AscA (YecA) is a molecular chaperone involved in Sec-dependent protein translocation in *Escherichia coli*

Running title: AscA is a Sec chaperone

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ABSTRACT.

Proteins that are translocated across the cytoplasmic membrane by Sec machinery must be in an unfolded conformation in order to pass through the protein-

conducting channel during translocation. Molecular chaperones assist Sec-dependent protein translocation by holding substrate proteins in an unfolded conformation in the cytoplasm until they can be delivered to the membrane-embedded Sec machinery. For example, in *Escherichia coli*, SecB binds to a subset of unfolded Sec substrates and delivers them to the Sec machinery by interacting with the metal-binding domain (MBD) of SecA, an ATPase required for translocation in bacteria. Here, we describe a novel molecular chaperone involved Sec-dependent protein translocation, which we have named AscA (for accessory Sec component). AscA contains a metal-binding domain (MBD) that is nearly identical to the MBD of SecA. *In vitro* binding studies indicated that AscA binds to SecB and ribosomes in an MBD-dependent fashion. Saturated transposon mutagenesis and genetics studies suggested that AscA is involved in cell-envelope biogenesis and that its function overlaps with that of SecB. In support of this idea, AscA copurified with a range of proteins and prevented the aggregation of citrate synthase *in vitro*. Our results suggest that AscA is molecular chaperone and that it enhances Sec-dependent protein translocation by delivering its substrate proteins to SecB.

IMPORTANCE.

This research describes the discovery of a novel molecular chaperone, AscA (YecA). The function of AscA was previously unknown. However, it contains a small domain, known as the MBD, suggesting it could interact with the bacterial Sec machinery, which is responsible for transporting proteins across the cytoplasmic membrane. The work described in this study indicates that the MBD allows AscA to bind to both the protein synthesis machinery and the Sec machinery. The previously function of the previously uncharacterised N-terminal domain is that of a molecular chaperone, which binds to

unfolded substrate proteins. We propose that AscA binds to protein substrates as they are still be synthesised by ribosomes in order to channel them into the Sec pathway.

INTRODUCTION.

In *Escherichia coli*, most newly synthesized periplasmic and outer membrane proteins are transported across the cytoplasmic membrane by the Sec machinery. During translocation, protein substrates of the Sec machinery pass through an evolutionarily conserved channel in the cytoplasmic membrane (composed of the integral membrane proteins SecY, -E and -G) in an unfolded conformation (1, 2). In addition, translocation usually requires the activity of SecA (3), an ATPase that facilitates translocation through SecYEG (4). The translocation of periplasmic and outer membrane proteins typically begins only after the substrate protein is fully (or nearly fully) synthesised (*i.e.* “posttranslationally”) (5, 6).

Because proteins must be unfolded to pass through SecYEG, folding of substrate proteins in the cytoplasm blocks Sec-dependent protein translocation, causing a protein to become irreversibly trapped in the cytoplasm (7). Furthermore, partially folded proteins that engage SecYEG can clog (or “jam”) the Sec machinery, which is toxic (8). As a result, cells have evolved multiple mechanisms to prevent premature folding of substrate proteins. For example, molecular chaperones can bind to unfolded Sec substrate proteins and hold them in an unfolded conformation until they can be delivered to the membrane-embedded Sec machinery. One such chaperone is SecB, which binds to a subset of unfolded Sec substrate proteins and delivers them to SecA for translocation across the membrane (9-13). Recognition of nascent substrates by SecB is dependent on SecA (14), suggesting that SecB requires an intermediary to recognise its substrate proteins.

The interaction of SecA with SecB is mediated by a small (~20 amino acid) metal-binding domain (MBD) near the extreme C-terminus of SecA (13, 15, 16). Recent work indicates that the MBD also binds to ribosomes and that ribosome binding is involved in coordinating binding of SecA to nascent polypeptides (17). As its name indicates, the MBD binds to a transition metal (zinc and/or iron) (15, 18), and binding to the metal ion is required for stable folding of the MBD (15). The amino acids responsible for metal binding are highly conserved (CXCXSX₆CH or CXCXSX₆CC) (15, 17, 19).

