Alternative Sigma Factors and Their Roles in Bacterial Virulence

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INTRODUCTION

Sigma factors are a class of proteins constituting essential dissociable subunits of prokaryotic RNA polymerase. The association of appropriate alternative sigma factors with core RNA polymerase provides a mechanism for cellular responses mediated through redirection of transcription initiation. Sigma factors provide promoter recognition specificity to the polymerase and contribute to DNA strand separation; they then dissociate from RNA polymerase core enzyme following transcription initiation (16). As the regulon of a single sigma factor can be comprised of hundreds of genes, sigma factors provide effective mechanisms for simultaneously regulating large numbers of prokaryotic genes. In some cases, the genes comprising a sigma factor regulon have a clearly defined primary function (e.g., genes regulated by the sporulation sigma factors in Bacillus subtilis [171]); in others, the genes comprising a regulon contribute to multiple functions (e.g., the stationary-phase and general stress response genes regulated by $\sigma^B$ in Listeria monocytogenes [100]). One newly emerging field is identification of the specific roles of alternative sigma factors in regulating expression of virulence genes and virulence-associated genes in bacterial pathogens.

Virulence and virulence-associated genes are those that contribute to at least one aspect of bacterial disease transmission and infection processes. Specifically, virulence genes encode proteins whose functions are essential for the bacterium to effectively establish an infection in a host organism. Examples of virulence genes are L. monocytogenes inlA, which encodes the internalin-A protein important for invasion of nonprofessional phagocytes (129), and the spv gene cluster of Salmonella enterica, which allows for bacterial growth inside macrophages (128). In contrast, virulence-associated genes can contribute to bacterial survival in the environment (e.g., the Ica operon of Staphylococcus aureus, which produces an adhesin important for biofilm formation on plastic surfaces such as those on indwelling medical devices [141]) or to survival in the host (such as bsh of L. monocytogenes, encoding bile salt hydrolase, which enhances bacterial survival in the intestinal environment prior to intracellular infection [48]). Therefore, activation of virulence-associated genes may enhance the capacity of the bacterium to spread to new individuals or to survive passage through a host organism. As alternative sigma factors have been shown to regulate expression of both virulence and viru-

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TABLE 1. Alternative sigma factors involved in virulence

<table>
<thead>
<tr>
<th>Family, class, and sigma factor</th>
<th>Bacterial species</th>
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<tbody>
<tr>
<td>σ^70 family</td>
<td>B. anthracis, L. monocytogenes, M. tuberculosis, S. aureus, S. epidermidis</td>
</tr>
<tr>
<td>Stress response</td>
<td>E. coli, P. aeruginosa, S. enterica serovar Typhimurium, S. enterica serovar Typhi</td>
</tr>
<tr>
<td>σ^54 family</td>
<td>M. tuberculosis</td>
</tr>
<tr>
<td>ECF</td>
<td>H. influenzae, S. enterica serovar Typhimurium, V. cholerae</td>
</tr>
<tr>
<td>AlgU</td>
<td>P. aeruginosa</td>
</tr>
<tr>
<td>PvdS, PfpI</td>
<td>M. tuberculosis</td>
</tr>
<tr>
<td>σ^N</td>
<td>M. tuberculosis</td>
</tr>
<tr>
<td>σ^I</td>
<td>M. tuberculosis</td>
</tr>
<tr>
<td>HrpL</td>
<td>Erwinia spp., P. syringae</td>
</tr>
<tr>
<td>σ^28</td>
<td>C. jejuni, H. pylori, S. enterica serovar Typhimurium, V. cholerae, Y. enteroxolitica</td>
</tr>
<tr>
<td>σ^54</td>
<td>C. jejuni, H. pylori, L. monocytogenes, P. aeruginosa, P. syringae, V. cholerae, V. parahaemolyticus</td>
</tr>
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</table>

a Sigma factors are grouped according to classes. The stress response, ECF, and σ^70 classes are all members of the σ^70 family of sigma factors.

b Species discussed in this review that contain the given sigma factors.

Virulence-associated genes, these sigma factors can contribute both directly and indirectly to bacterial virulence.

Virulence factor expression appears to be tightly regulated in bacterial pathogens. In some cases, pathogens have a "master regulator" of virulence gene expression, such as the positive regulatory factor A (PrfA) in L. monocytogenes. PrfA, a transcriptional activator, is required for expression of the majority of recognized L. monocytogenes virulence genes. Alternative sigma factors often function to regulate expression of virulence and virulence-associated genes in response to particular stimuli. Alternative sigma factors may regulate a small number of genes, each of which may be critical to infection (e.g., PvdS of Pseudomonas aeruginosa [discussed below] [147]), or they may regulate functions that contribute to virulence but also have additional physiological roles in the cell. For example, Salmonella enterica serovar Typhimurium σ^54 regulates genes that provide resistance to oxidative stress, which also aids bacterial survival in macrophages (82). This review focuses on both direct and indirect roles of selected alternative sigma factors in regulating virulence of bacterial pathogens of plants and animals.

Sigma factors can be classified into two structurally unrelated families: the σ^70 and the σ^54 families. Table 1 lists sigma factors in both the σ^70 and the σ^54 families that are currently recognized as contributing, either directly or indirectly, to bacterial virulence. For several alternative sigma factors, nomenclature in the literature has been inconsistent. In this document, in general, we refer to sigma factor families by number (e.g., the σ^70 family) and to specific sigma factors by letter (e.g., P. aeruginosa σ^N). For certain sigma factors, we use the predominant designation from the literature instead (e.g., FliA).

The σ^70 family includes primary sigma factors (e.g., Bacillus subtilis σ^E) as well as related alternative sigma factors (145, 164). Alternative sigma factors within the σ^70 family are further categorized by the physiological processes they control, e.g., stress response. In general, these groupings by function also correlate with phylogenetic relationships among the protein sequences (164). Within the σ^70 family of sigma factors is a large, phylogenetically distinct subfamily called the extracytoplasmic function (ECF) factors. These sigma factors are responsible for regulating a wide range of functions, all involved in sensing and reacting to conditions in the membrane, periplasm, or extracellular environment (70). Structurally, σ^70 family factors have four major regions, with the highest levels of conservation in regions 2 and 4. Subregions within region 2 are involved in promoter melting (region 2.3) and −10 sequence recognition (region 2.4). Region 4.2 is involved in −35 recognition. For a recent review on the σ^70 family of sigma factors, see references 164.

Although no sequence conservation exists between σ^54 and σ^70-like family members, both types bind to core RNA polymerase. However, the holoenzyme formed with σ^54 sigma factors has different properties than the σ^70 holoenzyme. While the C terminus (region III) of σ^54 enables DNA binding, all of σ^54 species require a separate activator protein along with the core RNA polymerase (RNAP) to form an open promoter complex. The σ^54 N terminus, which inhibits isomerization in the absence of the appropriate activator, stimulates initiation upon activation (19). Further, promoter structures recognized by σ^54-RNAP differ from those recognized by σ^70-RNAP. σ^54 promoters are highly conserved, short sequences that are located at positions −24 and −12 upstream of the transcription initiation site, whereas σ^70 promoter sites are typically located at −35 and −10 upstream. σ^54 promoters, which are called −24/−12 promoters, are almost completely invariant at the −24/−12 positions (GG and GC, respectively) and in their spacing in both gram-negative and gram-positive bacteria. For reviews on the structure-function relationships of σ^54, see references 19 and 142.

