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A Conserved Structure and Function of the YidC Homologous Protein Slr1471 from *Synechocystis* sp. PCC 6803

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In this article, we show that the *orf slr1471* from *Synechocystis* sp. PCC 6803 codes for a functional member of the YidC/Alb3/Oxa1 protein family, and the encoded protein has a transmembrane topology with a common core structure. Using specific antibodies raised against the *Synechocystis* YidC homologous protein, we further show that the *Synechocystis* YidC protein appears to be predominantly localized in the cyanobacterial cytoplasmic membrane. The impact of the described findings for synthesis of membrane proteins and for protein sorting within cyanobacterial cells is discussed.

Keywords: Albino3, cyanobacteria, Oxa1, *Synechocystis*, topology, YidC

As the Oxa1 protein in mitochondria [2, 7], Albino3 and YidC are proteins that are involved in membrane integration of proteins into thylakoid membranes or the bacterial cytoplasmic membrane, respectively [21, 23]. Although the exact functional mechanism of these proteins is not understood yet, it has been suggested that members of the Oxa1/YidC/Alb3 protein family not only chaperone membrane integration but also subsequent folding and assembly of transmembrane proteins [13, 14]. Computational analyses of various members of this protein family have suggested that the existence of five transmembrane helices is the only structural feature common to all members of this protein family [15, 26]. Interestingly, no amino acid is conserved throughout the entire protein family, and just the existence of five transmembrane helices appears to determine the protein function [8].

In the genome of the cyanobacterium *Synechocystis* sp. PCC 6803 (GenBank access code BA000022), the ORF *slr1471* encodes a protein with a weak sequence homology to members of the Oxa1/YidC/Alb3 protein family [11], and, although not proven yet, it has been suggested that this ORF codes for a functional Oxa1/YidC/Alb3 homologous protein [6, 17, 22]. The Slr1471 protein is only 14% and 12% identical to the *E. coli* YidC protein or the Albino3 protein from *Arabidopsis thaliana*, respectively (38% sequence similarity in both cases). Like chloroplasts, cyanobacteria contain thylakoid membranes as well as a cytoplasmic membrane. Since biogenesis of the cyanobacterial cytoplasmic and thylakoid membrane is not understood at all yet and the ORF *slr1471* could be involved in formation of inner membranes [22], assessing the function of Slr1471 could help understanding this process in more detail.

Despite the low sequence identity, all members of the YidC/Alb3/Oxa1 protein family are membrane integral proteins with a structural core of five transmembrane α-helices. Hydrophobicity analysis as well as a sequence comparison with other (cyano)bacterial YidC homologous and the *E. coli* YidC proteins, for which the membrane topology has been established experimentally [19], revealed that the *Synechocystis* Slr1471 protein spans the membrane with five α-helices (residues 20–45, 95–116, 265–283, 304–319, and 324–344). Besides the presence of the five transmembrane domains, the computational analysis indicated the existence of a large periplasmic domain in between transmembrane helices II and III (Fig. 1). To test whether the additional domain is unique to the cyanobacterium *Synechocystis* sp. PCC 6803, Slr1471 homologous proteins from several other cyanobacteria have been identified using the BLAST search tool [1], and the periplasmic domain of >100 amino acids appears to be highly conserved in between transmembrane helices II and III in other cyanobacteria as well. This domain does not show homology to any known protein domain and, in addition, no secondary structure or any structural and functional motifs were predicted by computational methods. Nevertheless, since this domain appears to be conserved in cyanobacteria, it is