We recently described a protein of unknown function in *E. coli* that contains a MBD that is nearly identical to the MBD of SecA (18), YecA, which we have re-named AscA (for accessory Sec component). AscA also contains a UPF0149-family domain at its N-terminus, the function of which has not been described. In this work, we investigated the function of AscA. The similarity of the AscA and SecA MBDs led us to investigate the interaction of AscA with SecB and ribosomes and the dependence of these interactions on the MBD. Genetic analysis suggested that AscA is involved in cell-envelope biogenesis and that AscA could be a molecular chaperone. Further studies indicated that AscA binds to cytoplasmic Sec substrate proteins and that it carries out its function in coordination with SecB *in vivo*. Our results suggest a potential model for how AscA could facilitate Sec-dependent protein translocation in *E. coli*.

RESULTS.

Binding of AscA to SecB. Many of the amino acids that mediate the interaction between the SecA MBD and SecB from *Haemophilus influenzae* are conserved in the MBD of AscA (**supplemental figure S1**) (18, 20). To investigate whether AscA can also bind to SecB, we examined the effect of AscA on the thermophoretic mobility of SecB using microscale thermophoresis. To this end, we fluorescently labelled SecB and incubated it with

unlabelled AscA. There was a large change in the thermophoretic properties of fluorescently labelled SecB at saturating concentrations of AscA (**figure 1A**), suggesting that SecB binds to AscA. However, the presence of a truncated variant of AscA, which lacks the MBD (AscA Δ MBD), did not affect thermophoresis of SecB (**figure 1A**). Purified AscA Δ MBD was fully folded even in the absence of its MBD (18), indicating that the interaction between SecB and AscA is dependent on the MBD. Analysis of the effect of increasing concentrations of AscA on the thermophoresis suggested an equilibrium dissociation constant (K_D) of approximately 150 nM (**figure 1B**).

Binding of AscA to ribosomes. We next investigated the interaction of AscA with ribosomes. To this end, we incubated AscA or AscA Δ MBD with purified non-translating 70S ribosomes and then separated ribosome-bound AscA from unbound AscA by sedimenting ribosomes through a 30% sucrose cushion by ultracentrifugation. Full-length AscA cosedimented with the 70S ribosomes, indicating that it can bind to ribosomes (**figure 1C**). Truncation of the MBD in AscA Δ MBD greatly reduced its ability to cosediment with ribosomes, indicating that binding is dependent on the MBD. Ribosome cosedimentation experiments in the presence of increasing concentrations of AscA suggested that the K_D of the AscA-ribosome complex was in the μ M range and that binding saturated at a 1:1 stoichiometry (**supplemental figure S2**).

Transposon-directed insertion-site sequencing of a Δ ascA mutant. To investigate the function of AscA, we determined which genes are essential for viability in a Δ ascA deletion mutant using transposon-directed insertion-site sequencing (TraDIS) (21, 22). Briefly, we created a library of ~500,000 independent mini-Tn5 transposon insertion mutants in a Δ ascA deletion mutant (23) (BW25113 Δ ascA) and then determined the location of each transposon insertion site using Illumina sequencing. Analysis of the location of the insertion sites revealed a set of ~143 genes that did not contain any insertions in BW25113 Δ ascA but did

contain insertions in the isogenic parent (**supplemental table S1**). These mutations affected a range of process but predominantly affected cell envelope biogenesis, cell-envelope stress responses, cell division, protein folding and protein synthesis. These results suggest that the effect of the $\Delta ascA$ mutation is pleiotropic, consistent with a role in cell envelope biogenesis.

