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Isolation of fusion proteins containing SecY and SecE, components of the protein translocation complex from the halophilic archaeon *Haloferax volcanii*

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Abstract By exploiting the salt-insensitive interaction of the cellulose-binding domain (CBD) of the *Clostridium thermocellum* cellulosome with cellulose, purification of CBD-fused versions of SecY and SecE, components of the translocation apparatus of the halophilic archaeon *Haloferax volcanii*, was undertaken. Following transformation of *Haloferax volcanii* cells with CBD-SecY- or -SecE-encoding plasmids, cellulose-based purification led to the capture of stably expressed, membrane-bound 68 and 25 kDa proteins, respectively. Both fusion proteins were recognized by antibodies raised against the CBD. Thus, CBD–cellulose interactions can be employed as a salt-insensitive affinity purification system for the capture of complexes containing the *Haloferax volcanii* translocation apparatus components SecY and SecE.

Keywords Archaea · Cellulose-binding domain · *Haloferax volcanii* · Halophiles · SecE · SecY

Introduction

Proteins destined to reside outside the cytoplasm cross the eukaryotic ER and bacterial plasma membranes at proteinaceous sites based on the trimeric Sec61αßγ and SecYEG complexes, respectively (Rapoport et al. 1996; Duong et al. 1997; Manting and Driessen 2000). At the core of these complexes lie the homologous Sec61α/SecY and Secγ/E proteins (Hartmann et al. 1994). While the oligomeric status of Sec61αßγ/SecYEG remains the subject of ongoing investigation (Hanein et al. 1996; Meyer et al. 1999; Manting et al. 2000; Yahr and Wickner 2000; Collinson et al. 2001; Bessonneau et al. 2002), it is believed that eukaryotic Sec61α and bacterial SecY, each spanning the membrane ten times (Akiyama and Ito 1987; Gorlich et al. 1992), form the actual pore through which the translocating protein passes (Joly and Wickner 1993; Mothes et al. 1994). In addition to these structural considerations, many of the mechanistic steps involved in bacterial and eukaryotic protein translocation are also well defined (Rapoport et al. 1996; Duong et al. 1997). In contrast, understanding of how proteins cross the membranes of Archaea, the third and most recently described domain of life, is limited (Pohlschroder et al. 1997; Eichler 2000a). Examination of sequenced archaeal genomes, as well as cloning of individual genes from other strains (Auer et al. 1991; Arndt 1992; Kath and Schäfer 1995), reveals the presence of archaeal homologues of translocation-related genes, including those encoding for archaeal SecY and SecE. Apart from such sequence-based analyses, however, little is known of the process of protein translocation across the archaeal plasma membrane.

Towards a better understanding of archaeal protein export, studies of *Haloferax volcanii* SecY and SecE were initiated at the protein level. Given their relatively simple growth requirements, halophilic archaea represent an excellent model system for addressing membrane-related processes in Archaea, including protein translocation (MacDonald and Lanyi 1975; Eisenbach et al. 1977; Garty et al. 1980; Steinert et al. 1997; Ring and Eichler 2001). Moreover, with the availability of molecular biology, biochemical and protein chemistry techniques designed for use with *H. volcanii*, this strain is a preferred strain for molecular studies in Archaea. In the following, we report the construction of plasmids containing the gene encoding the cellulose-binding domain (CBD) of the *Clostridium thermocellum* cellulosome (Morag et al. 1995) fused to the genes encoding for either *H. volcanii* SecY or SecE. These plasmids were

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used to transform *H. volcanii* cells to yield CBD-SecY or CBD-SecE chimeras, respectively. The presence of the CBD moiety allows for subsequent salt-insensitive cellulose-based purification, as well as for subcellular localization using appropriate antibodies (Ortenberg and Mevarech 2000). Accordingly, employing CBD–cellulose affinity purification, the following study reports the first purification of the translocation complex core components SecY and SecE from an archaeon. Moreover, these findings offer a strategy for the isolation of an archaeal protein translocation complex, possibly leading to the identification of novel elements involved in the archaeal protein export process.