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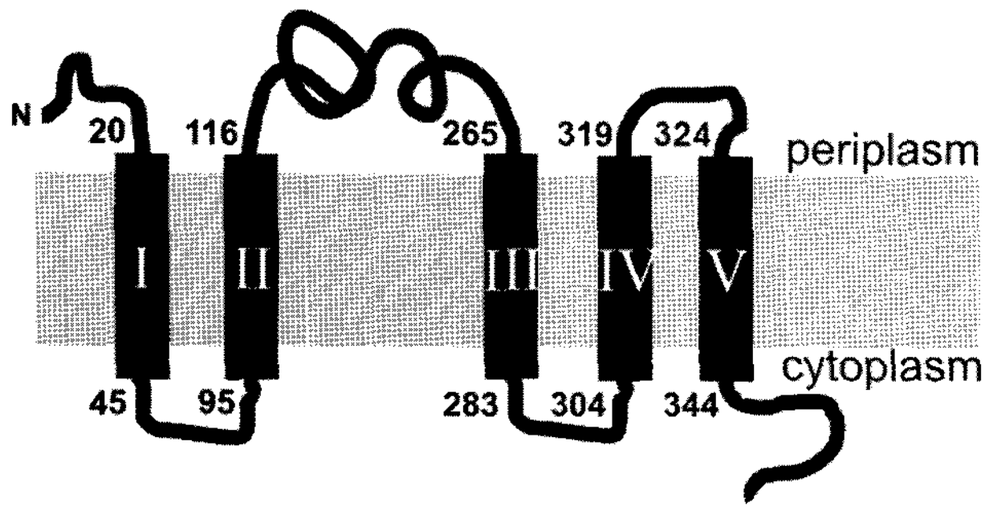

Fig. 1. Topology model of the YidC homologous protein from *Synechocystis*.
The predicted starts and ends of the transmembrane domains are indicated. A large soluble domain is localized in the periplasm between transmembrane helices II and III.

reasonable to speculate that the domain has a physiological function *in vivo*.

Members of the Oxa1/YidC/Alb3 protein family with a structural core of five transmembrane helices can be found in almost all organisms and have even been identified in archaea proteins, which show sequence homology to members of the Oxa1/YidC/Alb3 protein family [15, 26]. The functional complementation of an *E. coli* YidC depletion strain with homologous proteins from mitochondria or chloroplasts has been described [9, 24]. These observations encouraged us to test whether the Slr1471 protein from *Synechocystis* PCC 6803 can functionally complement an *E. coli* YidC depletion strain and to test whether Slr1471 is indeed a functional member of the Oxa1/YidC/Alb3 protein family, as previously suggested. To generate the plasmid used for the complementation analyses, the *Synechocystis* ORF *slr1471* was amplified by PCR from genomic *Synechocystis* DNA using the primers slr1471-5' and slr1471-3', which introduced a NdeI site at the 3'-terminus and a HindIII site at the 3'-terminus of the gene (Table 1). After restriction digestion, the gene was ligated to the equally restriction digested plasmid pMal-c2 (New England Biolabs) resulting in the expression plasmid pSynYidC.

