the β domain (residues 128 to 260), also within the periplasmic portion (250). Residues required for these interactions map to the final two strands of a central β sheet, at the interface of the crystallographic dimer.

The corresponding positions in *Acinetobacter* FtsQ proteins are relatively well conserved, suggesting the recruitment of FtsB and -L to share a mechanism with *E. coli*. Further studies of the structural basis of FtsQ interactions are required to identify potential targets for the rational design of novel antibacterials. In the future, it might then be possible to design compounds that disrupt the localization of FtsQ to the Z ring or its recruitment of downstream proteins such as FtsB and -L.

Peptidoglycan Recycling and Response to β-Lactam Antibiotics

Recent studies have revealed that *A. baylyi* strains carrying mutations in the peptidoglycan-recycling enzyme UDP-*N*-acetylmuramate:L-alanyl-γ-D-glutamyl-*meso*-diaminopimelate ligase (murein peptide ligase; Mpl) are hypersensitive to β-lactam antibiotics (91). In contrast, *mpl*-deficient strains of *E. coli* are no more sensitive to β-lactam compounds than wild-type strains (91). Deletion of the entire *mpl* gene results in nonviability in *A. baylyi* ADP1 under the growth conditions used in the high-throughput gene knockout study (61). The equivalent gene is dispensable for growth under similar conditions in *E. coli* (11, 170). Interestingly, an *A. baylyi* strain carrying an *mpl* mutation is also sensitive to UV-induced DNA damage (36). Aside from transport proteins, no proteins involved in cell envelope synthesis have been linked with the DNA damage response in *E. coli* (36).

Both UV damage and β-lactam compounds trigger the LexA-mediated SOS response in *E. coli* (80, 173). This leads to upregulation of the cell division inhibitor SulA, leading to temporary arrest of cell division, allowing time for DNA repair processes to occur (143). As discussed below, *Acinetobacter* spp. lack not only SulA but also the SOS regulator protein LexA. The sensitivity of *mpl*-deficient *A. baylyi* to both β-lactams and UV irradiation might therefore reflect an inability to arrest cell division in response to DNA damage in *Acinetobacter* spp. It has previously been shown that *Acinetobacter* spp. undergo reductive division following nutrient starvation; i.e., a large bacilliform cell divides to yield smaller coccoid cells (119). It is tempting to speculate that reductive division represents a general stress response in *Acinetobacter* and that the observed β-lactam/UV sensitivity of *mpl*-deficient strains might reflect a defect in remodeling peptidoglycan in the transition to a coccoid morphology. This is supported by the identification of mutations in other peptidoglycan-recycling enzymes that lead to β-lactam sensitivity in *Acinetobacter* spp. (91), although one cannot rule out more direct inhibition of each of these enzymes by β-lactam compounds.

It has been suggested that if suitable inhibitors of Mpl can be found, this might lead to novel antibiotics that are selective for *Acinetobacter* spp. and act to increase the efficacy of β-lactam compounds (91). The Joint Center for Structural Genomics has recently determined the crystal structure of *Psychrobacter arcticum* Mpl (Protein Data Bank accession no. 3HN7), highlighting the previously noted relationship with MurC enzymes (177). *P. arcticum* Mpl shows relatively high sequence conservation with *Acinetobacter* Mpl proteins (56% sequence identity). It might now be possible to rationally design inhibitors of *Acinetobacter* Mpl proteins based on the *P. arcticum* structure.

RNA TRANSCRIPTION

Bacterial transcription and transcriptional regulation have been the subjects of intense analysis since the concurrent isolation of DNA-dependent RNA polymerase (RNAP) from *E. coli* by four separate laboratories during the early 1960s (114). The process can be divided into three distinct phases: initiation, elongation, and termination (25). First, RNAP in complex with a promoter specificity component, or σ factor, recognizes and unwinds the template DNA at a specific promoter sequence upstream of the gene to be transcribed (25). RNAP then begins synthesizing mRNA, at first producing a series of short abortive transcripts before clearing the promoter and entering a highly processive elongation stage. Transcription is completed either by intrinsic termination at a specific sequence in the template DNA or by Rho factor-dependent termination, which occurs at poorly conserved Rho utilization sites (43). Each stage of transcription is highly regulated, either by RNAP-associated transcription factor proteins or by specific RNA or DNA sequences that interact with the RNAP transcription complex (74). By specifically regulating the transcription of individual genes (or groups of genes), the cellular levels of each gene product can be regulated and adjusted in response to a variety of cellular and environmental stimuli.

The *Acinetobacter* sp. genomes sequenced to date originate from strains that occupy three distinct environmental niches: a free-living soil organism, a human body louse symbiont, and a series of multidrug-resistant and drug-susceptible clinical isolates (4, 15, 115, 233, 248). Thus, as well as being able to assess the overall conservation of transcription components in *Acinetobacter* spp. (Table 3), we are now in a position to identify accessory factors likely to be involved with gene regulation during pathogenesis and antibiotic-induced stress.