Materials and methods

Materials

Ampicillin, cellulose, DNase I, novobiocin, and PEG-600 were obtained from Sigma (St. Louis, MO, USA). Yeast extract came from Pronadisa (Madrid, Spain) while tryptone came from USB (Cleveland, OH, USA). Molecular weight markers and goat anti-rabbit horseradish peroxidase (HRP)-conjugated antibodies were from BioRad (Hercules, CA, USA). FastStart *Taq* DNA polymerase was from Roche (Indianapolis, IN, USA). Restriction enzymes came from MBI Fermentas (Vilnius, Lithuania). Redivue [^{35}S] radiolabeling mixture (> 1,000 Ci/mmol) and an ECL kit came from Amersham (Buckingham, UK).

Organisms and growth conditions

Haloferax volcanii WR341 (Ortenberg et al. 2000) was grown aerobically at 40°C in medium containing: 3.4 M NaCl, 0.15 M $MgSO_4$·7H2O, 1 mM $MnCl_2$, 4 mM KCl, 3 mM $CaCl_2$, 0.3% (w/v) yeast extract, 0.5% (w/v) tryptone, 50 mM Tris-HCl, pH 7.2 (Mevarech and Werczberger 1985). *H. volcanii* SX/CBD bearing the pWL-CBD plasmid encoding for the CBD moiety of the *C. thermocellum* cellulosome was grown in the same medium to which 1 μg/ml novobiocin had been added (Ortenberg and Mevarech 2000). Both strains were obtained from Moshe Mevarech (Tel Aviv University, Israel).

Cloning of the *H. volcanii secY* gene

Two oligonucleotides, encoding well-conserved regions in numerous archaeal SecY sequences (CTSGGCATCGGYCCSATCGT, forward primer and RYCCATRYCSGTSGTYTCSACCCA, reverse primer), were used to isolate a 991-bp fragment of *H. volcanii secY* by PCR amplification from *H. volcanii* genomic DNA, prepared essentially as described previously (Rosenshine et al. 1987). To clone the 5′ region of the *secY* gene from *H. volcanii* genomic DNA, PCR was performed using oligonucleotides encoding a conserved region near the carboxyl-terminus of ribosomal L15 protein (TTCTCGGARGGCGCSMRSGARAAG, forward primer), shown to lie adjacent to *secY* in numerous species (see below), and a region within the cloned *secY* 991-bp fragment (CCCGACGAGCAGCTTCTGCAGTCC, reverse primer).

To identify the 3′ region of the *secY* gene, amplification by inverse PCR was performed (Ochman et al. 1988). In these reactions, *H. volcanii* genomic DNA was digested with three different restriction enzymes (*Pst*I, *Sph*I and *Sal*I), each shown to cleave the sequenced *secY* fragment obtained as above at a single site. Products of the digestion reactions were self-ligated and the product of one of these ligations (from the *Pst*I-based digestion) was used as a template for inverse PCR amplification containing back-to-back oligonucleotide primers encoding regions of *H. volcanii secY* contained within the same digestion product (GATGGCGATATCGAGCGGGTC, reverse primer and TCATCGACCTGATCTTCATGA, forward primer).