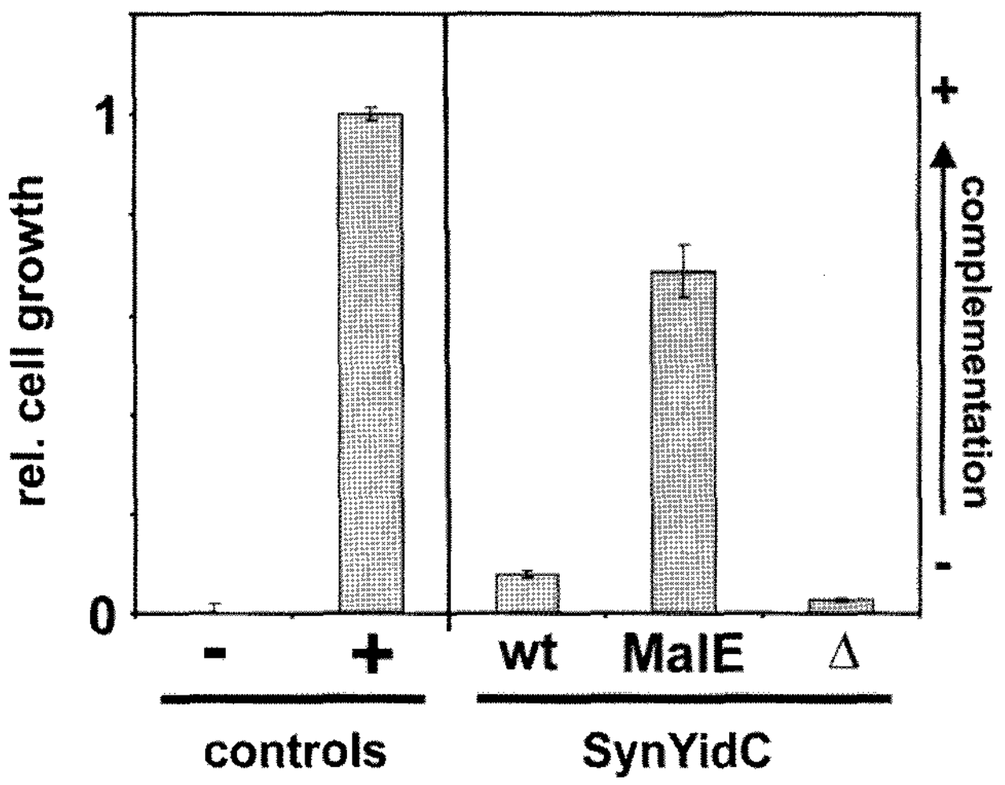

Fig. 2. Complementation of the *E. coli* YidC depletion strain.
The *E. coli* YidC depletion strain JS7131 [21] was transformed with plasmids encoding the full-length SynYidC protein without any fusion domain (wt) and the protein fused to the MalE protein from *E. coli* (MalE). As controls, the *E. coli* strain was transformed with the empty expression plasmid (negative control) as well as a plasmid encoding the *E. coli* YidC protein (positive control). Liquid LB medium with 0.2% glucose was inoculated with equal amounts of *E. coli* cells carrying the various plasmids and the strains were cultivated at 25°C for 12 h, and afterwards the OD_{600} of the different expression strains was determined. The growth of the cultures is shown relative to the growth of the strain complemented by expression of the *E. coli* YidC protein, which was set as 1. In contrast to the strain expressing the MalE-SynYidC fusion, expression of a truncated MalE-Slr1471 protein (Δ) did not result in functional complementation of the *E. coli* YidC depletion strain.

After transformation of the *E. coli* YidC depletion strain JS7131 [21] with this plasmid, cells were grown in LB medium containing 0.2% glucose. In the *E. coli* depletion strain, the expression of the *E. coli yidC* gene is controlled by the *ara* promoter and the cells are viable only in the presence of arabinose. If another protein that is also a functional member of the Oxa1/YidC/Alb3 family is expressed from a plasmid in this *E. coli* strain, the expressed protein can complement for the YidC depletion and the cells can survive on growth medium without arabinose [21]. However,

Table 1. Primers used for molecular cloning.

Primer	Sequence 5'→3'	Comment
slr1471-5'	tacgcatatggattttggtatcggttttatttc	Amplification of *slr1471*
slr1471-5'-2	tatgcgaattcatggattttggtatcggttttatttc	Amplification of *slr1471*
slr1471-3'	tagcggatcc ttacgaggtttttttccttcttttttac	Amplification of *slr1471*
YidCEC-5'	tacgcatatggattcgcaacgcaatc	Amplification of *E. coli yidC*
YidCEC-3'	tagcggatcctcaggatttttttcttctcgc	Amplification of *E. coli yidC*
bla1	cgcagtgttatcactcatgg	Construction of pMal1471Δins
bla2	gccaacttacttctgacaacg	Construction of pMal1471Δins
1471Δins1	ggtgtaattaatatcggaaaagg	Construction of pMal1471Δins
1471Δins2	ggctttctcttcatcaaggc	Construction of pMal1471Δins

Restriction sites, which were introduced by the primers and subsequently used for cloning, are underlined. Molecular cloning was carried out using standard techniques previously described [20]. All primers were purchased from Eurofins MWG GmbH (Ebersberg, Germany).

as can be seen in Fig. 2, expression of the wild-type Slr1471 protein could only marginally restore growth of the *E. coli* cells. Since it has been reported before that fusion of the N-terminus of an Alb3 protein to the *E. coli* MalE protein enhances the protein expression level and promotes complementation [9], we genetically fused the ORF *slr1471* to the MalE protein of *E. coli*. The plasmid pMal1471 was constructed by ligating the *slr1471* ORF to the plasmid pMal-p2 (New England Biolabs) after PCR amplification of the gene using the primers slr1471-5'-2 and slr1471-3' (Table 1) and restriction digestion with EcoRI and HindIII. After expression of the Slr1471 chimera, a restored growth of the *E. coli* depletion strain was indeed observed (Fig. 2), which strongly indicates that the MalE fusion enhances either the expression level of the fused protein and/or facilitates proper membrane integration. However, since expression of the *Synechocystis* Slr1471 protein can restore the growth of the *E. coli* YidC depletion strain and can thereby take over the function of the YidC protein *in vivo*, Slr1471 (SynYidC) can therefore be classified as a true member of the YidC/Alb3/Oxa1 protein family.