Architecture of Transcription Complexes and Suitability for Drug Design

The RNAP core contains five protein subunits, assembled in the order $\alpha \rightarrow \alpha_2 \rightarrow \alpha_2\beta \rightarrow \alpha_2\beta\beta' \rightarrow \alpha_2\beta\beta'\omega$ (24). To initiate transcription (152), the ~400-kDa core must first associate with a σ factor to form the RNAP holoenzyme ($\alpha_2\beta\beta'\omega\sigma$). The architecture of the RNAP complex has been elucidated through extensive biochemical and biophysical analyses in *E. coli* (reviewed in reference 24) and crystal structures of the *Thermus aquaticus* RNAP core (276) and *Thermus thermophilus* holoenzyme (251). The overall topology of RNAP resembles a "crab claw," in which the large β and β′ subunits comprise the "pincers" of the claw (276) (Fig. 5A). In addition to providing a structural brace for RNAP, the β and β′ subunits interact to create a cleft that opens into the main channel of the enzyme. Deep inside the base of this cleft resides the catalytically active center, which is marked by a stably bound Mg^{2+} ion.

Genes encoding RNAP subunits are readily identifiable in *Acinetobacter* spp. and maintain a relatively high level of sequence conservation (54 to 71% identity at the amino acid level) with equivalent *E. coli* subunits (Table 3). Within the identified α, β, β′, ω, and σ subunits, key functional regions (e.g., sequence motifs A to H of the β′ subunit [132]) are highly conserved. The relatively high conservation of RNAP subunits

TABLE 3. Conservation of RNA transcription proteins in *Acinetobacter* spp.

Protein	Function	Protein length (amino acids)[a]	% Sequence identity (residue range)			Essentiality[d]	Homologs with known structures[b] (residue range of sequence match; % sequence identity)	Known protein-protein interactions in *E. coli*	Reference(s)
			A. baylyi vs *E. coli*[b]	*A. baumannii* vs *E. coli*[c]	*A. baylyi* vs *A. baumannii*[b]				
RpoA	RNAP, α subunit	335	66 (1–324)	66 (1–324)	99 (1–335)	A, P, E	*E. coli* (1–235; 60), *Thermus aquaticus* (24–312; 39)	RpoB, RpoC, RpoZ	33, 276
RpoB	RNAP, β subunit	1,362	70 (1–1360)	70 (1–1360)	92 (1–1362)	A, P, E	*Thermus thermophilus* (13–219; 44/357–938; 52/1055–1357; 53)	RpoA, RpoC, RpoD, RpoH, RpoN, RpoZ, NusA, Mfd	60, 198, 269, 276
RpoC	RNAP, β′ subunit	1,413	71 (17–1386)	70 (1–1370)	97 (1–1380)	A, P, E	*T. aquaticus* (37–1044; 50/1145–1374; 47)	RpoA, RpoB, RpoD, RpoH, RpoN, RpoZ, NusG	174, 178, 276
RpoD	RNAP sigma 70 factor	629	60 (5–628)	59 (5–289)	95 (1–628)	A, P, E	*T. thermophilus* (97–182; 34/359–627; 61)	RpoB, RpoC	272, 276
RpoE	RNA polymerase sigma 70 factor, ECF subfamily	206	26 (31–197)	25 (55–195)	100 (1–206)	E	—[e]	RpoB, RpoC	94
RpoH	RNAP sigma factor 32	289	58 (5–288)	59 (5–289)	95 (1–288)	A, P, E	—	RpoB, RpoC	94
RpoN	RNAP sigma factor 54	482	46 (1–482)	47 (1–482)	81 (1–482)	D	*A. aeolicus* (429–480; 61)	RpoB, RpoC	68, 94
RpoS	RNAP sigma factor 38	—	—	—	—	D	—	RpoB, RpoC	94
RpoZ	RNAP, ω subunit	93	54 (1–64)	54 (1–64)	93 (1–93)	D	*T. thermophilus* (1–67; 30)	RpoA, RpoB, RpoC	276
GreA	Transcription elongation factor, cleaves 3′ nucleotide of paused mRNA	158	68 (1–155)	70 (1–146)	93 (1–146)	D	*E. coli* (1–158; 68)	RpoC	152, 238
GreB	Transcription elongation factor and transcript cleavage	157	61 (1–155)	67 (1–159)	58 (1–158)	D	*E. coli* (1–155; 61)	RpoC	152, 252
DksA	DnaK suppressor protein	179	62 (41–179)	63 (42–186)	87 (7–186)	D	*E. coli* (41–179; 62)	RpoC	201, 205
NusA	Transcription elongation factor NusA (N utilization substance protein A)	494	52 (1–493)	53 (1–493)	94 (1–494)	A, P, E	*T. maritima* (1–344; 40)	RpoB, phage λ N protein	212, 264, 269
NusB	Transcription termination, L factor (N utilization substance protein B)	149	44 (19–145)	44 (19–145)	91 (1–148)	P	*E. coli* (19–145; 44)	NusE	5, 152
NusE	30S ribosomal protein S10	108	83 (6–108)	83 (1–108)	99 (1–108)	A, P, E	*E. coli* (6–108; 83)	NusB	164
NusG	Transcription antitermination protein	177	61 (2–176)	59 (2–176)	96 (1–177)	A, P, E	*E. coli* (2–176; 61)	RpoC, Rho	153, 178, 232, 239
Mfd	Transcription–repair coupling protein	1,171	50 (30–1165)	50 (14–1147)	86 (1–1153)	D	*E. coli* (30–1163; 50)	RpoB	60, 198
Rho	Transcription termination factor Rho	422	77 (1–418)	77 (1–418)	98 (1–421)	E, P	*E. coli* (1–418; 77)	NusG	153, 232, 246