Plasmid construction and transformation

Vectors for the expression of the chimeric proteins CBD-SecE and CBD-SecY in *H. volcanii* were based on the pWL-Nov shuttle vector. This vector contains *E. coli* and *H. volcanii* replication origins as well as ampicillin and novobiocin resistance genes to allow for selection of transformants in *E. coli* and *H. volcanii*, respectively (Ortenberg and Mevarech 2000). A plasmid encoding for the chimeric protein CBD-SecE was constructed as follows: the *cbd* gene encoding for the *C. thermocellum* cellulosome cellulose binding domain was PCR-amplified from the pWL-CBD plasmid using primers designed to introduce a *Nco*I restriction site at the 5′-end of the gene and a *Nde*I site at the 3′-end (TTccatggCAAATACACCGGTA and TGATcatatgACCTCCTACTACACTGCCACCGGG, respectively). The *H. volcanii secE* gene was amplified from genomic DNA using primers to introduce *Nde*I and *Kpn*I sites at the 5′- and 3′-ends of the genes, respectively (TGTGGACCcatatgGGATGGAAGGACG and ATGggtaccCTACTCGGAGCCGAACAT, respectively). The 5′-end of the *cbd* gene was attached to the *H. volcanii* constitutive promoter PrR16 (Patenge and Soppa 1999), previously isolated by *BamH*I-*Nco*I cleavage of the pWL-CBD plasmid, while the 3′ end of the *cbd* gene was fused to the *secE* gene. The entire construct was cloned into the pWL-Nov shuttle vector between *BamH*I and *Kpn*I sites to yield the pWL-CBD-SecE plasmid. For the construction of the pWL-CBD-SecY plasmid, the *secY* gene was amplified using the following primers: GGACcatatgGACGTCAAGTACAATCT and ATGggtaccTCAGACCCCTCCGGGGAG, designed to introduce *Nde*I and *Kpn*I restriction sites, respectively. The *secE* gene of pWL-CBD SecE was then replaced by the cloned *secY* gene, using *Nde*I and *Kpn*I sites.

H. volcanii WR341 cells were transformed with the pWL-CBD-SecE and pWL-CBD-SecY plasmids essentially as previously described (Cline et al. 1989) and selected in *H. volcanii* medium supplemented with 1 μg/ml novobiocin (Ortenberg and Mevarech 2000). Stable propagation of the plasmids was verified by plasmid reisolation from the transgenic strains and characterization by PCR analysis and DNA sequencing.

Cellulose-based capture of CBD-SecE and CBD-SecY

To capture CBD or CBD-fused proteins, transformed *H. volcanii* cells were first metabolically [^{35}S] radiolabeled as follows: cells were grown aerobically at 40°C to mid-exponential phase in *H. volcanii* medium, harvested and resuspended in minimal medium (Ortenberg and Mevarech 2000) containing all standard amino acids, each at a final concentration of 40 μg/ml, to an OD_{550} = 0.6, and grown for 24 h. The cells were then transferred to minimal medium lacking methionine and cysteine for 1 h and then radiolabeled with 15 μCi [^{35}S] labeling mixture per milliliter for 90 min. One-ml aliquots of the labeled cells were harvested (at 3,000 rpm in a microfugefor 3 min at 4°C), the supernatants were removed and the pelleted cells were resuspended in 1 ml solubilization buffer (1% Triton X-100, 1.8 M NaCl, 50 mM Tris-HCl, pH 7.2) containing 3 μg/ml DNase. The solubilized mixture was nutated for 10 min at room temperature (RT), after which time 50 μl of a 10% (w/v) solution of cellulose was added. After a 60 min nutation at RT, the suspension was centrifuged (3,000 rpm for 3 min), the supernatant was discarded and the cellulose pellet was washed with 2 M NaCl, 50 mM Tris-HCl, pH 7.2. This washing procedure was repeated twice. After the final wash, the cellulose beads were centrifuged (5,000 rpm for 3 min), the supernatant was removed and the cellulose pellet was resuspended in 40 μl SDS-PAGE sample buffer (4% SDS, 20% (v/v) glycerol, 0.02% bromophenol blue, 1.5% β mercaptoethanol, 125 mM Tris-HCl, pH 6.8). The samples were boiled for 5 min, centrifuged (5,000 rpm for 5 min) and the

released cellulose-bound proteins were examined by 15% SDS-PAGE and fluorography using Kodak X-Omat film.