We present several examples of alternative sigma factors that have been shown to contribute to virulence in at least one organism. The text is organized by sigma factor to include the three subfamilies (stress response, σ^28, and ECF) within the σ^70 family, as well as those within the σ^54 family. For each sigma factor, when applicable, examples will be drawn from multiple bacterial species.

STRESS RESPONSE ALTERNATIVE SIGMA FACTORS

The ability to reproduce, or simply survive, under a wide variety of environmental conditions contributes to a microbial pathogen’s potential for transmission by various routes. For example, to establish a food-borne infection in a human host, a bacterium first must survive transit in a contaminated food. Following ingestion, the bacterium then must survive exposure to rapid and dramatic changes in environmental conditions, including the acidic pH within the stomach, followed by vastly differing conditions during intestinal passage and/or infection (e.g., exposure to bile, vacuolar stresses, etc.) Survival under these extreme and rapidly changing conditions
TABLE 2. Virulence genes and virulence-associated genes regulated by stress response sigma factors $\sigma^B$ and $\sigma^S$ and phenotypes of sigma factor null mutants in selected bacterial species

<table>
<thead>
<tr>
<th>Sigma factor and species</th>
<th>Genes regulated by sigma factor* (reference[s])</th>
<th>Phenotype* (reference[s])</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Virulence associated</td>
<td>Virulence</td>
</tr>
<tr>
<td>$\sigma^B$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td>bsh (100, 199, 200), gadA (100), opuCA (57, 100, 199, 200)</td>
<td>inlA (100, 103, 200), prfA (101, 151, 191)</td>
</tr>
<tr>
<td>S. aureus</td>
<td>cap genes (14), clfA (14), bhp (14), ebpA (14), icaA (177)</td>
<td>sarA (13, 14, 44), adiRS (14)</td>
</tr>
<tr>
<td>$\sigma^S$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. enterica serovar Typhimurium</td>
<td>Unknown chromosomal factors (51, 153)</td>
<td>spv (51, 68, 112, 157)</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>rhl (168, 218), las (168, 218)</td>
<td>Exotoxin A (201), alginate (196, 201), type IV pili (196, 201)</td>
</tr>
</tbody>
</table>

* Virulence-associated genes and virulence genes directly regulated by $\sigma^B$ or $\sigma^S$, as defined in the text.

<table>
<thead>
<tr>
<th>Phenotype* (reference[s])</th>
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Although all seven Rsb proteins identified in B. subtilis and L. monocytogenes are not conserved among all bacterial species bearing $\sigma^B$, two key proteins (RsbV and RsbW) are conserved among all species examined to date and thus appear to be minimally essential for regulating $\sigma^B$ activity (54). Specifically, in log phase, nonstressed B. subtilis cells, $\sigma^B$ is inactivated by its association with the anti-$\sigma^B$ antagonist protein, RsbW (i.e., the “anti-sigma factor”). In stressed cells, however, the unphosphorylated form of the anti-$\sigma^B$ antagonist protein, RsbV, (i.e., the “anti-anti-sigma factor”) competes for binding to RsbW. As the relative concentration of the RsbW-RsbV complex increases, the concentration of free $\sigma^B$ also increases, thus allowing $\sigma^B$ to bind to core RNA polymerase (47). In B. subtilis, both environmental and energy stress signals induce dephosphorylation of RsbV. Environmental stress signals specifically activate the B. subtilis RsbU serine phosphatase through involvement of RsbR, RsbS, RsbT, and RsbX (211, 212, 223). In addition to its role in partner-switching regulation under environmental stress, B. subtilis RsbX also functions as a feedback regulator for $\sigma^B$ activity (Fig. 1B) (12). While both energy and environmental stresses have been
shown to activate *L. monocytogenes* σ^B^ (25), specific interactions among the Rsb proteins have not yet been investigated. To date, specific activation mechanisms have been most extensively reported for *B. subtilis* σ^B^.

**Pathogenic Bacillus species.** At least two pathogenic species of *Bacillus* encode σ^B^ (55, 208). In *Bacillus anthracis*, only σ^B^, RsbV, and RsbW are encoded in the sigB operon (Fig. 1A). A third rsb gene, rsbY, encodes a protein with low similarity to *B. subtilis* RsbP. rsbY is located in close proximity to, but not within, the *B. anthracis* sigB operon (55). As in *B. subtilis* and *L. monocytogenes*, the sigB operon is autoregulated by σ^B^ and is induced by heat shock and entry into stationary phase (55). Through use of a combination of two-dimensional gels and Northern hybridizations, 15 *B. cereus* genes and proteins were determined to be σ^B^ dependent, including RsbV and the KatE catalase (209). The activity of currently recognized *B. cereus* virulence factors, including protease, lecinthinase, and hemolytic activity, as well as production of nonhemolytic enterotoxin, was not affected by disruption of sigB (208), suggesting that σ^B^ does not directly contribute to *B. cereus* virulence.

**Staphylococcus species.** *Staphylococcus aureus* was the first pathogenic bacterium in which sigB was identified (115, 224) (Fig. 1A). In *S. aureus*, the sigB operon is comprised of four genes, which are homologous to *B. subtilis* rsbU, rsbV, rsbW, and sigB. As in *B. subtilis*, all genes in the operon are expressed during exponential growth, presumably from the σ^B^ dependent promoter. The internal P_4 promoter was confirmed as σ^B^ dependent through in vitro transcription analyses (44). Transcriptional regulation of the *S. aureus* sigB operon is complex, generating multiple transcripts that appear to include a bicistronic sigB-rsbW transcript as well as a sigB monocistronic transcript. In support of an autoregulatory role for *S. aureus* σ^B^ under conditions of environmental stress, an rsbV'-W-sigB transcript was induced following exposure of cells to either 4% ethanol or a 48°C heat shock (115).

*S. aureus* σ^B^ activity is regulated posttranslationally by Rsb proteins. The open reading frame immediately upstream of *S. aureus* sigB encodes the anti-sigma factor, RsbW (146). As in *B. subtilis*, *S. aureus* σ^B^ also is activated via an RsbU pathway (62). An 11-bp deletion in rsbU in the NCTC8325 strain generated some phenotypic characteristics similar to those of a ΔsigB strain, (e.g., decreased H_2O_2 resistance [114]). Giachino et al. (62) confirmed that NCTC8325 does not produce a functional RsbU and that complementation of this strain with a complete rsbU allele restored phenotypes to those of the RsbU'-Newman wild-type strain. However, some NCTC8325 phenotypes were identical to those of other rsbU' strains (e.g., lipase production [see below]), suggesting the existence of multiple *S. aureus* σ^B^ activation pathways, including at least one that is RsbU independent (114). As with RsbU, loss of RsbV results in a dramatic decrease, although not complete loss, of *S. aureus* σ^B^ activity (165).

Through application of full-genome microarray screens for σ^B^-dependent genes in three *S. aureus* strains, as many as 251 genes have been identified as being σ^B^ regulated (14), including several genes encoding proteins involved in synthesis of capsular polysaccharides. A number of adhesins, which are involved in *Staphylococcus* virulence, are upregulated by σ^B^.

Multiple genes encoding exoenzymes and toxins (e.g., hla and nuc) are downregulated as σ^B^ is activated (14), which may reflect σ^B^'s role in controlling expression of *S. aureus* virulence gene regulators. For example, a number of the exoenzymes and toxins that are downregulated by σ^B^ depend on an effector RNA produced from the agr locus (RNAIII) for heightened expression (204). RNAIII levels are reduced when σ^B^ activity increases. The mechanism responsible for this phenomenon remains unclear (13, 14) but may involve the regulator SarA (8, 44, 79).