[a] *Acinetobacter baylyi* protein (15).
[b] Determined from BLAST output (6); residue range is for *A. baylyi* protein (15).
[c] Determined from BLAST output (6); residue range is for *A. baumannii* AYE protein (248).
[d] A, essential in *Acinetobacter baylyi* (61); P, essential in *Pseudomonas aeruginosa* (118); E, essential in *Escherichia coli* (11); D, dispensable gene.
[e] —, not present in *Acinetobacter* spp.

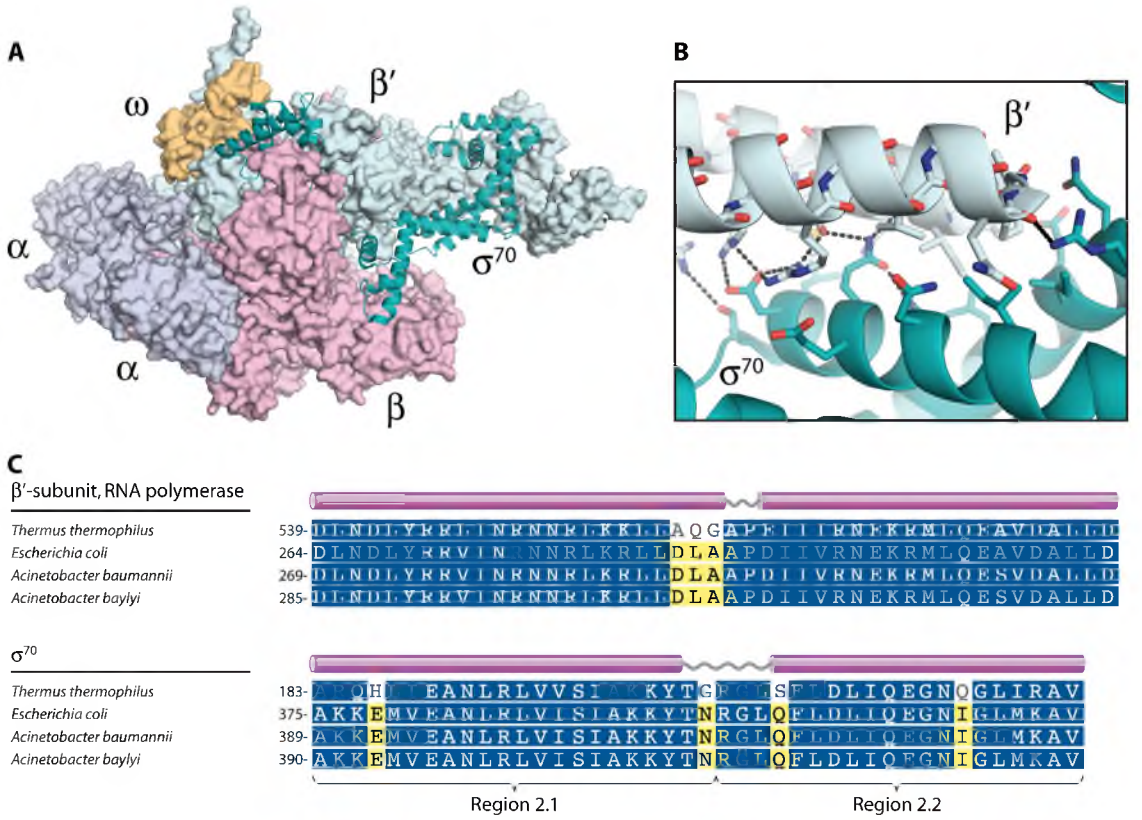

FIG. 5. Architecture of RNA polymerase and its interaction with σ^{70}. (A) Structure of the *Thermus thermophilus* RNAP holoenzyme (251). The solvent-accessible surface is shown for RNAP core subunits ($\alpha_2\beta\beta'\omega$), and σ^{70} is shown in ribbon representation. (B) Primary interaction surface between the coiled-coil region within the β′ subunit and conserved regions 2.1 and 2.2 of σ^{70}. Dashed lines indicate hydrogen bonds. (C) Sequence alignments for the coiled-coil region of the β′ subunit and conserved regions 2.1 and 2.2 of σ^{70}. White characters with blue shading indicate positions with homology across all sequences, while black characters with yellow shading indicate 60% conservation. Sequence alignments were produced using the MUSCLE algorithm (72) within the Geneious software package (Biomatters). (Panels A and B were produced with PyMOL [64] using data from Protein Data Bank entry 1IW7 [251].)

despite the relative genetic remoteness of the *Acinetobacter* genus within the gammaproteobacteria (Fig. 1) highlights the potential utility of RNAP as a target for broad-spectrum antibacterial compounds.