Immunoblotting

Subcellular fractionation was achieved by osmotic lysis of cells upon transfer into 1 ml water followed by addition of DNase (3 μg/ml). Soluble and membrane fractions of the cell were separated by ultracentrifugation [Sorvall Discovery M120 ultracentrifuge (S120ATS rotor, 73,000 rpm, 10 min, 4°C)]. Immunoblotting was performed using antibodies raised against the *C. thermocellum* CBD (obtained from Arie Admon, Technion Israel Technology Institute), against *H. volcanii* dihydrofolate reductase (DHFR) (obtained from Moshe Mevarech, Tel Aviv University, Israel) or against the *H. volcanii* S-layer glycoprotein (Eichler 2000b). Antibody binding was detected using goat anti-rabbit HRP-conjugated antibodies and enhanced chemiluminescence. The efficacy of the subcellular fractionation protocol employed was confirmed by immunoblotting the soluble and membrane fractions with antibodies raised against known cytoplasmic or membrane markers in *H. volcanii*, i.e., DHFR and the S-layer glycoprotein, respectively.

Results

Characterization of the *H. volcanii secY* gene and the encoded protein

As a first step in addressing the translocation complex components SecY and SecE in *H. volcanii*, the *secY* gene was cloned and sequenced. These studies revealed that the *H. volcanii secY* gene contains 1,467 bp and encodes for a 489-amino-acid residue protein. Nucleotide sequence data have been deposited in the DDBJ/EMBL/GenBank databases under the accession number AF336343. As shown in various bacteria and other archaea, including the haloarchaea *H. marismortui* and *H.* sp. NRC-1, the *H. volcanii secY* gene lies immediately downstream of the gene encoding the ribosomal L15 protein (Auer et al. 1991; Arndt 1992; Gu et al. 1994; Bult et al. 1996; Suh et al. 1996; Smith et al. 1997; Ng et al. 2000). The initiation codon of the *H. volcanii secY* gene lies only six nucleotides downstream of the final codon of the *L15* gene, leaving little space for regulatory elements. Accordingly, no putative transcription initiation sites for *H. volcanii secY* were identified. Thus, the *secY* promoter site is likely to be located elsewhere in the operon incorporating the *L15–secY* sequences. In the case of *H. marismortui*, the *secY* gene is believed to be the last gene in the *S10/spc* gene cluster, so that expression of *secY* is presumably under the regulation of the *S10/spc* promoter (Arndt 1992).

ClustalW-based alignment of the predicted SecY sequence from different haloarchaea reveals that the *H. volcanii* protein is highly reminiscent of its homologues in *H. marismortui* and in *H.* sp. NRC-1 (not shown). Moreover, *H. volcanii* SecY more closely resembles other euryarchaeal rather than crenarchaeal versions of SecY, confirming the distinctiveness of the two archaeal subdomains (Woese et al. 1990). The *H. volcanii* gene is also more similar to its eukaryal than its bacterial counterparts, as previously observed with other archaeal SecY proteins (Rensing and Maier 1994). Finally, hydropathic analysis predicts that the *H. volcanii* SecY protein spans the membrane ten times with both termini remaining in the cytosol, as reported for SecY/Sec61α in other species (Akiyama and Ito 1987; Görlich et al. 1992).

Cellulose-based capture of CBD-SecY and CBD-SecE

To express and then purify the product of the *H. volcanii secY* gene, *H. volcanii* cells were transformed with plasmid pWL-CBD-SecY, encoding for a *C. thermocellum* CBD–*H. volcanii* SecY chimera (Fig. 1A). Through its interaction with cellulose, the CBD moiety allows for affinity-based purification of halophilic proteins (Ortenberg and Mevarech 2000). In contrast to epitope-based purification systems that rely on salt-sensitive antibody binding reactions, the *C. thermocellum* CBD moiety is able to interact with cellulose in a salt-insensitive manner. Furthermore, relying on the sequence of the recently published *H. volcanii* SecE-encoding gene (Poplawski et al. 2000), plasmid pWL-CBD-SecE was