Multiple groups have described *S. aureus* ΔsigB mutants as having pigment loss and decreased peroxide resistance, but...
higher α-hemolysin, coagulase, clumping factor, and lipase activity, compared to the wild type (27, 62, 79, 114, 152). These characteristics have all been associated with \textit{S. aureus} virulence (61, 67, 98, 131, 150, 184). It is likely that optimal levels of virulence factor expression and activity are required for efficient \textit{S. aureus} infection and that too much or too little activity or expression at the wrong time is detrimental for the infection process. These hypotheses remain to be rigorously tested. In various animal models, wild-type \textit{S. aureus} and an otherwise isogenic \textit{ΔsigB} strain showed no difference in virulence (22, 152). In additional, conflicting studies, Horsburgh et al. (79) found no difference in virulence between \textit{rsbU} and \textit{rsbU} mutant strains in a murine skin abscess model, while Jonsson et al. (92) showed that both \textit{rsbU} and \textit{sigB} mutant strains displayed decreased virulence phenotypes compared to the wild-type strain in murine septic arthritis, including reduced mutant persistence in kidneys and reduced mouse mortality, weight loss, arthritis, and interleukin-6 production.

The contradictory evidence surrounding the role of \textit{σB} in \textit{S. aureus} virulence suggests that \textit{σB} contributions to virulence may be indirect or not detectable in some model systems. For example, \textit{σB} may contribute indirectly to \textit{S. aureus} virulence through regulation of biofilm formation. Biofilm formation can be a prerequisite for establishing infection by staphylococci, and \textit{σB} has been shown to enhance microcolony and biofilm formation in \textit{Staphylococcus} species (5, 177). Two studies have shown induction of \textit{S. aureus} biofilm formation in a \textit{σB}-dependent fashion (5, 177), although another showed that a \textit{ΔsigB} strain formed biofilms and produced PIA, the polysaccharide or produce PIA (106). As in \textit{S. aureus}, \textit{rsbU} of the adhesin PIA, but \textit{σB} regulates expression of virulence and virulence-associated genes in \textit{S. epidermidis} (Fig. 1A), signal transduction pathways differ in the two organisms. In \textit{B. subtilis}, environmental and energy stresses are conveyed to \textit{σB} through two interconnected but separate pathways. The environmental stimulus pathway is transmitted by regulatory proteins encoded in the \textit{sigB} operon (\textit{RsbT}, \textit{RsbU}, \textit{RsbV}, and \textit{RsbW}). In addition to requiring \textit{RsbV} and \textit{RsbW}, the \textit{B. subtilis} energy stress pathway also requires proteins encoded in a two-gene operon (\textit{rsbQ-rsbP}) that is physically separate from the \textit{sigB} operon (18). This operon is not present in \textit{L. monocytogenes}. Instead, both energy stress and environmental stress activation of \textit{σB} in \textit{L. monocytogenes} occurs through a single pathway, which includes \textit{RsbT}, \textit{RsbU}, \textit{RsbV}, and \textit{RsbW} (25).

A genome-wide search for predicted \textit{σB}-dependent promoters by using a hidden Markov model followed by application of a specialized, partial microarray identified 54 genes under positive control of \textit{σB} in \textit{L. monocytogenes}, although the full regulon is likely to be as large as that of \textit{B. subtilis} (100). \textit{σB} regulates expression of virulence and virulence-associated genes in \textit{L. monocytogenes} (Fig. 2A) and is directly regulated by \textit{σB} (100, 199). Another recently identified \textit{σB}-dependent virulence-associated gene is \textit{hfq}, which encodes an RNA-binding regulatory protein (29). Deletion of the \textit{σB}-dependent \textit{opuC} (57), which encodes an osmoprotector, also negatively affects \textit{L. monocytogenes} virulence (194, 217).

As in \textit{S. aureus}, \textit{L. monocytogenes} \textit{σB} also controls expression of virulence gene regulators (Fig. 2A). Of the two promoters directly upstream of the gene encoding positive regulatory factor \textit{A} (\textit{PrfA}), \textit{P2}_{\textit{PrfA}} is \textit{σB}-dependent. Dual deletion of \textit{sigB} and the \textit{σB}-dependent \textit{P1}_{\textit{PrfA}} promoter (leaving only the \textit{σB}-dependent \textit{P2}_{\textit{PrfA}}) reduced hemolytic activity and intracellular growth to the same low levels as deletion of both \textit{prfA} promoters (151). \textit{σB} activity at the \textit{P2}_{\textit{PrfA}} promoter was also directly confirmed, both by quantitative reverse transcription-PCR (101) and with β-glucuronidase reporter fusions of \textit{prfA} promoters, which demonstrated \textit{σB}- and growth phase-dependent expression from \textit{P2}_{\textit{PrfA}} (191).

Several \textit{PrfA}-regulated genes are also \textit{σB}-dependent, suggesting interplay between the two regulators (143) (Fig. 2A). For example, expression of the \textit{PrfA}-regulated \textit{inlA} gene, which encodes the cell surface protein \textit{internalin-A}, is also at least partially \textit{σB}-dependent (100, 200). \textit{Internalins} are cell wall-anchored proteins with important roles in the intracellular pathogenesis of \textit{L. monocytogenes}, and several members of the \textit{internalin} gene family show reduced expression in a \textit{sigB} mutant compared to in the wild type (100). \textit{Internalin-A}, specifically, is responsible for invasion of nonprofessional phagocytes (129). Loss of \textit{σB} reduced invasiveness of the mutant strain compared to that of wild-type \textit{L. monocytogenes} in two intestinal epithelial cell lines, Henle-407 and Caco-2 (103). In addition, \textit{inlA} transcription was greatly reduced in the \textit{ΔsigB} strain, and \textit{internalin-A} was undetectable by Western blotting (103). None of the effects of the \textit{sigB} deletion on \textit{inlA} were mediated through loss of \textit{σB}-dependent transcription of \textit{PrfA}, however, as a \textit{ΔP2}_{\textit{PrfA}} strain had the same levels of invasiveness, \textit{inlA} transcription, and \textit{internalin-A} concentration as the wild-type strain.

Wiedmann et al. (219) tested the effect of a \textit{sigB} deletion on virulence in a mouse model and found a small, but significant, decrease in spread of the mutant strain to the liver compared with that of the wild-type strain. Mouse infection experiments have been widely used to evaluate virulence characteristics of \textit{L. monocytogenes}, including the preliminary evaluations of the \textit{ΔsigB} mutant (151, 219). In recent years, however, increasing evidence suggests that the murine model does not appropri-
atly represent human *L. monocytogenes* infection by the oral route (121, 122). The gastric pH of mice is higher than the pH of the human stomach (97); thus, the role of *L. monocytogenes* acid tolerance is likely to be less important in mouse infection than in human infection. More importantly, in the human, *L. monocytogenes* has the ability to cross the intestinal barrier, the blood-brain barrier, and the fetoplacental barrier. Human E-cadherin acts as an *L. monocytogenes* internalin-A receptor, and the interaction between the receptor and the internalin-A surface protein contributes to the ability of *L. monocytogenes* to target and cross human intestinal and placental barriers (123). Murine E-cadherin, which differs in amino acid sequence from human E-cadherin, does not interact effectively with *L. monocytogenes* internalin-A, and hence mice show limited susceptibility to intragastric *L. monocytogenes* infection (121). In fact, in the mouse, translocation of *L. monocytogenes* across the intestinal barrier is typically no greater than that of the nonpathogenic *Listeria innocua*. Further, *L. monocytogenes* also does not appear to target the murine brain stem or the fetoplacental unit, even following intravenous injection (121, 122). *L. monocytogenes* strains do vary in their ability to cause systemic infection in intragastrically infected mice (38), and some strains of mice (A/J) are also more susceptible than others (C57BL/6) to intragastric infection (39). However, as a consequence of the biological differences in murine and human *L. monocytogenes* translocation across the intestinal barrier, data from mouse infection experiments may underestimate a given strain’s human virulence following oral infection.

