

of *Thermus* species (60–69%) dramatically reduces the frequency of UAA, UAG and UGA codons between genes and in non-coding reading frames. With these pre-conditions in place, a mutational event inactivating the translational start site of the *rnpA* gene in the ancestor of *Thermus* would have been tolerated, and selective pressure would result in a re-optimization of the sequences based on the new gene structure.

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Protein Sequence Motif

POTRA: a conserved domain in the FtsQ family and a class of β -barrel outer membrane proteins

Luis Sánchez-Pulido¹, Damien Devos^{1,4}, Stéphanie Genevrois², Miguel Vicente³ and Alfonso Valencia¹

¹Protein Design Group, Centro Nacional de Biotecnología (CNB-CSIC), Cantoblanco, E-28049 Madrid, Spain

²Research Unit in Molecular Biology (URBM), University of Namur (FUNDP), B-5000 Namur, Belgium

³Microbial Biotechnology, Centro Nacional de Biotecnología (CNB-CSIC), Cantoblanco, E-28049 Madrid, Spain

⁴University of California, San Francisco, Mission Bay Genentech Hall, 600 16th Street, San Francisco, CA 94143, USA

POTRA (for polypeptide-transport-associated domain) is a novel domain identified in proteins of the ShlB, Toc75, D15 and FtsQ/DivIB families. In most cases, the POTRA domain is associated with a β -barrel outer membrane domain and its function has been experimentally related to polypeptide transport in Toc75 (Tic–Toc protein import system in chloroplast) and ShlB families. In addition to potential key roles in protein transport across the outer membrane and in bacterial septation, the POTRA domain has attractive features for vaccine development in diseases such as cholera, meningitis, gonorrhoea and syphilis.

The 75-kDa subunit of the translocon at the outer envelope of chloroplasts (Toc)-75 is the most abundant protein of the chloroplast outer membrane with a key role in the translocon at the inner envelope of chloroplasts (Tic)–Toc protein import system [1]. *Serratia marcescens* hemolysin IB (ShlB)-related proteins are virulence-factor transporters that are present in the outer membrane of

Gram-negative bacteria [2–4]. The D15-related proteins are also found in the outer membrane of Gram-negative bacteria and their physiological function might be related to lipid transport [5] and/or to outer-membrane-protein assembly [6]. Similarity between these families has been proposed based on their transmembrane β -barrel C-terminal regions [7,8]. Furthermore, it has been postulated that Toc75 had its origin in an ancient prokaryotic channel protein of smaller size (ShlB-like family) and evolved by partial gene duplication in the N-terminal region of the protein [7,8].

Here, we characterize these N-terminal regions at the sequence level. We named this region the POTRA domain for polypeptide-transport-associated domain. We offer statistically significant evidence for its presence in the FtsQ/DivIB bacterial division protein family [9] (Figure 1), which is the only case we detected of a POTRA domain not associated with a transmembrane β barrel (Figure 2). This domain has also been found in other less characterized proteins that are associated with a transmembrane β barrel, for example, the YTFM and eukaryotic CGI51 families.

Corresponding author: Luis Sánchez-Pulido (sanchez@cnb.uam.es).