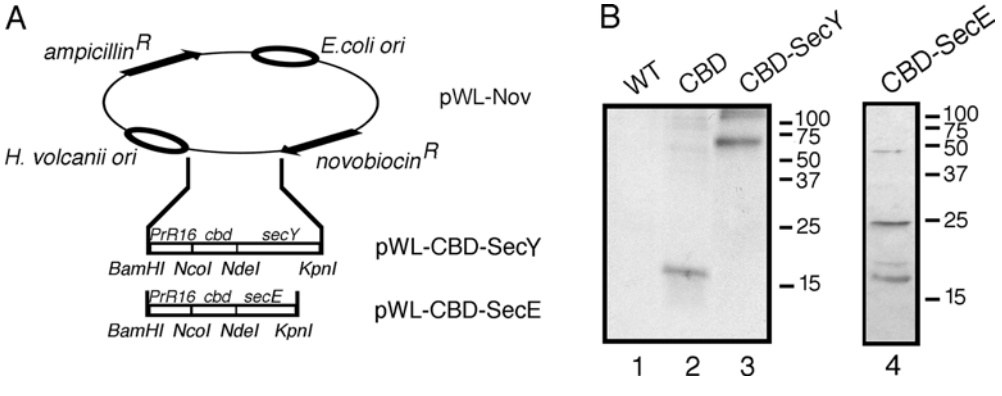

Fig. 1A, B Expression of the cellulose-binding domain (CBD), CBD-SecY and CBD-SecE. **A** Schematic representation of plasmids pWL-CBD-SecY and pWL-CBD-SecE; **B** cellulose-bound proteins from [^{35}S] radiolabeled wild type (*lane 1*), CBD- (*lane 2*), CBD-SecY- (*lane 3*), and CBD-SecE- (*lane 4*) expressing *H. volcanii* cells were examined by SDS-PAGE and fluorography. The positions of molecular weight markers are shown *on the right* of lanes 1–3 and lane 4

also constructed in order to employ cellulose-based affinity for isolation of this translocation complex component. In both pWL-CBD-SecY and pWL-CBD-SecE, the gene encoding the CBD moiety was placed at the 5′-end of the *secY* and *secE* genes, respectively. As the amino-termini of both the *H. volcanii* SecY and SecE proteins are predicted to remain in the cytoplasm, potential difficulties in translocating CBD across the membrane were thus avoided. Expression of the chimeric proteins by transformed cells does not interfere with cell growth, as compared with wild-type cell growth (not shown).

Wild type *H. volcanii* cells and *H. volcanii* cells transformed to express either CBD, CBD-SecY or CBD-SecE were [^{35}S] metabolically radiolabeled, and their protein contents were incubated with cellulose. Subsequent analysis of cellulose-bound proteins by SDS-PAGE and fluorography from the CBD-expressing cells revealed the capture of a 17-kDa species (Fig. 1B, lane 2). The cellulose beads captured a 68-kDa protein from the CBD-SecY-transformed cells (lane 3), in addition to a doublet migrating near the top of the gel. Cellulose treatment of [^{35}S] metabolically radiolabeled CBD-SecE-expressing cells revealed the capture of 48, 25, 18, and 17-kDa protein bands (lane 4). No bands were captured from non-transformed, wild-type cells (lane 1).

