Siderophores have been studied in great detail over the last 20 years, and although our knowledge of these special iron carriers is vast, new insights and new twists to this well-told story are regularly appearing. In this issue of *Journal of Bacteriology*, Voulhoux et al. (15) describe the effect of the twin-arginine translocation (Tat) system on the biosynthesis and the uptake of siderophores by *Pseudomonas aeruginosa*. The Tat pathway is a specialized transport system for the translocation of folded proteins across the cytoplasmic membrane. The signal sequences of proteins that are translocated via the Tat pathway almost invariably possess a twin-arginine (RR) motif and are usually longer than Sec secretion signal peptides. So, is it not possible to predict all Tat substrates from the primary sequence of the signal sequence? The article of Voulhoux et al. shows that this is not the case, and the reason is that some signal sequences just do not seem to obey the general rules. An example is the FpvA siderophore receptor of *P. aeruginosa*.

Siderophores are low-molecular-weight catechol or hydroxamate compounds that bind iron with high affinity and are therefore able to sequester and solubilize minute quantities of free iron present in the environment. These secreted iron scavengers have to be recaptured by the bacterium. For this, gram-negative bacteria have developed a special outer membrane transport system that consists of a dedicated outer membrane receptor and a cytoplasmic membrane complex composed of the ExbB, ExbD, and TonB proteins. The TonB system transduces the proton-motive force of the cytoplasmic membrane to the high-affinity outer membrane receptors, which are therefore known as TonB-dependent receptors. Siderophore-mediated scavenging of iron is a sufficient and widespread mechanism, but there is, however, a more favorable alternative: why waist energy on the production of siderophores that are secreted and might never return, if you could also steal them from your neighbors? In fact, that is exactly what happens. Some bacteria produce a large array of different TonB-dependent receptors, each with its own specificity, in order to steal their neighbors’ siderophores (heterologous siderophores) loaded with iron. The human opportunistic pathogen *P. aeruginosa*, for instance, possesses receptors for its own siderophores pyoverdine and pyochelin, but in addition it has the ability to produce 32 other TonB-dependent receptors. These additional receptors are used for the uptake of enteroactin produced by different *Enterobacteriaceae* (4, 6) and ferrioxamine or ferrichrome produced by several bacteria and fungi, respectively (9). The beauty of many of these receptors is that they are produced normally in tiny amounts and are induced only upon the presence of these heterologous siderophores in the environment. The most widespread regulatory mechanism for this involves the receptor itself in a transenvelope signal transduction pathway, also known as surface signaling (for a recent review, see reference 2). Sometimes a similar regulatory system controls the production of the receptor for the homologous siderophore, such as the pyoverdine receptor FpvA of *P. aeruginosa* (1). To distinguish this subclass of receptors, which are involved in both siderophore transport and signal transduction, from conventional TonB-dependent receptors, they have been called TonB-dependent transducers (7). These transducers sense an extracellular signal (i.e., the presence of their cognate siderophore), which is transmitted via an anti-sigma factor in the cytoplasmic membrane. This transduction pathway leads to the activation of a specific, alternative extracytoplasmic function sigma factor and to the subsequent transcription of a limited number of target genes, including the gene encoding the siderophore receptor itself and possibly genes encoding a periplasmic transport system (2). Interestingly, TonB-dependent transducers possess a unique N-terminal extension of about 70 to 80 amino acids compared to normal TonB-dependent receptors (7, 8, 9). This extension determines the specificity of the transduction pathway but has no effect on the binding and transport of the siderophore. Therefore, this domain is thought to interact with the anti-sigma factor.