Expression of AscA. The *ascA* gene is co-transcribed in a polycistronic message, which contains binding sites for the sRNAs RyhB and RybB immediately 5' to the *ascA* cistron (24). RyhB typically represses genes encoding iron-containing proteins, and RybB is involved in the σ^E cell-envelope stress response, suggesting that expression of AscA could be dependent on iron availability or cell envelope stress. To investigate this possibility, we examined the steady-state level of AscA in *E. coli* using by western blotting. Initial experiments indicated that the presence of ampicillin in the growth medium increased the steady state levels of AscA in the cells, suggesting that expression is dependent on cell envelope stress (**supplemental figure S3A**). Subinhibitory concentrations of chloramphenicol did not induce AscA expression (data not shown). In addition, BW25113 expressed AscA at higher levels when grown at lower temperatures (**figure S3B**). Finally, the presence of EDTA (which chelates transition metal ions with high affinity) completely repressed expression of AscA (**supplemental figure S3C**), consistent with the regulation of AscA by RyhB. These results suggest that expression of AscA is regulated by cell envelope stress and iron availability.

Construction of a $\Delta ascA$ $\Delta secB$ double mutant. Because AscA and SecB physically interact with one another and because the gene encoding SecB was among the set of 142 genes identified by TraDIS (**figure 2A**), we attempted to construct a $\Delta ascA$ $\Delta secB$ double mutant in order to investigate whether AscA and SecB have overlapping functions *in vivo*. Consistent with our TraDIS results, construction of a BW25113 $\Delta ascA$ $\Delta secB$ double mutant was only possible when the *ascA* gene was complemented from a high-copy number plasmid.

For reasons that are not clear, this strain grew much more poorly than the $\Delta secB$ single mutant under all conditions tested. However, it was possible to construct a $\Delta ascA \Delta secB$ double mutant in *E. coli* MG1655, which is derived from a different lineage of *E. coli* K-12. *E. coli* MG1655 and the $\Delta ascA$ single mutant grew normally under all conditions tested, and the $\Delta secB$ mutant displayed a mild cold-sensitive growth defect, consistent with previous studies (25). The $\Delta ascA \Delta secB$ double mutant grew normally at 37°C but was extremely cold sensitive for growth (**supplemental figure S4**), indicating that the $\Delta ascA$ mutation enhanced the phenotype of the $\Delta secB$ mutant. In addition, overproduction of AscA from a plasmid suppressed the cold sensitive growth defect of the $\Delta secB$ mutant at 21°C in both BW25113 (**supplemental figure S5**) and MG1655. Finally, the MG1655 $\Delta ascA \Delta secB$ double mutant formed filaments of 6-8 cell lengths before ceasing growth when grown at 21°C (**figure 2B**), consistent with a defect in cell envelope biogenesis. Taken together, these results suggested that AscA and SecB have overlapping functions *in vivo*.

Co-purification of proteins with AscA. To investigate whether AscA interacts with other proteins *in vivo*, we purified a fusion protein between AscA and the small ubiquitin-like modifier from *Saccharomyces cerevisiae* (SUMO), which was tagged at its N-terminus with a Strep(II) affinity tag (Strep-AscA). We then identified the co-purifying polypeptides using mass spectrometry (LC-MS/MS). SDS-PAGE analysis of purified Strep-AscA indicated that it copurified with polypeptides with a range of molecular weights (**supplemental figure S6**). LC-MS/MS analysis of the copurifying polypeptides revealed that full-length AscA copurified with ~134 different protein species (**figure 3A**), including both cytoplasmic and periplasmic proteins (**supplemental table S2**). However, Strep-AscA Δ MBD copurified with only 32 proteins, which was similar to the number of proteins (28) that copurified with a control protein (SUMO from *Saccharomyces cerevisiae*) (**figure 3A**). These results suggested

that AscA binds promiscuously to a range of proteins *in vivo* when overexpressed and that the MBD is required for efficient binding to these proteins.