To characterize the cellulose-captured proteins, immunoblotting experiments were performed. Probing of the protein contents of wild-type, CBD- and CBD-SecY-expressing cells with antibodies raised against CBD revealed staining of 17- and 68-kDa bands in the latter two strains, respectively (Fig. 2A, lanes 1–3). Thus, the labeled 17-kDa band corresponds to CBD and the 68-kDa protein to the CBD-SecY chimera. Immunoblotting of CBD-SecE cells with the anti-CBD antibodies revealed staining of a 25-kDa band in addition to the 17-kDa CBD moiety (Fig. 2B, lanes 1–3). As *H. volcanii* SecE has a predicted molecular weight of approximately 8 kDa (Poplawski et al. 2000), the 25-kDa species corresponds to the CBD-SecE fusion protein. In some experiments (e.g., Fig. 2B, lane 3), protein bands intermediate in size to CBD and CBD-SecE could be detected. Their appearance, however, was not consistent. Moreover, the failure of the antibodies to label the 48-kDa band captured by the cellulose (Fig. 1B, lane 4) suggests that this protein was non-specifically bound to the cellulose beads. To determine the subcellular distribution of CBD, CBD-SecY and CBD-SecE, cells were separated into soluble and membrane fractions and subsequently immunoblotted with the anti-CBD antiserum. As expected, in cells transformed to express CBD alone, the antibodies recognized a 17-kDa protein band restricted to the soluble fraction of the cells (Fig. 2A, compare lanes 5 and 8). The pattern of staining also revealed that the SecY-containing chimera was essentially localized to the plasma membrane (Fig. 2A, compare lanes 6 and 9). Similarly, subcellular fractionation studies revealed that the CBD-SecE fusion protein was localized to the membrane fraction of the cells (Fig. 1B, compare lanes 6 and 9). A minor degree of release of CBD from the chimera was observed, although

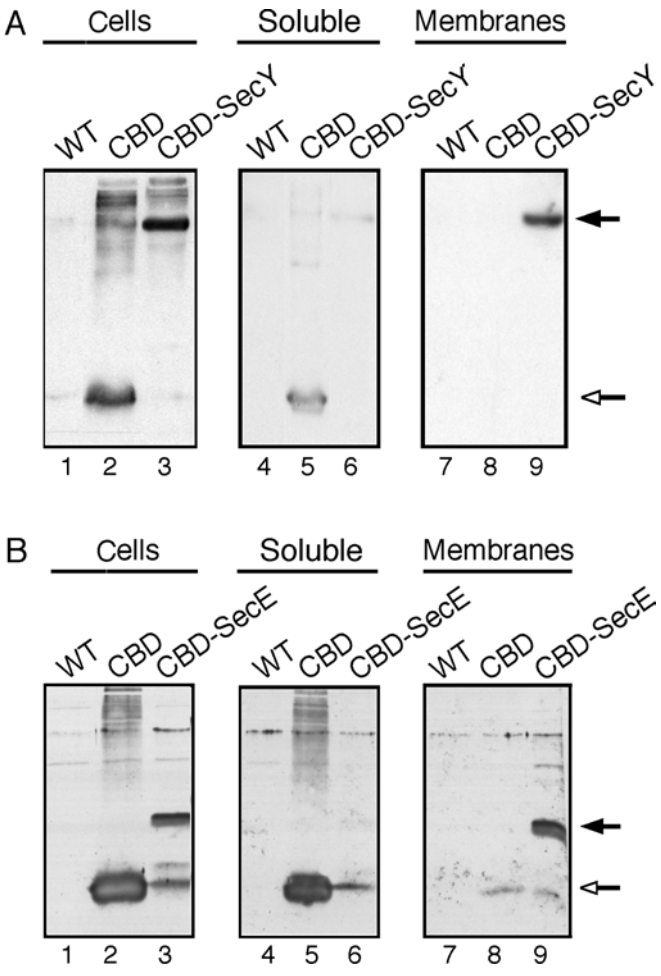

Fig. 2A, B CBD-SecY and CBD-SecE are localized to the plasma membrane. Aliquots of wild type, CBD-, CBD-SecY- and CBD-SecE-expressing *H. volcanii* cells were separated into soluble and membrane fractions and examined by immunoblotting using anti-CBD antibodies. **A** Wild type (*lanes 1, 4 and 7*), CBD-expressing cells (*lanes 2, 5 and 8*) and CBD-SecY-expressing cells (*lanes 3, 6 and 9*) were examined as intact cells (*lanes 1–3*) or separated into soluble (*lanes 4–6*) and membrane (*lanes 7–9*) fractions. **B** As in **A**, except that CBD-SecE-expressing cells were addressed. In both **A** and **B**, the *full arrows* indicate the position of the fusion protein, while the *open arrows* show the position of CBD

