S100B–p53 peptide complex structure demonstrates that there is no one mode of S100–target interaction, making the structurally homologous family of S100 proteins much more diverse than previously believed.

Calcium everywhere

The structure of S100B complexed with the p53 peptide provides the first glimpse of how an EF-hand protein recognizes a transcription factor in order to regulate transcriptional activity. Other examples of EF-hand proteins involving transcription regulation include the down-stream regulatory element antagonist modulator DREAM, which functions as a Ca^{2+}-dependent DNA-binding transcriptional repressor[24]. Clearly Ca^{2+} signaling has diverse cellular functions ranging from transmembrane and cytoplasmic signal transduction to gene regulation. Ca^{2+}–S100B-mediated regulation of p53 transcription activity provides a possible link between Ca^{2+} signaling and oncogenic processes in which the tumor suppressor p53 plays key roles. Future questions on the p53–S100B interaction include how S100B interacts with the tetramerization domain of p53 and how S100B binding influences the structural stability of p53 both *in vitro* and *in vivo*.

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TolC, a macromolecular periplasmic 'chunnel'

Kathleen Postle and Hema Vakharia

The crystal structure of TolC, one of the most mysterious proteins in the outer membrane of Gram-negative bacteria, suggests a mechanism for its role in secretion of proteins and efflux of toxic chemicals.

Two concentric membranes, the cytoplasmic (or plasma) membrane and an outer membrane fenestrated with protein-based pores, surround Gram-negative bacteria, with an aqueous compartment termed the periplasmic space between the two membranes. Due to the nature of these membranes, the trafficking of proteins and nutrients in and out of Gram-negative bacteria is much more complex than if only a single membrane were present. The crystal structure of the outer membrane protein TolC (tolerance to colicins) has just been reported[1] in a recent issue of *Nature* and has greatly enhanced our ability to understand these processes.

To address these complex modes of transport, it has been important to make mutations in transport protein genes and attempt crystallization of the transport proteins — a daunting task since they are integral membrane proteins. Although relatively few bacterial cytoplasmic membrane proteins have been crystallized, it has been fairly straightforward to isolate mutations in their corresponding genes in order to understand their function, and even make reasonable guesses about their structures. For virtually all, characteristic and specific functions have been established.

The transmembrane domains of the cytoplasmic membrane proteins are almost certainly α-helical, and their locations can be predicted with some reliability by examining the primary amino acid sequence with algorithms that identify long (~20 amino acid) hydrophobic stretches. The situation is quite different for outer membrane proteins, which thus far, at least, use β-strands to span the outer membrane. Because as few as six amino acids are needed to form a β-strand, only some of which must be hydrophobic in order to traverse the outer membrane, the 'signature' of a β-strand is much less apparent. Thus, from the very beginnings of sequence-gazing efforts, it has been difficult to make accurate predictions regarding the occurrence and placement of secondary structures in outer membrane proteins. However, like cytoplasmic membrane proteins, outer membrane proteins have been fairly straightforward to characterize mutationally.

Pore proteins were the first outer membrane proteins to be crystallized, and it was discovered that they have a rather simple structure — a trimer of β-barrels[2]. The temptation since has been to predict that simple β-barrels are the only types of proteins to be found in the outer membrane. The first examples to contradict this notion were FepA (ferric uptake) and FhuA (ferric hydroxamate uptake), where a globular signaling domain fills the hole in the β-barrel[3–5]. The globular domain

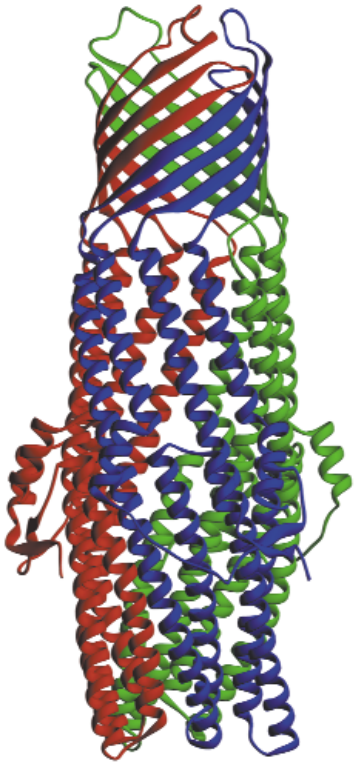

Fig. 1 The crystal structure of TolC. The three individual subunits are colored in red, blue, and gold. The external cell surface is at the top and the periplasm is at the bottom. The β-barrel sits in the outer membrane, which is not shown.

binds ligands at the external surface of the bacterium and signals their presence by means of large molecular movements on the periplasmic side of the membrane[4]. The globular domain must also somehow be the means by which FepA and FhuA actively transport ligands across the outer membrane. Given their functions, it makes sense that the structures of FepA and FhuA would be more complex.