Effect of AscA on protein aggregation. The promiscuous binding of AscA to multiple proteins *in vivo* suggested that it could be a molecular chaperone. To investigate this possibility, we examined the ability of AscA to inhibit the aggregation of citrate synthase (CS) at 50°C using light scattering, a widely used assay for molecular chaperone function (26) (**figure 3B**). The presence of AscA strongly inhibited aggregation of CS in a concentration-dependent manner, suggesting that it is a molecular chaperone (**figure 3B**). SecB also inhibited aggregation of CS, albeit to a lesser extent than AscA. However, hen-egg lysozyme, a thermostable control protein, did not inhibit aggregation of CS.

Effect of $\Delta ascA$ mutation on translocation of MalE-LacZ. To investigate the role of AscA in protein translocation, we examined the effect of a $\Delta ascA$ mutation on the activity of a reporter protein fusion between MalE and LacZ (MalE-LacZ) (27-29). The MalE portion of the protein targets it for Sec-dependent translocation across the cytoplasmic membrane. However, translocation results in inactivation of LacZ (β -galactosidase). A $\Delta secB$ mutation caused an increase in β -galactosidase activity, consistent with previous studies and consistent with the role of SecB in translocation of MalE-LacZ (29) (**figure 4A, black bars**). A $\Delta ascA$ mutation also caused an increase in β -galactosidase activity, suggesting that defect in AscA inhibits the translocation of MalE-LacZ. Expression of AscA from a plasmid complemented the translocation defect of the $\Delta ascA$ mutant, and overexpression of AscA in the parent strain reduced the residual β -galactosidase activity below background levels (**figure 4A, grey bars**). However, overexpression of AscA caused an increase in β -galactosidase activity in a $\Delta secB$ mutant (**figure 4A**), suggesting that AscA actually inhibits translocation in the absence of SecB. Despite apparently enhancing the translocation defect of the MM18 $\Delta secB$ mutant, overproduction of AscA suppressed the cold-sensitive growth defect.

Effect of AscA overexpression on Sec substrate proteins. To confirm the effect of AscA on Sec-dependent protein translocation, we examined the effect of AscA overproduction on the translocation of two native model Sec substrates, MalE and OmpF (28). Newly synthesised periplasmic and outer membrane proteins, including MalE and OmpF, contain an N-terminal signal sequence that allows it to be recognised by the Sec machinery (5, 30). Because the signal sequence is removed during translocation, the steady-state levels of precursor proteins (which contain a signal sequence) provides insight into the efficiency of translocation *in vivo*. AscA overproduction did not affect the relative levels of precursor and mature length MalE or OmpF in BW25113 (**figure 4B, lanes 1 - 4**), indicating that it does not cause a translocation defect when expressed on its own. Overproduction did cause a decrease in the steady state levels of MalE in BW25113, consistent with the reduced β -galactosidase activity of strains producing MalE-LacZ, but did not affect the levels of OmpF or thioredoxin-1 (a cytoplasmic control protein). However, overexpression of AscA enhanced the accumulation of precursor-length MalE and OmpF in a $\Delta secB$ mutant (**figure 4B, lanes 5 - 8**).

Effect of AscA overexpression of MalE-LacZ toxicity. To investigate the timing of the interaction of AscA with substrate proteins, we examined the ability of AscA to suppress the toxicity of high-level expression of MalE-LacZ. High-level expression of MalE-LacZ is toxic because the LacZ portion of the fusion protein “jams” the essential SecYEG complex (8, 27). We reasoned that AscA could suppress this toxicity if it binds to MalE-LacZ before it engages SecYEG. To this end, we examined the effect of inducing the expression of MalE-LacZ on minimal plates using maltose. Induction of MalE-LacZ in strains containing pTrc99A caused a severe growth defect, consistent with the MalE-LacZ toxicity. However, co-induction of AscA from a high-copy number plasmid relieved this toxicity (**supplemental**

figure S7), suggesting that AscA likely binds to its substrate proteins before they engage SecYEG.

DISCUSSION.