RNAP has been identified as the target for several classes of transcription inhibitors, including the clinically important rifamycin family (256). High-resolution crystal structures of RNAP in complex with members of the rifamycin, streptolydigin, sorangicin, and lipiarmycin inhibitor families have shed light on their modes of action (256). Each of these compounds was found to bind at distinct locations within the DNA-binding and secondary channels of RNAP, away from the active site. This suggests that they bind and interfere with regulatory sites within the enzyme, acting either to sterically block nucleoside triphosphates (NTPs) from entering the active site or by allosteric effects. Most compounds that inhibit RNAP activity exhibit a broad-spectrum bactericidal effect. It has been suggested that RNAP is an underutilized resource and remains a promising target for the discovery of new antibiotics (for reviews, see references 42 and 256).

While several antimicrobial compounds have been found to target RNAP directly, very few target the highly conserved protein-protein interactions that govern the formation of higher transcription complexes. *In vivo*, the RNAP core forms a series of transient complexes with other protein cofactors: σ^{70} and other σ factors, antitermination factors (NusA, -B, -E, and -G), transcript cleavage factors (GreA and -B), and regulatory factors (e.g., DksA) (152). Ultimately, these interactions underpin the regulation of gene expression and the transcription of rRNA and thus play important roles in cell proliferation.

The primary interaction surface between σ^{70} and the RNAP core has been mapped to the coiled-coil motif of the β′ subunit and the conserved region 2.2 of σ^{70} (272). The structural basis for the RNAP-σ^{70} interaction was demonstrated by the crystal structure of the *T. thermophilus* holoenzyme (251). The interaction surface (130) is relatively polar and is formed primarily through a network of side chain-side chain and side chain-main chain hydrogen bonds (Fig. 5B). If novel antagonists of this site could be found, they should interfere with assembly of the functional RNAP holoenzyme and thus inhibit transcription *in vivo*. The β′ and σ^{70} residues that mediate this interaction in *E. coli* are identical in the *Acinetobacter* sp. proteins (Fig. 5C) and are strongly conserved among all other bacteria (130).

Using an enzyme-linked immunosorbent assay (ELISA)-based approach, the Leonetti group has screened a library of small molecules for inhibitors of the assembly of σ^{70} onto the RNAP core (8). Several inhibitors of holoenzyme assembly

were identified, with some demonstrating bactericidal activity, particularly against Gram-positive organisms. Interestingly, while *E. coli* proteins were used for the initial screen and were inhibited *in vitro*, no antimicrobial activity was observed for *E. coli* or its fellow Gram-negative bacterium *P. aeruginosa*. Activity was seen, however, against an *E. coli* mutant strain with increased outer membrane permeability, suggesting that the lack of activity against the wild-type bacterium stems from limited cellular uptake of the compounds. Nonetheless, this work demonstrates the potential for protein-protein interactions within transcription complexes as targets for antimicrobial compounds.

High-throughput screening approaches could be similarly used to identify antagonists of other RNAP-interacting proteins such as NusA and -G, both of which are essential in *E. coli*, *P. aeruginosa*, and *A. baylyi* (Table 3). Traditionally these interactions would not be considered good targets, as the interaction surfaces lack clear grooves, within which an inhibitor might form more extensive protein contacts. However, recent developments in anticancer therapies with stapled peptide antagonists of NOTCH complex formation indicate that (i) interaction surfaces lacking deep clefts or grooves can be targeted and (ii) prevention of the formation of active transcription complexes represents a potentially valuable therapeutic approach (176).

Transcription complexes involved in rRNA synthesis, often called antitermination complexes, require recruitment of many protein transcription factors, such as NusA, -B, -E, and -G, and also require nucleic acid elements of the RNA leader of rRNA genes called *boxB*, *-A*, and *-C* (202). An antitermination complex cannot correctly form without the full complement of protein and RNA elements. The *boxA* sequences are highly conserved across the eubacteria (consensus, 5′-GCUCUUU[A/G][A/G]A; *Acinetobacter* consensus, 5′-GAUCAUUAAG) and are integral to the formation of the NusB-NusE heterodimer that binds RNA polymerase (93). More recently it has been shown that NusA binds *boxC* (consensus, 5′-UGUGU[U/G][U/G]; *Acinetobacter* consensus, 5′-UGUGUGG), which is involved in antitermination complex formation (10, 23). Targeting these protein-nucleic acid interactions may also be a valid approach for the development of new antibiotics, as preventing antitermination complex formation would severely disrupt cell proliferation. Indeed rRNA gene dosage is closely related to the rate of cell proliferation, with fast-growing species such as *B. subtilis*, *E. coli*, and *Vibrio natriegens* containing 7 to 13 rRNA operons per chromosome, while the slow-growing organism *Mycobacterium tuberculosis* contains a single rRNA gene (152). *Acinetobacter* species contain five to seven rRNA operons and are known to grow rapidly, suggesting that rRNA synthesis may be a valid target for bacteriostatic or bactericidal agent development. It may, for example, be possible to design nucleic acid mimetic drugs, such as peptide nucleic acid (PNA) or locked nucleic acid (LNA) (217) to competitively inhibit antitermination complex formation through interaction with *boxA* or *C* sequences. Although cell permeability remains a significant hurdle to the effective use of PNAs and LNAs as drugs, they can nevertheless be used to discover pharmacophores for the design of more cell-permeant compounds (217).