For the *Escherichia coli* TolC outer membrane protein, the situation has been decidedly confusing. Structure predictions have been unsatisfactory and divergent. While all predictions invoked some sort of β-barrel, they varied widely in predicted size, amount of α-helical content, and degree and type of extension into the aqueous periplasmic space between cytoplasmic and outer membranes[6–8]. To complicate matters, TolC mutations resulted in highly pleiotropic, and often mystifying, phenotypes. Thus, the TolC structure determination is especially satisfying since it resolves some of the confusion generated by the structural predictions and provides clues to understanding the observed pleiotropy.

The crystal structure of TolC

The structure of TolC is an obligate trimer of identical subunits (Fig. 1), and in that respect it is unique among the structures of other outer membrane proteins. Each subunit contributes only four of the β-strands that make up the final 12-stranded β-barrel. TolC is much more than just a simple β-barrel. Hanging below the β-barrel, like ordered jellyfish tentacles, are a series of α-helical coiled-coils, 100 Å long dipping into the periplasm. Koronakis *et al.*[1] refer to TolC as a 'channel-tunnel', leaving one with the irresistible urge to refer to it as the first ever macromolecular 'chunnel'. The α-helices taper at the periplasmic end, consistent with the observation that, while TolC can form pores in lipid bilayers, they are tiny ones[9]. Other proteins (see below) must be needed to expand the outer membrane-distal pore temporarily to allow its numerous (and large) substrates to pass through, and Koronakis *et al.*[1] have modeled what that may look like (Fig. 2*b*).

TolC mutants were originally isolated over 30 years ago, with the phenotype of resistance to the protein colicin E1 (ColE1), one of a wide variety of niche-warfare agents synthesized by some *E. coli* strains that are capable of killing neighboring bacteria that do not produce colicins themselves[10]. Because the mutants survived killing by ColE1, but still bound the colicin as well as wild type bacteria, they were considered to be tolerant, rather than resistant (non-binding), hence the mnemonic 'Tol'. Numerous other *Tol* genes have also been isolated, *TolA–TolZ*, some of which play a role in TolC-dependent entry of ColE1 into the bacteria[11]. However, over time, the accumulation of TolC-related phenotypes that are seemingly unrelated to the original phenotype or to each other in many cases, has been baffling (Table 1).

The role of TolC in secretion/efflux

The TolC structure helps explain the primary phenotype of the TolC mutants — the inability to secret several 'attack' proteins such as hemolysin and the inability to protect cells by efflux of noxious agents such as hydrophobic antibiotics (for example, tetracycline) out of the cell (Table 1). Bacteria are capable of synthesizing proteins in their cytoplasm that are then transported to the external environment in a process known as secretion. Bacteria are similarly protected from the effects of harmful environmental molecules that can leak past their membranes by their ability to transport the noxious agents back to the external environment in a process known as efflux. Periplasmic intermediates have never been detected for either of those processes, indicating that some sort of directed and sequestered path through the periplasm was present. Based on the crystal structure, TolC could potentially do the job by spanning the periplasmic space.

However, TolC cannot mediate secretion/efflux by itself. Two other classes of proteins are also known to be required[12]: (i) the integral cytoplasmic membrane protein components, which have homology to other traffic ATPases and are pre-

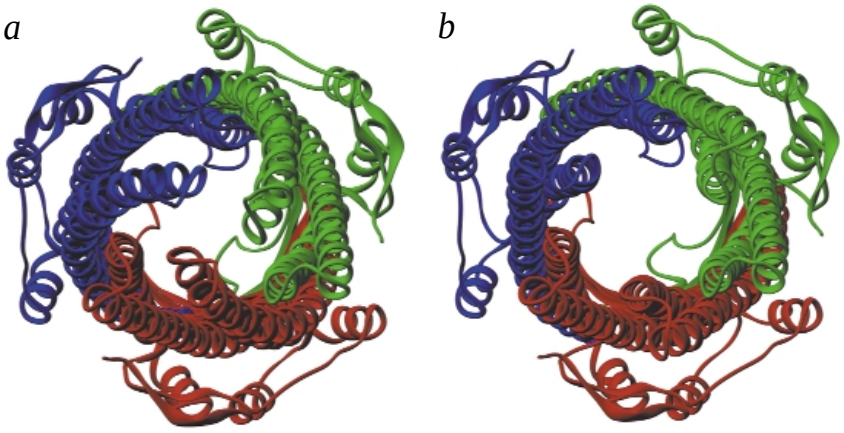

Fig. 2 Structure and model of TolC. ***a***, A view of crystallized TolC in the closed state, looking down the 3-fold axis of symmetry from the periplasmic end, where coiled coils close the channel. ***b***, A model of TolC in the open state, illustrating how the channel could open as the inner coiled coils move to the external boundary of the α-helical barrel. This motion is presumed to be initiated and/or stabilized by the ATPase/periplasmic proteins that are required for TolC function (Table 2).