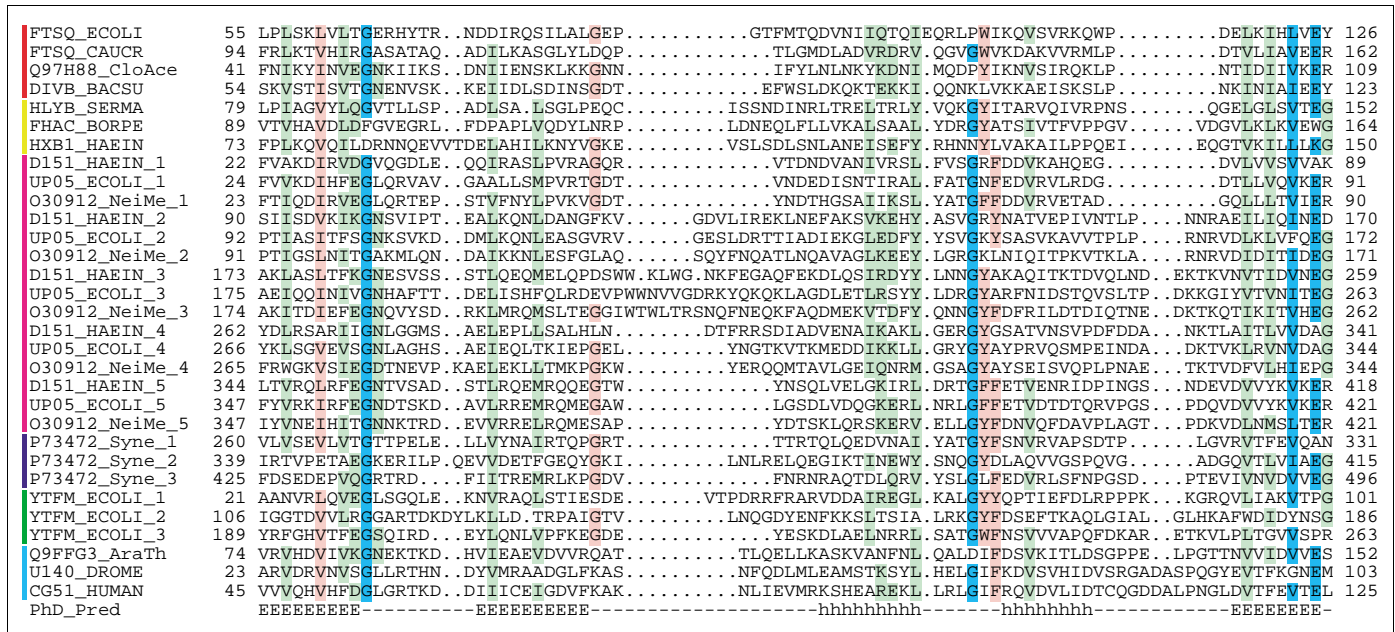


Figure 1. Representative multiple alignment of the POTRA (for polypeptide-transport-associated domain) domain. The alignment was produced with HMMer [10] and T-Coffee [23] using default parameters and was slightly refined manually. It is viewed with the Belvu program (http://www.sanger.ac.uk/Software/Pfam/help/belvu_setup.shtml). The colour scheme indicates the average BLOSUM62 score (correlated to amino-acid conservation) in each alignment column: cyan, >1.6; light red, 1–1.6; and light green, 0.3–1. The boundaries of the domains are indicated by the residue positions on each side. Consensus PHD secondary-structure prediction [11] is shown below the alignment, with E indicating a β strand and H an α helix, in upper and lower case for high and low accuracy, respectively. The sequences are named with their SWISSPROT or SPTREMBL identifications. Multiple alignments and trees for each family, profiles and other information are accessible at: <http://www.pdg.cnb.uam.es/POTRA>. A larger version of this multiple sequence alignment (alignment number ALIGN_000590) has been deposited at the European Bioinformatics Institute (http://ftp.ebi.ac.uk/pub/databases/embl/align/ALIGN_000590.dat). The species abbreviations are: AraTh, *Arabidopsis thaliana*; BACSU, *Bacillus subtilis*; BORPE, *Bordetella pertussis*; CAUCR, *Caulobacter crescentus*; CloAce, *Clostridium acetobutylicum*; DROME, *Drosophila melanogaster*; ECOLI, *Escherichia coli*; HAEIN, *Haemophilus influenzae*; NeiMe, *Neisseria meningitidis*; SERMA, *Serratia marcescens*; Syne, *Synechocystis sp.* The numbering after the protein name indicates the domain-repeat number when more than one is detected in the sequence. Different groups identified by sequence similarity are shown by coloured lines to the left of the alignment: red, FtsQ; yellow, ShIB; violet, D15; blue, Toc75; green, YTFM; and cyan, CGI51.