To further test whether the extra soluble domain of the SynYidC protein (see above) is critical for its function, we have deleted the gene region encoding a large part of this domain (residues 127–245) and have tested whether expression of the truncated protein can still complement the *E. coli* YidC depletion strain. Deletion of the DNA region coding for the extra cyanobacterial domain was accomplished by PCR using the primers 1471Δins-1, 1471Δins-2, bla1, and bla2 (Table 1), according to the method previously described [10]. The deletion resulted in generation of the plasmid pMal1471Δins.

As can be seen in Fig. 2, after expression of the truncated SynYidC protein, the *E. coli* YidC depletion strain was unable to grow. The extra soluble domain of SynYidC cannot be of any direct functional significance in *E. coli*, since the *E. coli* YidC protein does not contain a homologous domain. It is, however, possible that the soluble domain is of structural importance and is involved in organization of the SynYidC transmembrane structure. Besides a structural role, it is likely that the extra domain has a specific function in cyanobacteria, since this domain is highly conserved in these organisms.

In two recent reports, the YidC homologous protein has been localized to different internal membranes in cyanobacteria based on membrane separation techniques [6, 16]. To test the membrane localization of SynYidC, we have raised antibodies specifically recognizing the SynYidC protein (Fig. 3A) and we have used immunogold analyses to determine the subcellular localization of SynYidC. As