The guinea pig has emerged as a more appropriate model than the mouse for studying oral *L. monocytogenes* infection (121, 122). Like humans, guinea pigs exhibit gastroenteritis following *L. monocytogenes* infection by the oral route (122). Cultured guinea pig epithelial cells allow internalin-A-dependent *L. monocytogenes* entry, and both guinea pig and human E-cadherins bear a proline at critical amino acid position 16. When guinea pigs were inoculated orally with *L. monocytogenes* strain EGD or an otherwise isogenic internalin-A deletion mutant, significantly higher numbers of the wild type than of the ΔinlA strain were recovered from guinea pig liver and spleen. In contrast, in the mouse model, low, statistically indistinguishable wild-type and ΔinlA numbers were recovered from mouse organs (121). Lecuit et al. (121) also demonstrated that transgenic mice expressing human E-cadherin enable bacterial invasion of host cells. Taken together, these results illustrate the importance of appropriate internalin-A/E-cadherin interactions in the development of systemic listeriosis following oral infection with *L. monocytogenes*.

**Mycobacterium tuberculosis** $\sigma^B$ and $\sigma^F$. *Mycobacterium tuberculosis*, a high-GC-content bacterium, has 13 sigma factors (for a review, see reference 137). Two of these 13, $\sigma^B$ and $\sigma^F$, appear to share an evolutionary origin (54). *M. tuberculosis* $\sigma^B$ appears more similar to $\sigma^B$ of the low-GC gram-positive bacteria than to $\sigma^F$ of *B. subtilis*, which is a sporulation factor. Specifically, *M. tuberculosis* $\sigma^B$ is antigenically closer to *B. subtilis* $\sigma^R$ (43) and has the same consensus promoter recognition sequence (10, 60), and expression patterns for its encoding gene are similar to those of *B. subtilis* sigB (42, 133). As with *B. subtilis* $\sigma^R$, *M. tuberculosis* $\sigma^B$ is regulated posttranslationally by an anti-sigma factor and anti-anti-sigma factor partner-switching mechanism (10). The gene encoding *M. tuberculosis* $\sigma^F$ is immediately
downstream of the gene encoding its anti-sigma factor, UsfX, as is the case with B. subtilis σB and its anti-sigma factor, RsbW. M. tuberculosis sigB, on the other hand, is located 3 kb downstream of the gene for the primary sigma factor, σA, and is not flanked by genes encoding sigma factor regulatory proteins (45). The sigB genes in M. tuberculosis and in L. monocytogenes also share some characteristics. For example, expression of M. tuberculosis sigB is growth phase dependent, as is expression of sigB in other species (42, 80). The same studies also showed that sigB transcription is induced under a variety of stresses, including oxidative stress, heat shock, and cold shock. In spite of these observations on σB stress induction, no studies on contributions of this protein to either M. tuberculosis stress resistance or virulence have been reported.

Microarray analysis of the M. tuberculosis σS regulon identified abpC, a gene implicated in virulence, as greatly reduced in expression in a ΔsigF mutant (60). In addition, another sigma factor, sigC, which is required for M. tuberculosis lethality in mice (202), is also σS dependent (Table 3). Several studies have linked M. tuberculosis σS with virulence. Mice infected with a ΔsigF strain displayed a longer time to death than mice infected with the wild-type strain, and the weight loss caused by wild-type M. tuberculosis did not occur in mice infected with the mutant strain (26). In a separate study, CFU counts recovered from the lungs and spleens of infected mice were approximately 40 times higher for the wild type than for the ΔsigF strain. Histopathological analyses showed that the ΔsigF mutant caused fewer, smaller granulomas and less inflammation than the wild type (60) after 12 weeks. In summary, multiple lines of evidence support direct and indirect roles for σS in M. tuberculosis virulence.

**Sigma S (RpoS)**

In gram-negative bacteria, RpoS (σS) is functionally similar to σB in that it is responsible for stationary-phase and stress response gene expression. The chromosomal organizations of the rpoS and sigB loci, as well as the transcriptional and posttranscriptional regulatory mechanisms for these genes and proteins, are distinctly different, however. Regulation of σS expression and activity is extremely complex, relying on transcriptional, translational, and posttranslational mechanisms (for a thorough review, see reference 75). Further, a sequence comparison of 31 σS family sigma factors groups Escherichia coli σS separately from B. subtilis σB, indicating that while σB and σS may have similar functions, they are not highly homologous proteins (132).

**Escherichia coli**. A few reports have examined associations between σS and E. coli virulence, but little direct evidence of a link exists. Wang and Kim (214) demonstrated that E. coli K1 invasion of brain microvascular endothelial cells was higher for stationary-phase cells than for exponentially growing cells, possibly due to stationary-phase activity of σS. Indeed, complementation of rpoS into an rpoS mutant significantly increased invasion for one E. coli isolate but not for another (214). σS is not essential for murine urinary tract colonization (37) and actually appeared to be detrimental during competitive colonization experiments in the mouse intestine (113). It is also possible that the lack of an appropriate animal model for investigating all aspects of E. coli pathogenesis (e.g., the absence of an appropriate model for studying hemolytic uremic syndrome infections caused by enterohemorrhagic E. coli [193]) has impeded identification of a direct role for σS in E. coli pathogenesis.

It is likely that σS contributes indirectly to E. coli pathogenesis. E. coli O161:H7 strains tend to be acid resistant, and rpoS mutants show decreased acid resistance and fecal shedding in mice and cattle (175). Several studies have shown that rpoS transcription and σS activity are induced under stress conditions such as osmotic shock, heat, and low pH and that survival of rpoS mutants is reduced under these same conditions (3, 37, 59, 74, 215). Thus, in addition to enabling survival in high-acid
and high-salt foods, $\sigma^{28}$ may enhance *E. coli* host survival and transmission.

**Salmonella species.** *S. enterica* serovar Typhimurium $\sigma^{28}$ is highly similar to *E. coli* $\sigma^{5}$, in both function and regulation. In contrast with *E. coli*, however, numerous studies have shown the unequivocal dependence on $\sigma^{28}$ for full virulence of *S. enterica* serovar Typhimurium. For example, the plasmid-borne *spv* gene cluster is required for *S. enterica* serovar Typhimurium virulence, and several studies have demonstrated that transcription of this gene cluster is $\sigma^{28}$ dependent (51, 68, 112, 157) (Table 2). In fact, an *rpoS* mutant is up to 10-fold less virulent than an *rpoS*+, plasmid cured (*spv*-negative) strain, and the levels of plasmid-cured *rpoS*+ bacteria in the intestine were significantly higher than those of plasmid-cured *rpoS* mutants (51, 153), indicating that the effect of $\sigma^{28}$ on virulence is likely due to its role in regulating expression of chromosomal genes in addition to its effects on the plasmid-borne *spv* locus. In addition, mouse-based virulence assays show that in comparison to the wild-type strain, the *rpoS* mutant has a 3- to 4.5-log-unit higher 50% lethal dose (LD$_{50}$) (35, 51, 153). Similarly, an *rpoS aroA* strain was more virulence attenuated than an *aroA* strain, which has been used in vaccine candidate trials, as determined by spleen bacterial counts and time-to-death analyses (35). $\sigma^{28}$ does not contribute to levels of *S. enterica* serovar Typhimurium adherence, invasion, or intracellular survival, however (153).