Our results suggest that AscA is a ribosome-associated molecular chaperone that assists Sec-dependent protein translocation. When overexpressed, AscA binds promiscuously to a broad range of proteins, and the ribosome-binding activity of AscA suggests that it recognises these proteins while they are still nascent polypeptides. AscA also binds to SecB and facilitates protein translocation *in vivo*. However, overexpression of AscA enhances the translocation defect caused by disruption of the *secB* gene. These results suggest a pathway for AscA-mediated targeting (**figure 5**): (i) AscA recognises nascent substrate proteins as they emerge from the ribosome; (ii) AscA holds proteins in an unfolded conformation in the cytoplasm until (iii) it can target the bound protein to SecB; (iv) SecB then delivers the substrate protein to SecA for translocation through SecYEG across the cytoplasmic membrane.

Expression of AscA appears to be conditional, which could explain why AscA has not been identified in previous genetic screens for Sec components. The conditions required for *ascA* induction are not known, but our results suggest that expression could be regulated by the sRNA RybB, which is regulated by σ^E , and RhyB, which is regulated by iron availability (24). Our results suggest that AscA binds promiscuously to a range of substrate proteins, and it is possible that AscA recognises all or most nascent SecA substrates. This possibility would allow SecB to bind to nascent substrate proteins even under conditions that limit the availability of free SecA (14). However, the physiological substrates likely depend on the conditions under which AscA is normally expressed.

Overexpression of AscA simultaneously enhances the defect in protein translocation and suppresses the cold sensitive growth in strains defective for SecB, suggesting that the cold sensitivity of $\Delta secB$ mutants is an indirect effect (25, 31, 32). For example, it is possible that defects in SecB could increase SecYEG jamming at low temperatures, and binding of AscA to these proteins could prevent them from engaging SecYEG while simultaneously enhancing the apparent Sec defect caused by the absence of SecB. Alternatively, the reduction in the steady state levels of MBP upon overexpression of AscA suggests that AscA could be a quality control component that targets excess Sec substrate proteins for degradation. It is possible that AscA is involved in the turnover of Sec components and substrate proteins by Lon protease (32, 33). Finally, our results raise the possibility that the cold sensitive growth defects caused by defect in many Sec components are also an indirect effect of the defect in Sec-dependent protein translocation (31, 34).

The discovery of AscA highlights the importance of molecular chaperones in promoting Sec-dependent protein translocation. In addition to SecB and AscA, which facilitate translocation by retarding the rate of protein folding (9, 35), the ribosome-associated chaperone Trigger Factor prevents nascent outer membrane proteins from engaging the Sec machinery cotranslationally (36). Other housekeeping chaperones, such as the DnaKJ and GroEL/ES systems, can also facilitate Sec-dependent protein translocation when over expressed (37, 38). However, the physiological importance of these systems in Sec-dependent protein translocation is unknown. It has been suggested that some periplasmic chaperones assist protein translocation by inhibiting retrograde translocation (39). Our results raise the possibility the size and importance of the network of molecular chaperones that support Sec machinery is much greater than is currently known.

The high sequence conservation of SecA-like MBDs suggests that their function is to facilitate interactions with the Sec machinery and with ribosomes (18). Both SecA and AscA

MBD also binds to both SecB and ribosomes, and these interactions, except for the interaction of SecA with the ribosome, are dependent on the MBD (15, 17). Although AscA is not universally conserved, 92 of the 156 representative species used for a recent analysis of SecA produce an MBD-containing protein besides SecA (40). Many of these species produce multiple MBD-containing proteins besides SecA. For example, *E. coli* produces a third MBD-containing protein, YchJ (18), and *Salmonella typhimurium* produces five MBD-containing proteins, including SecA and AscA. Furthermore, in 17 of these species, the corresponding SecA protein does not contain an MBD. Besides SecA and AscA, the functions of these proteins are unknown, but bioinformatic analysis suggests at least 18 different functions associated with MBD-containing proteins (41-44). We speculate that SecA-like MBDs are part of an evolutionary toolkit that, when fused to a protein, can bring new functions to the bacterial Sec machinery. If true, MBD-containing proteins could belong to a network of accessory components that assist, modify and/or enhance Sec-dependent protein translocation.