Table 1 Phenotypes associated with *tolC* mutants

Phenotypes	Reference
Tolerance to Colicin E1	10
Anucleate cells	18
Loss of hemolysin ColV, heat-stable enterotoxins STIp, ST_B and microcin secretion	24–28
Hypersensitivity to hydrophobic antibiotics, dyes and detergents	29
Reduced levels of OmpF expression	20
Altered DNA supercoiling	19

sumed to provide the energy for the secretion/efflux; and (ii) the 'periplasmic' protein components, which are anchored to the cytoplasmic membrane by a protein or lipid tether, but primarily occupy the periplasm. These proteins exist as pairs dedicated to the secretion or efflux of specific ligands (Table 2). The periplasmic protein components have been hypothesized to serve as a bridge between the cytoplasmic membrane ATPase and TolC in the outer membrane. Whether this is true and to what extent they function as bridges, or have other functions, will largely depend on the size of the periplasm and the degree to which the ATPase proteins (which have not been crystallized) protrude into it. Unfortunately, the size of the periplasmic space is controversial[13], with estimates ranging from 70–250 Å between cytoplasmic and outer membranes. To fully understand the mechanism of secretion/efflux, it will be important to determine whether TolC can interact directly with the ATPase components. Based on the degree to which TolC occupies the periplasmic space, this becomes a real possibility.

Interestingly, for hemolysin secretion, the presence of secretable ligand allows detectable complex formation of TolC simultaneously with the ATPase and periplasmic protein partners[14,15]. Since TolC can function interchangeably in so many different systems (Table 2), transient complex formation may be a common theme. Reversible complex formation could be either an indication that Gram-negative bacteria cannot function optimally with membranes fused together at too many sites, or a reflection of multiple roles for a limiting component, or both.

Other roles of TolC

Bacteria divide by binary fission, so it is important for each daughter cell to receive at least one copy of the chromosome. To expedite orderly chromosome segregation, the origin of replication (*oriC*) appears to be tightly bound to regions of outer membranes[16], which would then grow and move apart as the cells grow and divide. A role of TolC in chromosome partitioning (the anucleate cell phenotype; Table 1) would be consistent with the crystal structure.

Chromosome segregation has been shown to involve the proteins SeqA (sequestration) and HobH (hemimethylated *oriC* binding), neither of which has a signal sequence that could direct it to the cytoplasmic or outer membranes[17]. TolC expression is six-fold down-regulated in the *seqA* mutants[17] and TolC mutations result in the production of anucleate bacteria[18]. Thus it is tempting to speculate that TolC could be one of the contact sites for the replicative origins in the outer membrane, especially since TolC protrudes so deeply into the periplasm.

Bacteria maintain their DNA in a highly negatively supercoiled state. Expression of a number of genes is sensitive to the level of DNA supercoiling. Absence of TolC increases expression from a supercoiling-sensitive promoter for ProU (proline uptake)[19] and leads to a decrease in the levels of OmpF (outer membrane protein F) in the outer membrane[20]. OmpF expression can also be regulated by changes in DNA supercoiling[19]. In contrast to the effects on secretion/efflux and chromosome segregation, the effect of TolC on DNA supercoiling (Table 1) is probably indirect since TolC does not protrude through the cytoplasmic membrane and into the cytoplasm where gene regulation takes place. Thus it is reasonable to suspect that TolC mutants somehow create or respond to environmental conditions in such a way as to affect supercoiling[19]. Perhaps TolC mutants alter membrane integrity such that cells are duped into responding to nonexistent stimuli. In any case, this change in the level of DNA supercoiling found in *tolC* mutant strains suggests that there may be other, as yet undiscovered, TolC roles in maintaining normal cell physiology.

TolC and colicins

Colicins are large proteins, 300–700 amino acids in length. They can be divided into three functional domains, with a receptor binding domain bordered on the N-terminal side by a translocation domain and on the C-terminal side by an activity domain, which kills sensitive bacteria by a number of different means, depending on the colicin. The crystal structure of colicin Ia shows a largely α-helical, highly elongated molecule, with the receptor binding domain at one end and, ~200 Å away, the activity domain and the translocation domain[21]. Based on amino acid similarities, it is not unreasonable to assume that other colicins will have similar overall structures. Somehow the colicins must deliver the activity domains to the appropriate target sites.