Alternative Sigma Factors and Their Regulation

Bacteria respond to changing environmental conditions by (often dramatically) altering the expression of stress response genes. This regulation is effected primarily at the level of transcription, in particular through the differential use of σ factors within the RNAP holoenzyme (167). During exponential growth in rich media, σ^{70}, the housekeeping σ factor, directs transcription of most essential genes. Following application of a particular cellular stress, an alternative σ factor with specific affinity for a certain promoter sequence can replace σ^{70} and thus direct transcription toward the specific set of genes required under the stressed conditions. Under normal conditions, these alternative σ factors are repressed, either by rapid degradation or by their sequestration by anti-σ factors.

A wide disparity is observed in the number of σ factors encoded by bacteria; e.g., *E. coli* has 7 different σ factors, *B. subtilis* has 17, and *Streptomyces coelicolor* has 63 (152). Alternative σ factors identified in *E. coli* include σ^{28}, σ^{38}, σ^{32}, σ^{54}, and the extracytoplasmic function σ factors (ECFs) σ^{E} and σ^{FecI} (80, 175). Of these, only homologs of σ^{32} and σ^{54} are identifiable in *Acinetobacter* spp., along with one to five ECF σ factors (Table 4).

σ^{32} (RpoH) is the primary regulator of the heat shock response in bacteria. In *E. coli*, a temperature shift to >42°C results in the induction of a subset of more than 20 σ^{32}-regulated heat shock genes, including the molecular chaperones DnaJ and -K and GroEL and -ES. These proteins serve to stabilize expressed proteins in the cell cytoplasm, preventing misfolding and aggregation, which are especially common during periods of heat stress. It has been shown that the strain *A. calcoaceticus* 69-V responds to heat shock by upregulating a similar set of proteins, including DnaK, GroEL, and HtpG (18). This suggests that the σ^{32} regulon in *Acinetobacter* spp. is probably similar to that in *E. coli*.

The σ^{54} family is unusual in that, unlike all other σ factors, its members display no sequence homology with the housekeeping factor σ^{70}. In *E. coli*, σ^{54} was originally implicated in the regulation of nitrogen metabolism. However, many additional regulatory roles have since been attributed to this protein (211, 219). While no systematic attempt has yet been made to characterize the σ^{54} regulon in *Acinetobacter* spp., σ^{54} has been shown to be required for the utilization of phenol and benzyl alkanoates as metabolic substrates in *A. baylyi* (73, 133). The expression of ComP, a pilin-like protein essential for transformational competence in *A. baylyi* ADP1, is present at the highest levels during late stationary phase and also is likely to be regulated by σ^{54} (210). It was recently found that genes under the control of the σ^{54}-regulated *Pu* promoter from *Pseudomonas putida* could be expressed exogenously in *A. baylyi* ADP1 (111). Expression patterns mirrored those seen in its natural host, suggesting that there is at least some compatibility between the σ^{54} regulatory systems of *Acinetobacter* spp. and *Pseudomonas* spp.

ECF sigma factors are extremely divergent, and in general sequence analysis alone is insufficient to enable prediction of their function (106). *E. coli* contains two σ factors which group within the ECF subclass: σ^{E} and σ^{FecI}. Analysis of the genome sequence of *A. baylyi* ADP1 reveals that this organism contains three genes with some similarity to *E. coli* σ^{FecI}, which is

TABLE 4. *Escherichia coli* alternative σ factors and regulators and their homologs in *Pseudomonas aeruginosa* and *Acinetobacter* spp.