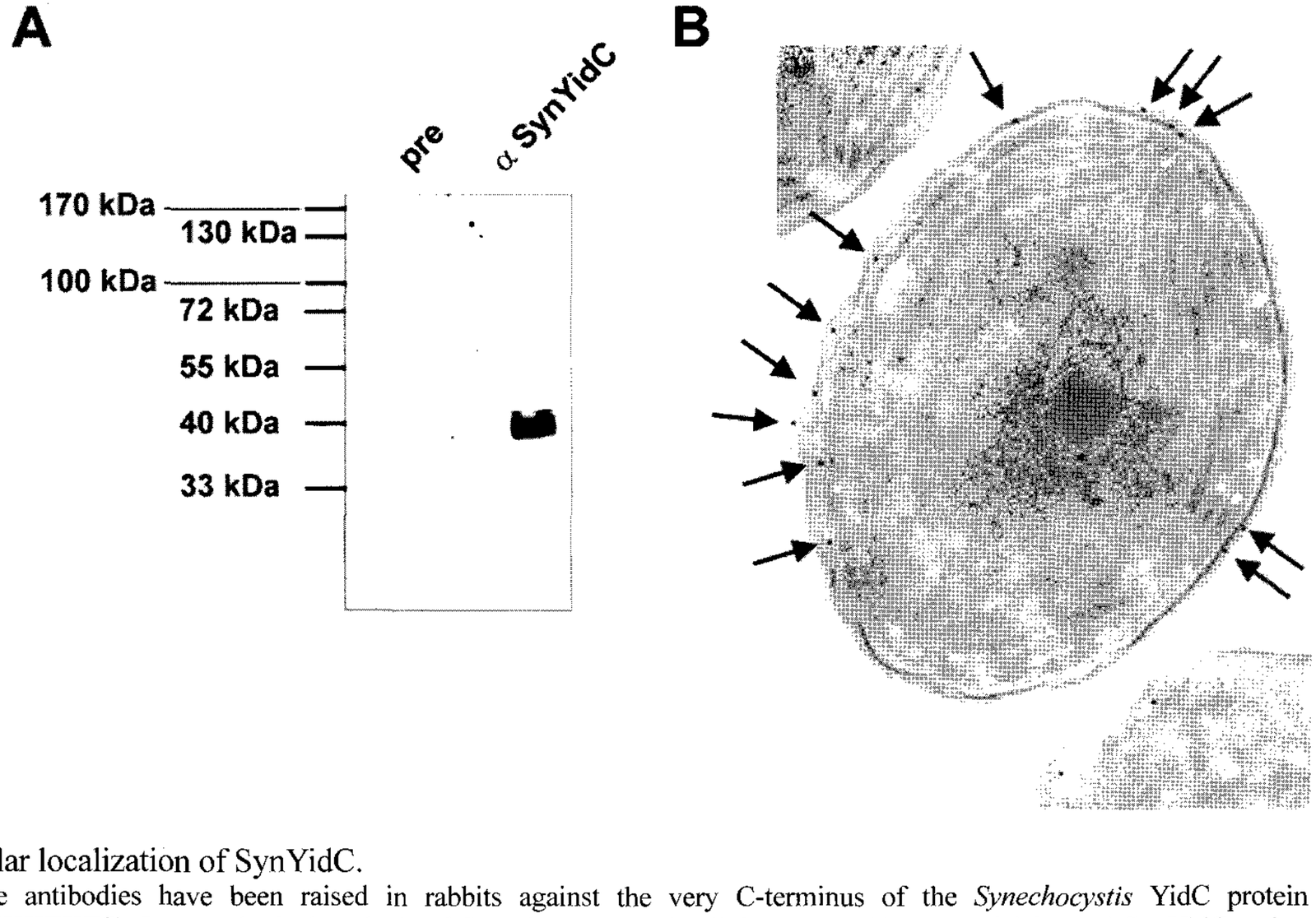

Fig. 3. Subcellular localization of SynYidC.
A. Specific peptide antibodies have been raised in rabbits against the very C-terminus of the *Synechocystis* YidC protein (peptide sequence: GRESLPFEKKSSKKKEKTS). Immunological analysis of *Synechocystis* total cellular extract (5 μg protein) by anti-SynYidC antiserum and pre-immune serum. Only the anti-SynYidC serum specifically recognizes the SynYidC protein with a molecular mass of about 40 kDa. **B**. Electron micrograph of *Synechocystis* cells immune-stained with an anti-SynYidC antiserum (dilution 1:100) followed by treatment with gold-conjugated anti-rabbit IgG. The results indicate a preferential localization of SynYidC within the cytoplasmic membrane. *Synechocystis* PCC 6803 wild-type and mutant strains were grown at 30°C in BG11 medium [18] under constant illumination of 40 μmol photons m^{-2} s^{-1}. A cell pellet obtained from a 10-ml cell suspension was washed three times with EM buffer (50 mM KH_2PO_4/Na_2HPO_4, pH 7.0). The ultrastructural and immunocytochemical investigations were performed as described previously [4].

can be seen in Fig. 3A, the pre-immune serum did not cross-react with any protein from *Synechocystis*, whereas the anti-SynYidC serum showed a clear cross-reactivity with a protein of about 40 kDa, which is the predicted molecular mass of SynYidC. Furthermore, SynYidC was exclusively found in close proximity to the cytoplasmic membrane of *Synechocystis* and we could not detect any gold particles closely associated with thylakoid membranes (Fig. 3B). When the pre-immune serum was used instead, no cells were labeled, as expected (data not shown). This observation strongly suggests that SynYidC is predominantly localized within the *Synechocystis* cytoplasmic membrane, although we cannot completely exclude that a minor part, which has not been detected in the present analysis, is also present in thylakoids.

In a recent study, it has been described that SynYidC is essential for membrane integration of the photosystem II reaction center precursor protein pD1 [17]. It has further been proposed that the initial steps of photosystem II biogenesis occur exclusively in the cytoplasmic membrane of *Synechocystis* [28], and in line with these observations, a factor involved in the biogenesis of photosystem II has also been identified in the *Synechocystis* periplasm [12]. The observation that the SynYidC protein is predominately localized within the cytoplasmic membrane of *Synechocystis* is completely in line with the above-mentioned observations and further supports the proposal that the initial steps of the photosystem II biogenesis occur in the cyanobacterial cytoplasmic membrane. However, in *E. coli*, it has been shown that YidC is also involved in membrane integration of other proteins, like the mechanosensitive channel MscL [5], the CyoA subunit of cytochrome bo_3 oxidase [3], the F_1F_0 ATP synthase subunit c [25], and the SecE subunit of the SecYEG translocase [27]. Therefore, our localization studies may suggest that not only early steps of the photosystem II biogenesis but also assembly of other transmembrane protein complexes take place, at least partly, in the cyanobacterial cytoplasmic membrane. However, since the SynYidC localization data are the only hint so far, such a conclusion has to be taken with caution without any further experimental evidence.

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