The different proteins used by colicins to gain access to their intracellular targets is worthy of a review in itself, just to catalog all the variations on a theme. We actually know very little about the actual mechanisms of access, and in particular, the means by which they get across the outer membrane, although it is clear that they must first bind a receptor. Translocation of colicin across the outer membrane is the second step, and for two colicins, ColE1 and Col10, it is here that TolC is apparently required[10,22]. Like the secretion/efflux role for TolC, sets of three cytoplasmic membrane proteins (TolQ,R,A for ColE1 or TonB (bacteriophage T1 resistance),

Table 2 TolC partner proteins in secretion/efflux

Substrate	ATPase	Periplasmic protein	Reference
HlyA of *Escherichia coli*	HlyB	HlyD	24
ColV of *Escherichia coli*	CvaB	CvaA	25
Haemophore HasA of *Serratia marcescens*	HasD	HasE	30
Protease C of *Erwinia chrysanthemi*	PrtD	HasE	30
Protease SM of *Serratia marcescens*	PrtD	PrtE	31
Heat-stable enterotoxin Ip of *E. coli*	?*	?*	26
Hydrophobic antibiotics, dyes and detergents	AcrB	AcrA	29
Fluoroquinolone resistance	MexY	MexX	32
Microcin J25 secretion	?	?	28

? indicates that the ATPase and periplasmic components have not been identified.
* STIp is very small (18 amino acids), has a periplasmic intermediate and appears to use TolC to cross the outer membrane.

ExbB (excretion of ColB inhibitor), and ExbD (excretion of Col10 inhibitor) are also involved in the translocation step.

Interestingly, the interaction of these cytoplasmic membrane proteins with TolC is outside of what is considered to be the normal scope of their action. For instance, TonB/ExbB/ExbD serve to transduce energy to the active transport proteins FepA and FhuA in the outer membrane, but have not been hypothesized to form channels across the periplasmic space. Unlike the ATPase and periplasmic proteins that partner with TolC for secretion/efflux, the cytoplasmic membrane proteins required for colicin translocation through TolC are thus more likely to cause conformational changes in TolC (as TonB/ExbB/ExbD do to FepA[23]) than to provide direct and sequestered access to the cytoplasmic membrane.

Clearly there is a lively dance at the outer membrane as the receptor is bound and then the translocation and activity domains are somehow stuffed through TolC, which must interact with these new sets of cytoplasmic proteins to accomplish the translocation. It is not yet clear how closely the receptor proteins and TolC associate in the membrane, but a picture of many transiently formed interactions is beginning to emerge, if only to handle all the different players. Thus, even if we had adequate explanations for the indirect effects of *tolC* mutants, TolC might still be the most versatile and interactive protein in the outer membrane.

Although many questions remain, the TolC crystal structure[1] has now illuminated the mysteries that stood between those scientists who study TolC and their ability to interpret diverse mutational data. With this conceptual barrier removed, the floodgates through which mechanistic answers should flow are now open.

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Signaling through sigma

Jay D. Gralla

Some prokaryotic transcriptional activators act by binding to enhancers and directly changing the conformation of a specialized sigma factor in the RNA polymerase holoenzyme. This mechanism has interesting parallels in other transcription systems.

Cells respond to physiological need in large part by using complex networks of signal transduction pathways to alter patterns of gene expression. The nuclear output of these pathways is typically the engagement of particular promoters by RNA polymerases to begin the process of transcription. Mammalian cells integrate diverse signals by using multiple DNA enhancer elements, located far from the polymerase binding sites. While compact bacterial genomes use enhancers less often, they nevertheless have a striking mechanism to deal with this situation. A specialized sigma factor (σ^{54}) interacts with the common core RNA polymerase (which is composed of four subunits, $\alpha_2\beta\beta'$) to form the holoenzyme. This interaction directs the holoenzyme to bind selected promoters at which it rests in an inactive but ready to respond state[1]. Diverse enhancer binding proteins, through their ATPase activities, activate the σ^{54} holoenzymes in these complexes[2]. However, the transient nature of the presumably weak protein–protein interactions has hindered discovery of exactly how the activation occurs. Now, on page 594 of this issue of *Nature Structural Biology* Cannon *et al.*[3] identify σ^{54} as the direct target of activation by these activators and show that the activator and its ATPase work by triggering a critical conformational change involving the N-terminus of σ^{54}.

The σ^{54} protein can be viewed as a receptor for an enhancer binding protein — specifically, a receptor that directs a transcription mechanism that is a hybrid of prokaryotic and eukaryotic mechanisms. As in eukaryotes, promoter engagement relies on enhancers and ATP hydrolysis, but this happens in a prokaryotic cell using its far simpler RNA polymerase and associated machinery. Remarkably, σ^{54} bears no more than a passing resemblance, at the level of amino acid sequence, to the large family of other sigma factors, even though they all bind to the same core RNA poly-