Function(s)	*E. coli* proteins		Homologs (% sequence identity[a])					
			P. aeruginosa		*A. baylyi*		*A. baumannii*	
	σ factor	Anti-σ factor(s)/ regulator(s)	σ factor	Anti-σ factor(s)/ regulator(s)	σ factor	Anti-σ factor(s)/ regulator(s)	σ factor	Anti-σ factor(s)/regulator(s)
Cell envelope stress response, mucoid phenotype development	RpoE (σ^E)	RseA	PA0762 (AlgU) (65)	PA0763 (MucA) (34)	ACIAD2627 (24)	—[b]	ABAYE0897 (24)	—
		RseB		PA0764 (MucB) (30)	ACIAD0935 (26)	ACIAD0936 (17)		
Regulation of flagellum assembly	RpoF (σ^{28})	FlgM	PA1455 (53)	PA3351 (23)	—	—	—	—
Heat shock response	RpoH (σ^{32})	—	PA0376 (59)	—	ACIAD1311 (58)	—	ABAYE1217 (59)	—
Regulation of nitrogen metabolism, metabolism of aromatic compounds, pathogenesis	RpoN (σ^{54})	RpoX (Hpf)	PA4462 (54)	PA4463 (54)	ACIAD0657 (46)	ACIAD0658 (34)	ABAYE3136 (47)	ABAYE3135 (32)
Regulation of stationary phase and stress response proteins	RpoS (σ^{38})	—	PA3622 (76)	—	—	—	—	—
Iron uptake, unidentified functions	FecI	FecR	PA3899 (52)	PA3900 (36)	ACIAD1161 (34)	ACIAD1162 (19)	AB57_0985/ACICU_00873 (35)	AB57_0986/ACICU00874 (30)
			PA1912 (49)	PA1911 (37)	ACIAD1778 (43)	ACIAD1779 (27)		
			PA0472 (46)	PA0471 (30)	ACIAD1469 (33)	ACIAD1471 (25)		
			PA2468 (50)	PA2467 (37)				
			PA2050 (45)	PA2051 (28)				
			PA4896 (42)	PA4895 (36)				
			PA2093 (38)	PA2094 (26)				
			PA1300 (36)	PA1301 (28)				
			PA2387 (34)	PA2388 (24)				
			PA3410 (28)	PA3409 (41)				
			PA2426 (31)	—				
			PA0149 (26)	PA0150 (35)				
			PA2896 (31)	—				
			PA0675 (25)	PA0676 (26)				
			PA1776 (23)	—				

[a] Sequence identity with equivalent *E. coli* proteins.
[b] —, not applicable or protein not present in organism.

involved in a signaling cascade leading to the cellular uptake of iron, in the form of ferric citrate (Table 4). In the *A. baylyi* ADP1 chromosome, all three of these sequences reside upstream of a gene that encodes a FecR-like anti-σ factor and a membrane receptor protein. In contrast, *A. baumannii* strains contain either none or only one of these proteins, suggesting that alternative iron uptake pathways are important in its pathogenesis (279).

In *E. coli*, σ^E is induced following heat shock and during the early stationary phase and regulates the expression of around 200 genes, overseeing the cell envelope stress response (57). It is essential in *E. coli*, even under apparently nonstressed conditions, and loss of σ^E activity has been shown to result in loss of cell envelope integrity (105). Remarkably, *Salmonella enterica* serovar Typhimurium σ^E, which shares 99% sequence identity with *E. coli* σ^E, is nonessential but plays a critical role in virulence (113). The equivalent protein in *P. aeruginosa*, AlgU, regulates genes involved with the mucoid phenotype associated with cystic fibrosis (211). Clearly, there is much variation in the cellular functions regulated by σ^E regulons in different bacterial species, although all are linked by their site of action at the cell envelope.

The scope of the σ^E regulon in *Acinetobacter* spp. has yet to be examined. Interestingly, however, it has been noted that the AdeABC multidrug efflux pump, which is associated with resistance to several classes of antibiotics in *A. baumannii*, is likely to be transcribed from an ECF-regulated promoter (166). Given that some strains of *A. baumannii* do not contain any discernible σ^{FecI} homologs, it is highly likely that the *adeABC* operon is transcribed by RNAP-σ^E in *Acinetobacter* spp. No homologs of the anti-σ factor RseA/MucA or the periplasmic protein RseB/MucB, which negatively regulate the activity of σ^E (273), were found in the published *Acinetobacter* sp. genomes. The genome of *A. baylyi* ADP1 contains a second homolog of σ^E (ACIAD0935); the function of this protein is not yet clear (Table 4).

The absence of σ^{28} (or RpoF) from the genomes of *Acinetobacter* spp. comes as no surprise. The σ^{28} factor is a regulator of flagellar biosynthesis (200), and as members of a nonmotile genus, *Acinetobacter* spp. do not carry genes for flagellum assembly. Intriguingly, the σ^{38} (or RpoS) sigma factor, which is thought to be the master stress response regulator in gammaproteobacteria (107), is also missing from *Acinetobacter* spp. This σ factor controls the induction of a subset of more than 100 genes involved in stress resistance in *E. coli*, including those whose products resist oxidative stress, acidic pH, ethanol, and hyperosmolarity (107). Although σ^{38} is undetectable in exponential-phase cultures, its levels reach 30% of that of σ^{70} in stationary phase, allowing it to more effectively compete with σ^{70} for binding to core RNAP (129). This is further enhanced by the concurrent induction of Rsd, an anti-σ factor that binds to σ^{70}, preventing it from interacting with the RNAP core (274). The gene encoding Rsd is similarly absent from the genomes of *Acinetobacter* spp. The apparent lack of a σ^{38} system in *Acinetobacter* (and the related genus *Psychrobacter*) is unique within gammaproteobacteria (excluding symbiotic organisms with reduced genomes). The potential implications of a lack of σ^{38} in the global stress response and adaptation to antibiotics in these organisms are discussed below.