Further evidence for the role of $\sigma^{28}$ in *Salmonella* virulence was obtained through analysis of *rpoS* alleles from recognized avirulent or virulence-attenuated strains. For example, the *Salmonella enterica* serovar Typhi vaccine strain Ty21a contains an *rpoS* sequence that generates a nonfunctional $\sigma^{28}$ (181). Virulence attenuation in the *Salmonella enterica* serovar Typhimurium LT2 strain may be a consequence of low levels of *rpoS* mRNA translation due to the presence of a rare UUG start codon on the transcript (124, 203). As in laboratory-generated *rpoS* mutants, the LT2 strain is greatly decreased in its ability to reach the spleen and liver of mice (221).

*Pseudomonas aeruginosa*. *P. aeruginosa* produces many exotoxins that contribute to its pathogenesis. $\sigma^{28}$ appears to have multiple regulatory roles in *P. aeruginosa*. In some cases, $\sigma^{28}$ positively regulates *P. aeruginosa* toxin expression; in others, it negatively regulates expression; and in still others, it appears to have no effect at all. For example, in an *rpoS* mutant, both exotoxin A and alginate production are approximately 50% of that of the wild type (196, 201) (Table 2). However, both reduced *rpoS* expression (109) and loss of $\sigma^{5}$ (196, 201) resulted in increased expression of pyocyanin, an antibiotic that also inhibits lymphocyte proliferation. In two studies, loss of $\sigma^{28}$ was shown to have little to no effect on production of elastase or LasA protease (196, 201). Some of the phenotypic effects on *P. aeruginosa* virulence factor production that are associated with loss of $\sigma^{28}$ may be indirect, for example, resulting from reduced expression of quorum-sensing systems (Table 2). $\sigma^{28}$ contributes to expression of members of the *P. aeruginosa rhl* and *las* quorum-sensing systems (168, 218). These quorum-sensing gene products are responsible for regulating production of several virulence factors, including lectins (190, 222); aminopeptidase, endoprotease, and lipase (158); and rhamnolipid (166, 232). Several studies have shown quorum-sensing mutants to be avirulent or less virulent than the wild-type strain in mouse (167, 185, 195, 232), amoeba (34), and rat models (126). Finally, the role of $\sigma^{28}$ in *P. aeruginosa* virulence is highly dependent on the model system in which it is assessed. For example, while an *rpoS* mutant was as virulent as the wild type in a rat chronic lung model (201), it was approximately half as virulent as the wild-type strain in a *Galleria mellonella* (silkworm) larva model (196).

**SIGMA 28 SUBFAMILY**

$\sigma^{28}$ is a subfamily of the $\sigma^{70}$-like sigma factors. Members of this subfamily are structurally and functionally related and span many genera of both gram-positive and gram-negative bacteria. While the primary regulatory role of $\sigma^{28}$ in many bacterial species is to transcribe genes required for flagellar synthesis and bacterial motility (69, 144), it also contributes to other functions. For example, in the nonmotile *Streptomyces coelicolor*, $\sigma^{28}$ contributes to expression of a diverse set of genes, including those responsible for sporulation and agarase production (96). Examples of $\sigma^{28}$ factors are FliA of enteric bacteria and $\sigma^{D}$ of *B. subtilis*.

**FliA**

*Salmonella enterica* serovar Typhimurium. As in many enteric bacteria, the genes for flagellar biosynthesis and function in *S. enterica* serovar Typhimurium are divided into three hierarchical classes based on their temporal order of transcription (116). One operon, *flhDC*, is categorized into class I. The *flhDC* operon encodes activators required for transcription of the class II operons, including *fliA*, which encodes the $\sigma^{28}$ subfamily sigma factor responsible for expression of the class III genes (84, 161), and *flgM*, which encodes the FlgM anti-sigma factor that regulates activity of FliA (63, 162). The remaining class II genes encode proteins responsible for formation of the flagellar basal body and hook apparatus. Through an additional posttranslational regulatory mechanism, following formation of the flagellar structure, FlgM is secreted through the basal body/hook assembly, which enables depression of FliA and allows subsequent transcription of the class III genes (81, 117). Inactivation of any of the class II genes interrupts complete formation of the flagellum, and the accumulated FlgM prevents further flagellar filament formation. Loss of FlgM results in an approximately sixfold increase in transcription of the FliA-dependent class III genes (118). Interestingly, while *flgM* mutants are virulence attenuated, an additional mutation that inactivates FliA function restores virulence to the strain (187). The mechanism for this phenomenon is still unknown. Many studies have shown the importance of flagella for virulence of *S. enterica* serovars (87, 182, 188, 197), although the specific aspect of flagellar function that contributes to virulence remains unclear.

**Other species.** Regulation of flagellar gene expression in *Yersinia enterocolitica* is similar to that in *S. enterica* serovar Typhimurium. *fliA* encodes a $\sigma^{28}$ factor responsible for motility of the bacterium, and the master regulators FliC and FliD are required for expression of all genes encoding proteins active in subsequent flagellar synthesis (85). Motility is also required for full *Y. enterocolitica* invasion efficiency (228). However, another group of enteric bacteria has a different strategy for regulating
flagellar gene expression, Helicobacter pylori, Campylobacter jejuni, and Vibrio cholerae do not have the flhDC master operon. Instead, early flagellar gene expression is carried out via a $\sigma^{70}$ factor, while later genes are transcribed by FliA (89, 105, 154). These species also encode one or more $\sigma^{54}$ activator proteins, such as FlgR. Virulence in these species is linked to production of flagella. In C. jejuni, virulence proteins are secreted through the flagella, and full virulence requires a complete flagellar export apparatus (110). Multiple studies have shown H. pylori virulence to be dependent both on expression of flagellin proteins and on flagellar motility (94, 140).

**ECF SIGMA FACTORS**

Members of the extracytoplasmic function subfamily of $\sigma^{70}$ sigma factors regulate functions related to sensing and responding to changes in the bacterial periplasm and extracellular environment. These sigma factors are conserved in both gram-positive and gram-negative species. The first ECF sigma factor identified was E. coli $\sigma^{E}$, which was recognized as a second heat shock sigma factor in this organism (213). Although $\sigma^{E}$ does not appear to affect virulence in E. coli, other ECF sigma factors contribute to regulation of virulence genes and virulence-associated genes in a number of bacteria, including S. enterica serovar Typhimurium, Pseudomonas aeruginosa, and Mycobacterium tuberculosis. A recent review covers some aspects of ECF sigma factors and their involvement in pathogenesis (4).

**Sigma E (RpoE)**

rpoE contributes to oxidative stress resistance in S. enterica serovar Typhimurium. To illustrate, inactivation of rpoE diminishes bacterial survival and growth inside host macrophages (21, 82). Further, while an rpoE mutant is severely attenuated in virulence in a mouse model of infection (82, 205), an rpoE mutant strain appears to be fully virulent in gp91phox$^{-/-}$ mice, which are defective in phagocyte oxidative burst (205). Expression of htrA, a gene required for oxidative stress resistance, macrophage survival, and S. enterica serovar Typhimurium virulence (7, 23, 91), is dependent on $\sigma^{E}$ (50, 130). However, the survival and virulence defects in rpoE mutants are not entirely due to loss of htrA expression, because the attenuated virulence phenotype of an htrA mutant is less severe than that of the rpoE mutant (82).