METHODS.

Chemicals and media. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise indicated. Rabbit anti-AscA antiserum was produced using purified AscA by Eurogentec (Liège, Belgium). Rabbit anti-OmpF antiserum was a kind gift from T. Silhavy. Rabbit anti-thioredoxin-1 antiserum was purchased from Sigma-Aldrich. Horseradish peroxidase (HRP)-labelled anti-rabbit antibody was purchased from GE Healthcare. Strains were typically grown in lysogeny broth (LB). Where indicated, strains were grown in M9 minimal medium containing the indicated carbon source (45). Maltose was used at a final concentration of 1% or 0.2%, as indicated, and glycerol was used at a final concentration of 0.5%. Kanamycin and ampicillin were used when required at a final

concentration of 30 µg/ml and 200 µg/ml, respectively. When used, 100 µl of a 50 mg/ml solution of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (x-gal) was spread directly onto plates. IPTG and chloroamphenicol were added to the growth media at the indicated concentrations.

Strains and plasmids. Strains and plasmids were constructed using standard methods (45, 46) and are listed **supplemental table S3**. Plasmids pDH585, pDH963, pCS070, pCS071 and pCS163 were constructed by amplifying the DNA encoding AscA, AscAΔMBD or SecB by PCR and ligating into plasmid pCA528 or pCA597 using the *BsaI* and *BamHI* restriction sites (47). Plasmid pAscA was constructed by amplifying the DNA encoding AscA containing *PciI* and *BamHI* sticky ends and ligating into plasmid pTrc99a (Stratagene) between the *NcoI* and *BamHI* sites. Strain MM18 was a kind gift from J. Beckwith. Single-gene replacement mutations Δ*ascA*::Kan and Δ*secB*::Kan were obtained from the Keio collection(23) and confirmed by PCR.

Protein purification. Unfused full-length AscA, AscAΔMBD and SecB were purified as described (14, 18). BL21(DE3) containing plasmid pCS070 or pCS071 was grown to mid-log phase in LB containing kanamycin at 37°C, and expression of His₃-SUMO-AscA (or His₃-SUMO-AscAΔMBD) was induced using 1 mM isopropyl-thio-galactoside (IPTG; Bioline) overnight at 18°C. Cells were lysed by cell disruption, and lysates were cycled over a 5 ml His-Trap column (GE Healthcare) at 4°C overnight. The column was washed using 25 ml of high-salt wash buffer (20 mM potassium HEPES pH 7.5, 500 mM potassium acetate, 10 mM magnesium acetate, 50 mM imidazole) followed by 25 ml low-salt wash buffer (20 mM potassium HEPES pH 7.5, 25 mM potassium acetate, 10 mM magnesium acetate, 50 mM imidazole). The bound protein was eluted from the column using elution buffer (20 mM potassium HEPES pH 7.5, 25 mM potassium acetate, 10 mM magnesium acetate, 500 mM imidazole). The eluted protein was dialysed by anion-exchange chromatography using a 1ml

ResourceQ column (GE Healthcare) equilibrated with 20 mM potassium HEPES pH 7.5, 25 mM potassium acetate, 10 mM magnesium acetate and raising the potassium acetate concentration to 500 mM using a linear gradient over 25 column volumes. The fusion protein was cut with purified His₆-Ulp1 protease, passaged over a His-Trap column to remove the His-tagged SUMO moiety and concentrated using a VivaSpin20 concentrator with a 10 kDa molecular weight cut-off. Finally, the purified, concentrated protein was cleaned up using a Superdex S-200 column equilibrated with 20 mM potassium HEPES pH 7.5, 25 mM potassium acetate, 10 mM magnesium acetate.