Pathogenic *Acinetobacter* spp. Are Enriched in Transcription Factors

A recent comparative genomics study of *Acinetobacter* strains has revealed a set of 475 genes that are found in the genomes of all completely sequenced pathogen strains but not in the nonpathogenic *A. baylyi* ADP1 genome (4). This set, termed the pan-*A. baumannii* accessory genes, includes 59 that encode proteins predicted to be transcription factors based on sequence homology. The LuxR, TetR, MerR, AraC, MarR, GntR, AcrR, ArsR, BadM/Rrf2, XRE, and HxlR families of transcription factors are represented, suggesting that *Acinetobacter* pathogenesis involves a complex transcriptional network that regulates the expression of proteins with diverse biochemical functions. Further investigation is required to examine the precise roles of these transcription factors in pathogenesis.

AN UNUSUAL DNA DAMAGE RESPONSE

Several genes associated with DNA replication, transcription, and cell division are not found within the genomes of *Acinetobacter* spp., despite being conserved in virtually all other gammaproteobacteria. In particular, the error-prone polymerase DinB (DNA polymerase II), the stress response factor σ^{38}, and the cell division proteins SulA, FtsE, and FtsX appear to be uniquely missing in *Acinetobacter* and *Psychrobacter* spp. (Table 5).

While σ^{38} is best known for its roles in stationary phase and global stress response regulation, its role in the response to DNA damage in *E. coli* was recently described (171). In the same organism, *dinB* and *sulA* are both induced as part of the SOS DNA damage response mediated by the transcriptional regulator LexA. Together with the fact that acinetobacters also lack LexA, these missing genes indicate an unusual DNA damage response in *Acinetobacter* spp.

The SOS response has been well characterized in *E. coli* (143). The response is triggered by DNA damage, some classes of antibiotics, or the disruption or overexpression of certain genes (14, 143, 195). Detection of one of these triggers leads to RecA-dependent autoproteolysis of LexA, a repressor that governs the transcription of around 30 genes in *E. coli* (29). While some variation is seen among the members of the LexA regulon in different species, secondary DNA polymerases (e.g., DinB and UmuD) and cell division inhibitors, such as SulA, are almost always upregulated (2, 44, 45, 56, 128, 143). Promoters governing the expression of these proteins are derepressed, and therefore upregulated, upon cleavage of LexA (143). As a result, cell division is temporarily arrested and DNA damage is repaired with the aid of repair and recombination systems.

Thus far, only two DNA damage-inducible loci have been identified in *Acinetobacter* spp. (Table 5). Not surprisingly, DNA damage in *A. baylyi* ADP1 (then known as *A. calcoaceticus*) was found to result in upregulation of the *recA* gene, which is associated with DNA repair and recombination in bacteria (216). Unlike in most other organisms, however, this regulation was independent of the RecA protein, suggesting a novel mechanism for the DNA damage response in this organism. The second gene found to be upregulated following DNA damage in *A. baylyi* ADP1 is *ddrR*, which has no identifiable

TABLE 5. DNA damage response proteins in *Acinetobacter* spp.

Protein	Function and comment
E. coli DNA damage proteins not found in *Acinetobacter* spp.	
DinB	DNA polymerase II; error-prone polymerase involved in SOS mutagenesis
σ^{38}	Stationary phase/stress response σ factor
SulA	Cell division inhibitor; induced during SOS response
FtsEX	Transporter of unknown function; induced during SOS response
LexA	Transcriptional regulator; primary regulator of SOS response
Recognized DNA damage response proteins in *Acinetobacter* spp.	
UmuC	DNA polymerase V subunit; truncated to UmuC* (first 64 residues of UmuC) in *A. baylyi* ADP1
UmuDAb	DNA polymerase V subunit; 50% longer than *E. coli* UmuD protein, contains LexA-like protease recognition site, regulates expression of DdrR in response to DNA damage
DdrR	Unknown function; upregulated following induction of DNA damage in *A. baylyi* ADP1, found only in *Acinetobacter* spp.
RecA	Recombination protein A; upregulated following induction of DNA damage in *A. baylyi* ADP1, required for DNA damage-inducible regulation of DdrR

sequence homologs in the current sequence databases and is of unknown function (102). Regulation of *ddrR* was found to be RecA dependent and appears to involve an unusual UmuD (DNA polymerase V) protein, UmuDAb, that contains a LexA-like domain at the N terminus. Disruption of this novel regulator led to a decrease in the expression of *ddrR*, suggesting that it acts as an activator rather than a repressor, in contrast to LexA. In *A. baylyi* ADP1, the *umuDAb* gene forms part of an unusual *umuDC* operon in which the *umuC* gene has been truncated, apparently as a result of transposon activity (102). *umuDAb* and the truncated *umuC* gene are constitutively expressed from this operon. *A. baumannii* strains each appear to contain an *umuDAb*-like gene in an operon with a full-length *umuC* gene.