$\sigma^{E}$ also appears to contribute to oxidative stress resistance in other gram-negative pathogens. In Haemophilus influenzae, rpoE expression was discovered to increase 102-fold inside macrophages, and survival of an rpoE mutant was reduced relative to that of the wild type in the macrophage (36). Vibrio cholerae rpoE mutants are virulence attenuated, exhibiting a reduced ability to colonize the mouse intestine and an LD$_{50}$ that is 3 log units higher than that of the wild type (111). Although $\sigma^{E}$ is not essential for growth in H. influenzae or V. cholerae, interestingly, it is essential for growth in Yersinia enterocolitica (76). $\sigma^{E}$ in Y. enterocolitica also appears to regulate htrA, which is important for virulence in this bacterium as well (77, 127).

AlgU of P. aeruginosa (also called AlgT) is homologous and functionally equivalent to $\sigma^{E}$ of E. coli (139, 229). As in the pathogens mentioned above, P. aeruginosa algU mutants have increased sensitivity to oxidative stress (139) and reduced survival in macrophages and neutrophils (230). Additionally, AlgU regulates biosynthesis of alginate, a major virulence factor in P. aeruginosa infections of cystic fibrosis patients. Expression of the major alginate biosynthesis gene algD and production of alginate are dependent on algU (138, 189). Thus, while regulation of oxidative stress resistance is functionally conserved between P. aeruginosa AlgU and $\sigma^{E}$ in multiple bacterial species, AlgU also has an additional role in P. aeruginosa virulence through the regulation of alginate production.

**PvdS and FpvI**

In P. aeruginosa, secretion of the siderophore pyoverdine, a virulence factor, is required for in vivo growth and virulence. Pyoverdine is released when cells experience iron-limiting conditions, which is common during host infection. Pyoverdine enables P. aeruginosa to sequester iron from the environment. The secreted pyoverdine chelates extracellular iron, and the resulting ferri-pyoverdine complex is transported back into the bacterial cell (210), as described below.

The genes involved in pyoverdine synthesis are located in three clusters on the P. aeruginosa chromosome, with the major genes comprising the pvd locus. Among these genes is pvdS, which encodes an alternative sigma factor. PvdS appears to be predominantly responsible for regulating genes in the pvd locus as well as other pyoverdine synthesis genes (147, 160, 198). The binding of iron by pyoverdine, which occurs outside of the cell, initiates a signaling cascade that leads to enhanced expression of pvd genes and additional secretion of pyoverdine and other virulence factors. Upon forming a complex with iron, pyoverdine binds to the FpvA cell surface receptor protein. FpvA is responsible for transporting the pyoverdine into the cell, but it also triggers a signal cascade to the membrane-bound anti-sigma factor, FpvR, which releases PvdS and allows it to transcribe the pvd genes. FpvR also controls the activity of another sigma factor, FpvI (9). The signal from bound pyoverdine also results in release (and hence activation) of this factor, which is responsible for expression of fpvA.

In addition to increasing pyoverdine synthesis and secretion, free PvdS also activates transcription of genes encoding two more virulence factors, those encoding exotoxin A and PrpL endoprotease. Expression of genes responsible for pyoverdine, exotoxin A, and PrpL production is also controlled by the regulator PtxR; expression of ptxR is also controlled by PvdS. A pvdS deletion mutant generates less PrpL (220) and only 5% of the exotoxin A produced by a wild-type strain (159).

Loss of PvdS results in decreased P. aeruginosa virulence in a rabbit aortic endocarditis model (226). The PrpL endoprotease contributes to the ability of P. aeruginosa to persist in a rat chronic pulmonary infection model (220). PvdS is required for virulence and appears to regulate only virulence-related genes.

**Mycobacterial ECF Sigma Factors**

*Mycobacterium tuberculosis* has 13 recognized sigma factors; among these, 10 are ECF sigma factors. At least six *M. tuberculosis* sigma factors affect virulence, including the primary sigma factor (31), $\sigma^{E}$, and four ECF sigma factors, $\sigma^{C}$, $\sigma^{D}$, $\sigma^{E}$,
and σ^H (Table 3). The regulons of many of these sigma factors (σ^C, σ^D, σ^E, σ^F, and σ^H) have been identified through application of M. tuberculosis genome arrays (20, 60, 99, 134, 135, 179, 202). M. tuberculosis ECF sigma factors do not appear to control many currently characterized virulence genes. For example, σ^D does not appear to directly regulate any virulence-associated genes (20, 179), although it does control the putative transcriptional regulator Rv1856. It is possible that this putative regulator is responsible for direct control of virulence gene expression, but no evidence currently exists to support this hypothesis (179). σ^C, σ^F, and σ^H each control a relatively small number of virulence or virulence-associated genes, as well as some regulatory genes that may influence expression of other virulence genes. Several other ECF sigma factors also regulate a number of known or putative regulatory genes (Table 3). Interestingly, in some cases, this group of sigma factors contributes to regulation of other sigma factors within the group. For example, sigB expression is affected by σ^C, σ^F, and σ^H (99, 134, 135, 202). σ^E activates expression of hspX, mtrA, and senX3 (202), three genes shown to be required for virulence. mtrA and senX3 are examples of two-component system regulators. Other virulence-associated genes regulated by M. tuberculosis ECF sigma factors include genes for heat shock proteins and oxidative stress response proteins. For example, the heat shock genes hsp and hspX are σ^E dependent (134), and hsp, dnaK, and clpB are regulated by σ^H (99, 135). A number of putative thioredoxins and other oxidative stress genes are controlled by σ^H (99) (Table 3), and sodA, encoding the superoxide dismutase, is regulated by σ^E (134). The contributions of these ECF sigma factors to expression of oxidative stress resistance genes may explain reduced survival of the respective null mutant strains under oxidative stress conditions or inside macrophages (134, 135).

Recently, deletion of sigC was shown to render M. tuberculosis unable to cause death in infected mice (202). Deletion of another sigma factor gene, sigH, also produced a nonlethal strain (99). Interestingly, despite the inability to cause fatalities, both sigC and sigH mutants grew to wild-type numbers in macrophages and murine tissues (99, 135, 202). Although the reasons for the similar phenotypes in the two different mutant strains are unknown, it is possible that a subset of virulence-associated genes are regulated by both factors. Alternatively, σ^C and σ^H may provide similar contributions to M. tuberculosis, but through different mechanisms. σ^E appears to affect M. tuberculosis virulence differently than σ^C and σ^H. As with sigH, sigE expression is induced inside macrophages (64, 90). Loss of σ^E, however, does result in decreased survival strain in macrophages and a greater susceptibility to killing by activated macrophages (134). In mouse infection models, the sigE mutant is delayed in its ability to cause lethality but is not completely compromised, as with the sigC and sigH mutant strains (2, 136). Manganelli et al. (136) reported a lower number of sigE mutants in the lungs compared to the wild type, while Ando et al. (2) reported no difference. This discrepancy may be due to differences in mouse strains used in the two studies.

Multiple studies suggest that σ^D also contributes to M. tuberculosis virulence. Deletion studies of sigD show the mutant to be less virulent than the wild type in BALB/c and C3H:HeJ mouse infections, allowing substantially longer mouse survival (20, 179). The ΔsigD strain did not show a difference in time to death in SCID mice, which lack T and B cells (20), suggesting that σ^D regulates pathogenicity in a manner that is dependent on cell-mediated immunity. In addition, loss of σ^D resulted in much milder tissue damage and granuloma formation in lung tissue histopathology in BALB/c mice (179).