since a small amount of CBD was also present in the membrane fraction, it is possible that cleavage occurred during sample preparation (Fig. 2B, lane 9). No bands were recognized in control wild-type *H. volcanii* cells (Fig. 2A, B, lanes 1, 4 and 7). Finally, as described in the Materials and methods section, control experiments confirmed the efficiency of the subcellular fractionation (not shown).

CBD-SecY and CBD-SecE are stably expressed

Pulse chase radiolabeling experiments were next carried out to determine the stability of the CBD-SecY and CBD-SecE proteins. Transformed cells were metabolically pulse-labeled with [^{35}S] methionine and then

challenged with an excess of unlabeled methionine added to the growth medium. Aliquots were then removed at 10-min intervals and subjected to cellulose treatment. As shown in Fig. 3A, the level of CBD-SecY remained relatively constant over 40 min of chase. When pulse chase radiolabeling of CBD-SecE cells was performed, the level of the fusion protein remained relatively unchanged throughout the course of the chase (Fig. 3B). Low levels of CBD- and intermediately-sized bands could be detected at some time points in the CBD-SecE sample, however, given that these did not increase in intensity over the course of the chase, the relative stability of CBD-SecE can be concluded. Indeed, densitometric analysis confirms that the levels of CBD-SecY and CBD-SecE did not significantly change over the period of the experiment (Fig. 3C).

Discussion

In Archaea, a variety of proteases, glycosidases, transporters, surface layer glycoproteins, and other proteins must be inserted into and across the plasma membrane. Unlike the well-characterized bacterial and eukaryal protein export systems, less extensive study has been devoted to understanding the archaeal protein translocation system. Thus, towards a molecular characterization of the archaeal system, the present study reports the first purification of the translocation apparatus components SecY and SecE from Archaea. In this study, membrane-localized CBD-SecY and CBD-SecE were captured from transformed *H. volcanii* cells using salt-insensitive cellulose-based affinity.