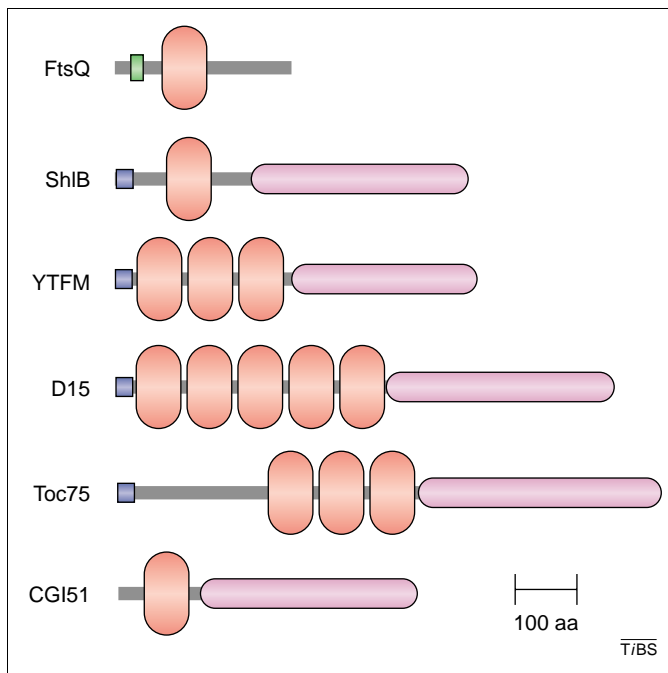


Figure 2. Schematic representation of the domain architectures of a representative set of POTRA (for polypeptide-transport-associated domain) domain-containing proteins. Corresponding to SWISSPROT identifiers: CGI51, SW:Q9Y512; FtsQ, SW:P06136; FtsQ_ECOLI; ShIB, SW:P15321; HLYB_SERMA; Toc75, SPTREMBL:P73472; YTFM, SW:P39320; YTFM_ECOLI. The proteins are drawn approximately to scale and colour coded as follows: transmembrane region, green; signal peptide, blue; POTRA domain, pale red; β barrel, pink. Hypothetical signal peptides were predicted with Signal P [24]. Transmembrane β -sheets were predicted using B2TMPRED [25].

Characterization of the POTRA domain

For sequence searches, independent profiles were generated for the appearance of the domain in each family [10]. Searches with the corresponding global hidden Markov models (HMM; using `hmmsearch` of HMMer version 2.2 g; <http://hmm.wustl.edu/>) produced statistically significant *E*-values that connected all POTRA-domain-containing families. Initial characterizations of the N-terminal region of the D15 family gave the following results: the HMM profile from repeat 1 detected repeat 5 with *E*-values of 4.2×10^{-5} and the combined HMM profile from repeats 1 and 5 detected repeat 2 with an *E*-value of 0.0086. The repeat 2 profile detected repeats 3 and 4 with *E*-values of 3.5×10^{-6} and 0.031, respectively. The profile of the POTRA domain of the ShIB family detected one of the repeats of SynToc75 (Toc75 from *Synechocystis sp.*) with an *E*-value of 9.7×10^{-8} and the fourth repeat of the D15 family with an *E*-value of 2.6×10^{-6} . The profile of the third POTRA domain of the YTFM family detected repeat 5 of the D15 family with an *E*-value of 0.0056. The profile of the POTRA domain of the FtsQ/DivIB family detected repeat 1 of the D15 family with an *E*-value of 0.0063. And finally, the profile of the CGI51 POTRA domain detected repeat 5 of the D15 family with an *E*-value of 0.015.

As none of these HMMer profile searches retrieved new unrelated sequences and reciprocal searches produced convergent results, we conclude that POTRA is a previously undetected, conserved domain that is commonly found in members of the FtsQ/DivIB, ShIB, CGI51

(with one repeat), Toc75, YTFM (with three repeats) and D15 (with five repeats) protein families (Figures 1 and 2).

Secondary-structure predictions using PhD [11] on each family suggest three β strands with the second and third strands separated by two α helices. The α helices are predicted with a low score (less-conserved region) and are separated by a loop conserved around the 'GYF' motif (Figure 1).

Putative role of the POTRA domain

The precise functional role of the POTRA domain remains to be elucidated. The lack of invariant residues in the multiple alignment renders a catalytic function improbable. However, interesting hypotheses can be formulated from the analysis of the experimentally characterized protein families bearing the POTRA domain.

FtsQ is one of the ten proteins known to be essential for cell division in the Gram-negative bacterium *Escherichia coli* [9]. FtsQ and DivIB (the FtsQ homolog in *Bacillus subtilis*) are inner transmembrane proteins with a short cytoplasmic N terminus, a single helical transmembrane region and a periplasmic (in Gram-negative) or extracellular (in Gram-positive) C terminus. FtsQ and DivIB are localized at the septal ring and have a central role in the recruitment of division machinery. FtsQ was initially characterized as essential in bacterial division [9], a quality that DivIB presents only at higher temperatures. However, this requirement for DivIB can be suppressed by the overexpression of FtsL (another division-machinery protein), indicating that one of the roles for DivIB in cell division is maintaining the stability of FtsL. This led to the hypothesis that FtsQ/DivIB proteins might have chaperone-like roles, protecting FtsL from degradation [9].