Microscale thermophoresis. Purified SecB was randomly labelled using an NT-647-NHS labelling kit (Nanotemper Technologies). 160 nM NT-647-labelled SecB was incubated with the indicated concentrations of unlabelled AscA or AscAΔMBD in buffer (20 mM potassium HEPES [pH 7.5], 100 mM KOAc, 10 mM MgOAc, 0.05% Tween). The thermophoretic properties of the labelled SecB were then determined with a Monolith NT.115 (Nanotemper Technologies) using MST Premium Coated Capillaries at 100% MST power. Binding constants were determined by fitting the data using the Nanotemper software.

Ribosome cosedimentation. Cosedimentation of AscA with ribosomes was assayed as described previously (14). Purified AscA was incubated with ribosomes at the indicated concentration in 10 mM HEPES potassium salt, pH 7.5, 25 mM potassium acetate, 10 mM magnesium acetate for >10 minutes. The reaction mixture was then layered on top of a 30% sucrose cushion made with the same buffer and centrifuged at >200,000 x g for 90 minutes. The concentration of ribosomes in the pellet fractions were normalised using the absorbance at 260 nm. Samples were resolved by SDS-PAGE and the amount of AscA in the pellet was determined by western blotting (46).

Identification of AscA-copurifying proteins. BL21(DE3) containing plasmid pDH963, pCS163 or pCA597 were in LB containing kanamycin grown at 37°C. Expression of Strep-

SUMO-AscA, Strep-SUMO-AscA Δ MBD and Strep-SUMO was induced at OD₆₀₀ 1.0 with 1 mM IPTG for 2 hours at 30°C. Cells were resuspended in buffer containing 10 mM potassium HEPES (pH 7.4), 100 mM potassium acetate, 10 mM magnesium acetate, 1 mM phenylmethylsulfonylfluoride (PMSF) and 10 mg bovine Dnase I and lysed by sonication. The cell debris was removed from the lysate by centrifugation (16000 x g for 5 minutes at 4°C), and 1 ml of the clarified lysate was incubated with 50 μ l Strep(II)-Tactin Sepharose resin (IBA) for 5 minutes at 4°C. The bound resin was then washed three times with 1 ml 10 mM potassium HEPES (pH 7.4), 100 mM potassium acetate, 10 mM magnesium acetate, three times with 1 ml 10 mM potassium HEPES (pH 7.4), 1000 mM potassium acetate, 10 mM magnesium acetate and one time with 100 mM Tris (pH 8.0) to remove the excess salt. The resin was then dried using a SpeedVac, resuspended in 50 μ l SDS-PAGE sample buffer and boiled for 5 minutes. The eluted protein was resolved by SDS-PAGE and Coomassie staining. The co-purifying proteins in the sections of each lane corresponding to molecular weights of greater than ~80 kDa, ~60-80 kDa and less than ~60 kDa were identified using LC/MS-MS.

Citrate synthase aggregation assay. 200 nM porcine citrate synthase (Sigma-Aldrich) was incubated in 10mM potassium HEPES (pH 7.40, 100 mM potassium acetate, 10 mM magnesium acetate in the presence of the indicated concentration of AscA, SecB or hen egg lysozyme (Sigma-Aldrich) at 50°C. After 30 minutes, samples were returned to room temperature, and side scattering of light at 320 nm was determined using a fluorometer.