Several alternative sigma factors present in M. tuberculosis affect virulence, whether through direct, indirect, or both types of strategies. In addition, some alternative sigma factors of M. tuberculosis autoregulate transcription of their own genes. Many sigma factors also activate transcription of other alternative sigma factors (Fig. 2C). In all, M. tuberculosis appears to have control over expression of its virulence genes via a complex network of multiple alternative sigma factors.

HrpL

Psuedomonas syringae is a plant pathogen with several pathovars that display selective host specificity. Infection of a plant by a specific pathovar will cause disease in susceptible host species, while eliciting a programmed cell death termed the hypersensitive response (HR) in resistant plants. The groups of genes responsible for both of these reactions have been termed hrp and avr. These gene products encode either the type III secretion machinery that translocates proteins into host plant cells or the effector proteins that are delivered and that interact with host elements. Most of the hrp genes are regulated by the alternative sigma factor HrpL, which has been shown by microarray analysis to be almost exclusively responsible for virulence functions (56). Strains with mutations in hrp genes cannot elicit disease or HR in plants (for a review, see reference 120). Likewise, inactivation of HrpL decreases P. syringae pv. Phaseolicola growth in leaves (178).

Another important phytopathogen, Erwinia amylovora, also utilizes an hrp-encoded type III secretion system. As in P. syringae, E. amylovora HrpL is an alternative sigma factor that directs transcription of several hrp genes (104). Inactivation of HrpL prevents E. amylovora from causing disease in susceptible plant species or HR in resistant plants (216). E. amylovora also has a dsp, or “disease-specific,” gene cluster which is homologous to the avr genes of P. syringae (15), dspA is dependent on HrpL for expression and is required for virulence (58). In addition to E. amylovora, several other members of the Erwinia genus carry hrpL and other hrp genes, including the tumorigenic pathogen Erwinia herbicola (149, 155, 156) and the soft-rot pathogens Erwinia carotovora (24, 125, 180) and Erwinia chrysanthemi (6). The hrp-encoded type III secretion system is thus a common virulence mechanism among plant pathogens and is widespread among several types of pathogens, including tumorigenic, macerating, and soft-rot-causing species.

SIGMA 54

σ^4 forms a distinct subfamily of sigma factors, apart from the σ^70-like family. In almost all species, the σ^4 factor is called σ^54. σ^54 has been identified in many species, spanning a diverse phylogeny, including Legionella pneumophila (88), Pseudomonas spp. (72, 86, 108), Enterococcus faecalis (40), Campylobacter jejuni (89), and Listeria monocytogenes (183). A physiological theme for σ^54-dependent genes has not yet emerged, as the regulated genes described to date control a wide diver-
sity of processes (Table 4). Often nitrogen metabolism is controlled by $\sigma^N$, but other functions of $\sigma^N$-dependent genes can be found in several organisms.

**Sigma N**

*Pseudomonas aeruginosa*. Evidence of $\sigma^N$ involvement in bacterial pathogenesis and virulence is well documented for *P. aeruginosa*. Alginate has been identified as a virulence factor that is important in strains colonizing cystic fibrosis patient lungs. $\textit{algD}$ and $\textit{algC}$, two important genes for the biosynthesis of alginate, are controlled by $\sigma^N$ (17, 233). In addition, through gene fusion and microarray studies, expression of a large number of flagellar structural genes was shown to be dependent on $\sigma^N$ (41).

Flagellar motility and pilus-mediated attachment are established virulence factors in *P. aeruginosa* (148, 186). Pili are external structures that are responsible for adhesion to host cells and interactions such as internalization. *P. aeruginosa* $\textit{rpoN}$ mutants do not produce pilin or form pili (206), and they demonstrate drastic loss of adhesion to multiple cell types (28, 32, 172). Wild-type *P. aeruginosa* also is internalized by host cells more efficiently than an $\textit{rpoN}$ mutant (172), suggesting an enhanced capacity of the wild-type strain to invade host cells. Reduced virulence due to loss of flagellar motility is also possible in $\textit{rpoN}$-disrupted strains, as mutants are decidedly non-motile (73, 206). $\textit{rpoN}$ mutants also do not produce the proteinaceous flagellin subunit or form flagella (206). Several studies have shown that *P. aeruginosa* strains lacking flagella are severely virulence attenuated (46, 52, 148).

*P. aeruginosa* $\textit{rpoN}$ mutants are also less virulent than wild-type strains in multiple infection models. An $\textit{rpoN}$ mutant strain showed diminished cytotoxicity to Madin-Darby canine kidney (MDCK) cells (32) and reduced virulence in several mouse models specifically developed to study *P. aeruginosa* pathogenicity; compared to the wild type, $\textit{rpoN}$ mutants cause lower mortality rates in infected mice (32, 73) and reduced fecal carriage and recovery from gastrointestinal tissues (170). In addition, no pathology was observed following infection with an $\textit{rpoN}$ mutant in a murine corneal scratch model (173). Cohn et al. (30) reported that $\textit{rpoN}$ mutants did not readily colonize human tracheal epithelium xenografts implanted in mice, although the difference in bacterial numbers of the mutant and wild-type strains was not statistically significant. In general, the defects associated with the $\textit{rpoN}$ mutation were greater than with strains that were specifically pilin negative, indicating the existence of an additional, pilus-independent mechanism through which $\sigma^N$ also contributes to virulence (28, 32, 170, 172).

*Pseudomonas syringae*. $\sigma^N$ of *P. syringae* controls $\textit{hrp}$ gene expression and influences virulence. Regulation occurs via a short regulatory cascade, wherein $\sigma^N$ and its enhancer-binding proteins HrpR and HrpS direct transcription of $\textit{hrpL}$, the product of which is the alternative sigma factor required for expression of the $\textit{hrp}$ and $\textit{avr}$ genes (83) (Fig. 2B). Xiao et al. (225) showed that while expression of $\textit{hrpL}$ and $\textit{hrpL}$-dependent genes requires $\textit{hrpR}$ and $\textit{hrpS}$, constitutive expression of $\textit{hrpL}$ can provide full expression of $\textit{HrpL}$-dependent genes with or without $\textit{hrpR}$ and $\textit{hrpS}$. In addition, $\textit{avrD}$, which is transcribed from an $\textit{HrpL}$-dependent promoter, requires $\textit{rpoN}$, $\textit{hrpL}$, and $\textit{hrpS}$ for its expression (192). Characterization of *P. syringae* pv. Maculicola $\textit{rpoN}$ mutants identified a more severe phenotype than in $\textit{hrpL}$ mutants, however (72). $\textit{rpoN}$ mutants were nonmotile, displayed nitrogen utilization defects, and were unable to produce the phytotoxin coronatine, cause disease or HR, or induce host defense mRNAs. Complementation of $\textit{hrpL}$ into this strain partially restored some phenotypes but did not restore coronatine production. Other studies have also shown that $\sigma^N$ is required for production of coronatine biosynthetic intermediates (synthesized by the $\textit{cft}$/CMA and $\textit{cmaABT}$ gene products) as well as $\textit{hrpL}$ transcription and HrpL-dependent gene expression (1, 71). Thus, $\sigma^N$ regulates a range of virulence factors in *P. syringae*, some via $\textit{hrpL}$ activation and others by HrpL-independent mechanisms.