Another peculiar characteristic of these TonB-dependent transducers is that many of them contain long and unusual signal sequences. For instance, the signal sequences of the *P. aeruginosa* pyoverdine receptor FpvA and the recently identified ferrioxamine receptor FoxA are 43 and 47 residues, respectively (9, 11). These signal sequences have a normal cleavage site preceded by a normal hydrophobic stretch, but the positively charged N-terminal region of the signal sequence is significantly enlarged (Fig. 1). What could be the function of these long signal sequences? In bacteria, protein translocation across the cytoplasmic membrane occurs via two major routes, the Sec and the Tat pathways. Whereas the Sec pathway is used by a whole array of different proteins that are destined for the cell envelope, the Tat pathway, as mentioned before, seems to be dedicated to transport folded proteins. Most of these folded proteins contain redox cofactors, such as iron-sulfur clusters, molybdopterin, and copper, that have to be assembled in the cytoplasm. The initial stage in determining which pathway a protein will use to cross the membrane is generally dictated by the protein’s N-terminal signal sequence (14). In light of this, one might predict that signal sequences directing traffic to separate pathways would themselves be fairly distinct. However, Tat and Sec signal sequences appear to be remarkably similar: both possess positively charged residues in the N-terminal region, followed by a hydrophobic stretch and a pep-
tidase cleavage site. But Tat signal sequences have additional targeting information, as in the presence of a twin-arginine motif. Some of the unusual signal sequences of the TonB signal transducers, such as that of the FpvA receptor, are potential Tat-like signal sequences (5, 10). Based on this prediction and on experimental evidence showing that a P. aeruginosa tat mutant does not produce pyoverdine and is also not rescued by the presence of pyoverdine in the medium, it has been claimed that FpvA is translocated across the cytoplasmic membrane via the Tat system (10). In their report, Voulhoux et al. (15) have now clearly demonstrated that FpvA translocation is not, in fact, Tat dependent. In contrast with the previous study (10), Voulhoux and colleagues, using a combination of biochemical and biophysical experiments, provide direct evidence for the correct localization and full functionality of FpvA in the outer membrane in a tat mutant. This is in agreement with the data obtained from the recently elicited crystal structure of FpvA that shows that this receptor is similar to other TonB-dependent receptors (i.e., a 22-stranded beta-barrel transmembrane domain with a plug-forming N-terminal domain), and does not contain a bound redox cofactor; therefore, there is no clear reason for Tat-dependent transport (3). In addition, the newly released TatP 1.0 prediction program does not predict a Tat motif for FpvA. However, this new prediction program does not solve all problems, because it still predicts that other TonB-dependent transducers, such as FoxA and Pseudomonas putida PupB (8), do have a potential Tat signal sequence. Based on the work of Voulhoux et al. (15) and knowledge of the general structure of TonB-dependent receptors, it is not likely that these receptors are translocated via Tat; this finding shows the importance of experimental analyses of these predictions. Voulhoux et al. also show that, although FpvA translocation is not Tat dependent, the production of pyoverdine is, as was also described previously (10, 15). The authors convincingly demonstrate that at least one of the proteins involved in the biogenesis of pyoverdine, PvdN, a still unknown but essential component in pyoverdine biogenesis, is transported into the periplasm by the Tat pathway. The investigators’ results further support the idea that this enzyme is probably part of a multiprotein complex involved in the synthesis of pyoverdine.

So, if the unusually long signal sequences of the TonB-dependent transducers are not directing these proteins to the Tat pathway, what is their function? Recently, two papers have been published that could give some clues. In these papers, Sijbrandi et al. (12) and Szabady et al. (13) study another group of large outer membrane proteins, the autotransporters. A subset of autotransporters also contains unusually long signal sequences that are, in fact, similar in composition to those of the TonB-dependent transducers (Fig. 1). Sijbrandi et al. (12) showed that these long signal sequences interact with the Sec translocon and with the signal recognition particle. This means that these proteins are transported cotranslationally. Furthermore, Sijbrandi et al. also show that SecB is not required for targeting of these autotransporters. Szabady et al. (13) also found Sec-dependent translocation. However, they also noticed that these long signal sequences transit slowly through the Sec machinery. Remarkably, normalization of the signal sequence did not affect transport but did result in misfolding of the mature protein in the periplasm. Based on these results, it is proposed that these unusual signal peptides allow the correct folding of the passenger domain in the periplasm, perhaps by delaying the translocation. Since TonB-dependent transducers are also very large outer membrane proteins, incorrect periplasmic folding might be anticipated if normal signal sequences are used, a hypothesis that can now be tested.

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FIG. 1. Signal sequences of E. coli EspP autotransporter and of the P. aeruginosa FpvA and PvdN proteins. The characteristic N, H, and C regions found in typical signal peptides are indicated as well as the long N-terminal extension of EspP and FpvA. The twin-arginine (RR) motif of PvdN is underlined.


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