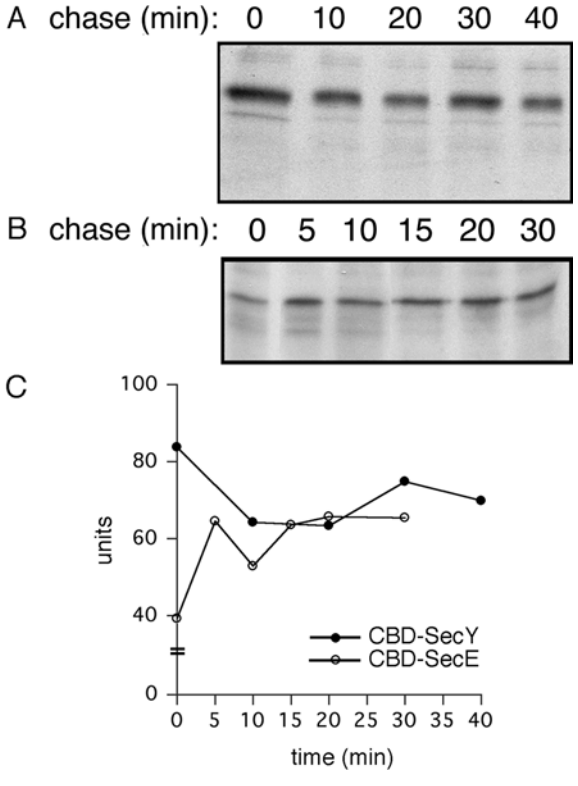

Fig. 3A–C CBD-SecY and CBD-SecE are stably expressed. Pulse chase radiolabeling experiments were performed by [^{35}S] radiolabeling transformed *H. volcanii* cells followed by chase with excess cold methionine. Aliquots were removed at various intervals, incubated with cellulose and examined by SDS-PAGE and fluorography. **A** CBD-SecY; **B** CBD-SecE. **C** The intensities of CBD-SecY (*full circles*) and CBD-SecE (*open circles*) were densitometrically assessed

As the levels of native SecY or SecE were not directly modified in either the CBD-SecY or CBD SecE-expressing cells, it is likely that the CBD-bearing versions of these proteins would have to compete with their native counterparts in the formation of putative SecYE-based complexes. As such, only a given percentage of the chimeric SecY or SecE proteins would be expected to exist in such complexes. Excess CBD-SecY and CBD-SecE proteins might, therefore, be degraded, exist as uncomplexed forms, or alternatively, be incorporated into complexes of incorrect stoichiometry. Pulse-chase radiolabeling and immunoblotting revealed that CBD-SecY and CBD-SecE do not experience significant breakdown. However, as complexed and uncomplexed versions of the fusion proteins could not be differentiated, it is difficult to determine whether uncomplexed CBD-SecY or CBD-SecE were degraded more rapidly than our pulse chase experiment would detect. In *E. coli*, uncomplexed SecY is rapidly degraded by the FtsH protease (Kihara et al. 1995). *H. volcanii* has been shown to contain intracellular proteases, although it is not known whether this group includes a FtsH homologue (Patenge and Soppa 1999; Wilson et al. 1999). In the case of CBD-SecE, some breakdown of the SecE portion of the chimera was noted, although such degradation was limited, varied between experiments, and did not increase during the course of pulse-chase radiolabeling. The observed stabilities of CBD-SecY and CBD-SecE are in marked difference to the results of previous attempts at synthesizing chimeric membrane proteins in *H. volcanii*, where protein degration was observed (Patenge and Soppa 1999). In contrast to the present report, however, these earlier studies involved fusion proteins in which the membrane protein component was of non-archaeal origin. Finally, translocation complexes containing incorrect SecY or SecE stoichiometry would not be expected to function properly and, as such, their presence should interfere with normal cellular physiology, assuming them to be present in sufficient quantity. Given that CBD-SecY or CBD-SecE expression did not significantly interfere with normal cell growth, it thus seems unlikely that CBD-fused SecY or SecE exist in SecYE complexes of incorrect stoichiometry.

With the availability of *H. volcanii* cells capable of expressing CBD-fusion proteins, separately cellulose-purified CBD-SecY and CBD-SecE could be combined into proteoliposomes to yield a reconstituted *H. volcanii* SecYE translocation complex. In this context, separately purified bacterial SecY, SecE and SecG have been reconstituted into translocation-competent

proteoliposomes (Hanada et al. 1994). Alternatively, efforts can now focus on capture of an intact archaeal Sec complex via an incorporated CBD-SecY or -SecE component. Such efforts could address the putative association of archaeal SecYE with additional translocation-related components present in Archaea, such as SecDF. These auxiliary components are elements of the bacterial, but not the eukaryal, translocation system (Pohlschroder et al. 1997; Eichler 2000a). Given that archaeal SecYE proteins, including those of *H. volcanii*, are more similar to their eukaryal than their bacterial counterparts (Pohlschroder et al. 1997; Eichler 2000a), it will be of interest to define the interaction between SecYE and SecDF. Moreover, capture of the archaeal Sec complex could serve to confirm the identify of the recently proposed archaeal Sec61β homologue (Kinch et al. 2002). Together, such reconstitution and purification approaches will allow for the dissection of the molecular mechanism of archaeal protein export.

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