The high sensitivity to trypsin cleavage of inactive ShlA (the ShlB-specific export substrate) suggests that the secretion-competent conformation of ShlA is not tightly folded. N-terminal ShlB deletion mutants in the POTRA domain-containing region were reported to uncouple ShlA secretion from ShlA activation, which suggest that the POTRA domain of ShlB has a chaperone-like function over ShlA [3].

Similarly to ShlB, it is postulated that the C-terminal regions of Toc75 form a transmembrane β -barrel channel through which precursor proteins are transported across the outer membrane of the chloroplast. The pore size is predicted to be small, which means that the transported pre-proteins would have to be largely unfolded during import [12]. POTRA domains of ShlB and Toc75 could interact with their substrates in a secretion-competent conformation during transport through the β barrel, analogously to the bacterial type III secretion SicP chaperone [13]. This would explain why no chaperones are required for the Toc-core complex-translocation event [14]: Toc75 and ShlB could function as both a channel and a chaperone during the translocation of their substrates. The number of POTRA domains found in each porin family might be related to the selectivity of the transporter (Figure 2).

The hypothetical chaperone role for POTRA is in agreement with the 'cork hypothesis' proposed for the N-terminal region of FhaC protein (from the ShlB family) [4],

where the POTRA domain would fold back into the C-terminal β -barrel membrane channel, as observed for the N-terminal domain of FepA [15]. This would explain why N-terminal deletion mutants for ShlB (in the POTRA domain-containing region) produce channels with much higher single channel conductance than wild-type ShlB protein [2]. Furthermore, POTRA domains could cross the membrane through the barrel channel, presenting an external localization. This hypothesis conciliates the apparent contradiction between the second rule of folding in transmembrane β barrels (both the N and C termini are periplasmic) [16] and the external localization of the N terminus, experimentally determined for different POTRA domain-containing proteins (e.g. ShlB and FhaC) [2,4]. In addition, peptides derived from the N-terminal half of *Hemophilus influenzae* D15 were strongly recognized by the antisera raised against the full-length D15 protein, indicating that the N-terminal region of D15 (POTRA repeats 1–3) contains most of its immunodominant B-cell epitopes and that this region is a potential candidate vaccine development in diseases such as cholera [17], meningitis [18, 19], gonorrhoea [19] and syphilis [20,21,22].

Concluding remarks

The identification of the POTRA domain could help in the design of further experimental work to investigate its association with the transport-related process in Gram-negative bacteria (ShlB, OMP85 and YTFM families), chloroplast (Toc75 family) and eukaryotic (CGI51 family), and to analyze its precise role in bacterial cell division (FtsQ/DivIB family). The hypothesis that the POTRA domain could have a chaperone-like function creates an evolutionary picture in which an ubiquitous bacterial domain, POTRA from FtsQ/DivIB family, was incorporated by a class of β -barrel outer-membrane proteins, ShlB-like, which are characteristic of Gram-negative bacteria, to regulate their channel properties as a solution to the complex problem of translocation across biological membranes.

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Erratum

Erratum to: “Phototransduction: crystal clear” [Trends Biochem. Sci. 28 (2003) 479–487]★

Kevin D. Ridge¹, Najmoutin G. Abdulaev¹, Marcelo Sousa² and Krzysztof Palczewski³

¹Center for Advanced Research in Biotechnology, National Institute of Standards and Technology and the University of Maryland Biotechnology Institute, Rockville, MD 20850, USA

²Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO 80309, USA

³Departments of Ophthalmology, Chemistry, and Pharmacology, University of Washington, Seattle, WA 98195, USA

In the article: ‘Phototransduction: crystal clear’ by Kevin D. Ridge *et al.*, published in the September issue of *Trends in Biochemical Sciences*, there was an error in the text of Box 1. The currently undetermined protein structures and those determined at low resolution are colored different shades of purple in Figure I, not gray. The sentence describing this in the first paragraph of the Box 1 text should have read: ‘Outstanding protein structures or those available only at low-resolution are shown in different shades of purple, including the rhodopsin photointermediates (such

as R* and others that are not shown [38]), the cGMP-gated channel holoenzyme and constituent α and β subunits, rhodopsin kinase (RK), PDE6 holoenzyme and constituent α , β , γ and δ subunits, guanylate cyclase (GC), G β 5L and R9AP’.

TiBS apologizes to the authors and the readers for this error.

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Corresponding author: Kevin D. Ridge (ridge@carb.nist.gov).