*Vibrio species*. The contributions of $\sigma^N$ to virulence in *Vibrio* species are similar to its contributions in *P. aeruginosa*. *V. cholerae* $\textit{rpoN}$ mutants lack flagella and are completely non-motile (105). In a competitive infant mouse colonization trial, an $\textit{rpoN}$ mutant was 10- to 20-fold less able to colonize the intestine than the wild type (105). This defect is not entirely due to lack of flagella, because a $\textit{flaA}$ mutant, while inhibited in intestinal colonization, was still superior to the $\textit{rpoN}$ mutant in colonization. Prouty et al. (176) also demonstrated the involvement of $\sigma^N$ in expression of several *V. cholerae* flagellar structural genes. $\sigma^N$ is required for flagellin production and motility in the fish pathogen *Vibrio anguillarum* as well. A mutant lacking $\sigma^N$ was also severely impaired in its ability to infect fish immersed in contaminated water but was not virulence attenuated in an intraperitoneal injection model (163).

Other species. $\sigma^N$ contributes to virulence in a number of gram-negative pathogens. In addition to the examples provided above, the uropathogen *Proteus mirabilis* is 1,020-fold less virulent than the wild type when $\sigma^N$ is inactivated but remains identical to the wild type with respect to growth, glu-

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TABLE 4. Virulence genes regulated by $\sigma^N$ in multiple bacterial species

<table>
<thead>
<tr>
<th>Species</th>
<th>Virulence mechanism</th>
<th>Gene(s)</th>
<th>(reference[s])</th>
</tr>
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<tbody>
<tr>
<td><strong>H. pylori, C. jejuni, V. cholerae</strong></td>
<td>Flagella</td>
<td>Class II flagellar genes (flaA and structural) (89, 154, 176)</td>
<td></td>
</tr>
<tr>
<td><strong>P. syringae, E. carotovora</strong></td>
<td>Type III secretion</td>
<td>$\textit{hrpL}$ (24, 83)</td>
<td></td>
</tr>
<tr>
<td><strong>P. aeruginosa</strong></td>
<td>Flagella</td>
<td>Class II flagellar genes (regulatory and structural) (41)</td>
<td></td>
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<tr>
<td></td>
<td>Alginate</td>
<td>$\textit{algD}$, $\textit{algC}$ (17, 233)</td>
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<td></td>
<td>Pili</td>
<td>$\textit{pilA}$ (206)</td>
<td></td>
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</tbody>
</table>

$^a$ Virulence systems regulated by $\sigma^N$-dependent genes.

$^b$ Specific genes or types of genes within a virulence system that are regulated by $\sigma^N$. 

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Downloaded from mmbr.asm.org at Albert R. Mann Library on August 11, 2009
amine synthesis, and fimбриa production (231). $\sigma^N$ does not share a common role among all pathogens, however. For example, the plant pathogens, *Pseudomonas syringae*, *Erwinia carotovora*, and *Xanthomonas campestris* all use type III secretion systems to cause disease in host organisms, and $\sigma^N$ has a substantial effect on virulence and *hrc* gene expression in *P. syringae* and *E. carotovora* (24) but is not required for expression of *hrc* genes or for virulence in *X. campestris* (78). As with the low-GC bacterial $\sigma^B$, $\sigma^N$ appears to be an alternative sigma factor that has evolved to regulate virulence determinants in some species but not in others.

CONCLUSIONS

Bacteria utilize alternative sigma factors to regulate a wide range of physiological processes. In pathogenic bacteria, alternative sigma factors often affect virulence. Virulence effects can be mediated either through direct virulence gene regulation or indirectly, by regulating genes that increase fitness of the bacterium during transmission and infection. Direct effects on virulence genes include $\sigma^B$ activation of the *L. monocytogenes* virulence genes *inlA* and *prfA* and $\sigma^D$-dependent expression of the *S. enterica* serovar Typhimurium *spv* genes. Indirect effects of sigma factors on virulence may be more difficult to identify, but alternative sigma factors frequently have roles in virulence by regulating virulence-associated genes that aid in a bacterium’s survival during infection. For example, $\sigma^E$ enhances survival of oxidative stress and hence aids in bacterial survival of the oxidative burst within macrophages. The stress response sigma factors $\sigma^B$ and $\sigma^D$ contribute to survival of multiple stresses (e.g., acid and osmotic stresses) important for bacterial survival of passage through a host stomach and gastrointestinal tract. In addition, $\sigma^A$ and $\sigma^S$ contribute to environmental survival, and thus transmission, of food-borne pathogens in foods and food-processing environments. Another alternative sigma factor role that contributes to environmental survival, and has virulence implications, is regulation of biofilm formation, e.g., by $\sigma^B$ in *S. aureus* and *S. epidermidis*.

Functional roles for alternative sigma factors can be clearly defined and highly specific (e.g., sporation sigma factors) or multifunctional. While *Pseudomonas syringae* *HrpL*’s role is predominantly virulence related, most alternative sigma factors contribute to multiple, diverse functions in a cell. In some cases, sigma factors are conserved across pathogenic and non-pathogenic species, with virulence genes constituting a relatively small subset of the total regulon in the pathogenic species. For example, $\sigma^A$ is present and contributes to stress resistance in the nonpathogenic *B. subtilis* and *Listeria innocua* (S. Raengpradub, unpublished data), both of which are closely related to the pathogenic *L. monocytogenes*. It is possible that virulence gene incorporation into the *L. monocytogenes* $\sigma^A$ regulon is a relatively recent evolutionary event. Likewise, as no evidence currently supports a direct role for $\sigma^A$ in *E. coli* virulence gene regulation, the inclusion of virulence genes in the regulatory network of *S. enterica* serovar Typhimurium $\sigma^A$ may have occurred after the species divergence of *S. enterica* serovar Typhimurium and *E. coli*.

A comparison of homologous sigma factor functions among different bacterial genera reveals that the roles of sigma factors vary greatly among bacterial species, even for closely related species such as *E. coli* and *S. enterica* serovar Typhimurium. In some cases, as with *M. tuberculosis* $\sigma^R$, distinct virulence-related phenotypes have been observed in alternative sigma factor null mutants. For others, such as *S. aureus* $\sigma^H$, while virulence genes are directly transcribed by the sigma factor, $\Delta\sigma^B$ strains are not severely virulence attenuated. Even more apparent are the different roles for $\sigma^A$, $\sigma^N$ is required for virulence in *S. enterica* serovar Typhimurium and yet does not demonstrate a pronounced role in *E. coli* pathogenesis.

A common mechanism of virulence regulation by alternative sigma factors involves coordinated networks of sigma factors along with other transcriptional regulators. Alternative sigma factors may regulate not only individual genes involved in virulence but also other sigma factors or transcriptional regulators that in turn regulate virulence genes and virulence-associated genes (Fig. 2). For example, $\sigma^D$ of *L. monocytogenes* not only directly regulates *bsh* and *inlA* but also contributes to expression of PrfA, which is required for transcription of almost all of the currently recognized *L. monocytogenes* virulence genes. $\sigma^D$ of *S. aureus* also affects expression of a virulence gene regulator, RNAIII, $\sigma^N$ and HrpL of *P. syringae* present a different type of regulatory network, in which one sigma factor controls expression of another. HrpL also controls expression of the HrpR and Hrps two-component system regulators. Regulatory networks can be very complex, as in the multiple sigma factor interactions of *M. tuberculosis*.

Finally, to extrapolate bacterial pathogen research findings to ensure relevance in human infection, the importance of identifying and applying suitable model systems that accurately mimic interactions between pathogen and humans is essential. This point is illustrated by the significantly reduced traversal of the intestinal barrier by *L. monocytogenes* in wild-type versus (human) E-cadherin transgenic mice (121). In addition, pathogens such as *P. aeruginosa* that can infect a multitude of different hosts are likely to respond differently and to have different virulence requirements depending on the host species. Significant efforts are still needed to identify or develop appropriate model systems for exploration of virulence mechanisms that are important in human infection.

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