The σ^{38}-mediated stress and LexA-mediated SOS responses are activated not only by DNA damage but also by common drug classes: β-lactams, quinolones, and folate biosynthesis inhibitors (143, 184). Activation of these responses leads to upregulation of mutation-inducing error-prone DNA polymerases and increased rates of lateral gene transfer (80). The σ^{38} and SOS responses are now thought to play a significant role in the evolution of antibiotic resistance in bacteria by increasing the scope for adaptive evolution through increased rates of mutation and gene transfer (75, 80, 81, 143). This sets up an apparent paradox for some *Acinetobacter* spp.: they lack the σ^{38} and LexA stress responses yet have acquired resistance to all antibiotics in clinical use. In fact, it has been shown that unlike most other bacteria, *A. baylyi* ADP1 does not show an induction of mutagenesis following irradiation with UV light (19). This suggests either that alternate uncharacterized stress response mechanisms support the acquisition of drug resistance determinants in *Acinetobacter* spp. or that the pathways leading to mutation and/or lateral gene transfer are constitutively enabled. The high level of transformability associated with the laboratory strain *A. baylyi* ADP1 could be taken as evidence for the latter scenario; however, high-level natural competence has not yet been demonstrated for any other *Acinetobacter* strain. Given the rapid emergence of drug resistance in *Acinetobacter* spp. and their increasing significance as pathogens, it is important to develop an understanding of antibiotic-induced stress responses in these organisms, as they are clearly different from those that have been studied to date.

PERSPECTIVES

This review examines three essential biological systems within *Acinetobacter* spp., i.e., DNA replication, cell division, and RNA transcription, with a view to future exploitation of these systems as targets for rational design of novel antimicrobial compounds. Of course, these are not the only potential targets for drug design in *Acinetobacter* spp. Ribosomal protein synthesis, for example, has not been covered here, yet it is one of the most common targets for antibacterial compounds. Compared with replication, cell division, and transcription proteins, ribosomal proteins and rRNA molecules are very highly conserved across the bacteria and in fact even within the eukaryotes and archaea. *Acinetobacter* spp. show little variation within these components relative to other, better-studied bacteria. Primary metabolism and cell wall structures, which are also common drug targets, are not covered here, as information about these systems is difficult to extract from genome sequence information. Our analysis has, however, demonstrated the merits of using genome sequence data to extrapolate information about fundamental biological processes from model organisms to clinically relevant, but more poorly understood, bacteria. This analysis has allowed us to identify highly conserved aspects of DNA replication, cell division, and transcription in these organisms, which could have potential as targets for the rational design of novel antimicrobials. We recognize, of course, that a great deal more work is required to assess the actual drug susceptibilities of these systems, even before the challenges of developing inhibitors that are nontoxic to humans, are available at the site of infection, are capable of being taken up by bacterial pathogens, and then exhibit a bacteriostatic effect can be addressed.

While research into the fundamental aspects of *Acineto-*

bacter biology is still in its infancy, it is already clear that these bacteria have many unusual attributes. Here we have brought to the surface some peculiar characteristics of their DNA replication machinery. These observations imply that chromosomal replication in these organisms might involve subtle mechanistic differences from *E. coli* and other bacteria. Within the cell division proteins, interruption of the gene encoding the peptidoglycan-recycling enzyme Mpl has been shown to produce a β-lactam-sensitive phenotype in *A. baylyi* ADP1. Such readily observable phenotypes are rare for knockouts of peptidoglycan-recycling genes (199). Although the reason for this is currently unknown, it does suggest that *A. baylyi* ADP1 might prove to be a useful model organism for studying peptidoglycan recycling in Gram-negative bacteria.

RNAP and other components of the basic transcription machinery in *Acinetobacter* spp. are similar to those found in other bacteria. Some variation is seen, however, in the use of transcriptional regulators. *Acinetobacter* spp. appear to contain a somewhat minimized set of alternative σ factors, with some strains containing only σ^E, σ^{32}, σ^{54}, and one ECF-type σ factor. Interestingly, *Acinetobacter* spp. also lack many other canonical stress response proteins, including the DNA damage response regulator LexA. They do, however, contain a large number of putative DNA-binding repressors and activators, many of which are of unknown function. The numbers and types of these transcriptional regulators vary among *Acinetobacter* species, suggesting that they play specific roles in adaptation of each species to its environment. This is evident in the fact that the genomes of pathogenic *Acinetobacter* spp. are highly enriched for genes encoding transcriptional regulators. Further research into transcriptional stress responses in individual *Acinetobacter* species is vital, as such responses are likely to play a central role in the development of antibiotic resistance in these organisms, which is perhaps the foremost problem associated with treating *Acinetobacter* sp. infections in the clinical setting.

Already it is clear that *Acinetobacter* spp. have diverged significantly from other gammaproteobacteria over time and have developed unique solutions for coping with evolutionary pressures. While this presents challenges for conducting *Acinetobacter* research, the tremendous utility of *A. baylyi* ADP1 as a laboratory organism provides an exciting opportunity to understand many processes and pathways in these organisms, thereby increasing our understanding of bacterial evolution in the context of a group of bacteria that are still emerging as human pathogens.

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