TraDIS. TraDIS experiments were conducted as described previously (21, 22). A library of ~500,000 independent mini-Tn5 transposon mutants was created by transforming BW25113 Δ ascA with an EZ-Tn5 <Kan-2> Tnp transposome kit (Epicentre) by electroporation. Kanamycin-resistant transformants were pooled by flooding the transformation plates with LB. After extracting the genomic DNA from the pooled colonies

using a RTP Bacteria DNA Mini Kit (Strattech, Ely, UK), the DNA concentration was determined by Qubit (Invitrogen) and sheared by sonication. The sheared genomic DNA was prepared for sequencing using a NEBNext Ultra DNA Library Prep Kit for Illumina (New England Biolabs) with an additional PCR step to amplify for transposon containing fragments. The PCR products were purified using the Agencourt AMPure XP system by Beckman Coulter. The products were sequenced using Illumina Miseq V3 (150 cycle) cartridges on an Illumina MiSeq sequencer. The locations of the sequences were mapped to the *E. coli* reference genome CP_009273.1 (*E. coli* K-12 BW25113). Files containing the locations of the mapped sequences can be found at doi: 10.6084/m9.figshare.12676739.

Microscopy. Cells were grown overnight in LB at 37°C, subcultured into LB, and grown at 21°C for 4 hours to OD₆₀₀ 0.4. Cells were fixed by pelleting and resuspending in PBS containing 2.5% paraformaldehyde. The cells were stained with DAPI and immobilised on a poly-L-lysine-coated glass slide. Cells were imaged using a Zeiss Axio Observer.Z1/7 inverted microscope with a Plan Apochromat 100X Oil DIC M27 objective (numerical aperture 1.4). DIC images were produced using a TL Halogen light source, and epifluorescence images were produced using an Albireo LED lightsource at 405 nm using a 90HE DAPI/GFP/Cy3/Cy5 reflector (maximum emission = 465 nm; maximum excitation = 353 nm). Images were captured using an Axiocam 503m detector and subsequently processed using Zeiss ZEN 2.3 software and ImageJ 1.51j8 (48).

β-galactosidase assays. Because the β-galactosidase activities of cells grown in culture were variable, strains were streaked onto the indicated plates containing x-gal (which was included to screen against the spontaneous loss of the *malE-lacZ* gene) and IPTG and grown overnight at 37°C. Samples were prepared by scraping cells from the plate using a wire loop and resuspending in Z-buffer (45). Otherwise, the β-galactosidase activities of strains producing MalE-LacZ were determined as described (45).

Western blotting. To determine the steady-state levels of MalE, OmpF and thioredoxin-1, cells were streaked onto LB plates containing 1% maltose to induce the *mal* regulon and grown at 37°C. After overnight growth, cells were scraped from the plate using a wire loop and resuspended in LB. The samples were then normalised to OD₆₀₀ 1.0 by spectrophotometry, pelleted by centrifugation and resuspended in 100 µl SDS-PAGE sample buffer. The samples were resolved by SDS-PAGE and analysed by western blotting using antisera specific to the indicated protein (46).

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Figure 1.

Figure 1. Binding of AscA to SecB and to ribosomes is dependent on its C-terminal MBD. (A & B) Fluorescently labelled SecB was incubated in the absence or presence of AscA or AscAΔMBD, and the effect of AscA on the thermophoretic mobility of SecB was determined by microscale thermophoresis. (A) The magnitude of the effect of 100 μM

unlabelled AscA or AscAΔMBD on the thermophoretic mobility of 160 nM SecB was determined by microscale thermophoresis. Confidence intervals represent one SD. (B) To determine the approximate dissociation constant of the AscA-SecB complex, 160 nM fluorescently labelled SecB was incubated in the presence of AscA at concentrations between 6 nM and 200 μM, and the K_D was determined by fitting the data. Thermophoresis was determined at least three time for each AscA concentration. Confidence intervals are one standard deviation. (C) The indicated concentrations of AscA or AscAΔMBD were incubated in the absence or presence of 1 μM vacant 70S ribosomes. After equilibration at 30°C, the binding reactions were layered on a 30% sucrose cushion and centrifuged at >200,000 x g. The pellet fractions were resolved by SDS-PAGE and subjected to western blotting using anti-AscA antiserum (above). As a loading control, a 1:10 dilution of the binding reaction prior to centrifugation was also analysed by western blotting (below).

Figure 2.

Figure 2. Deletion of the *ascA* gene enhances the cell envelope biogenesis defect of *secB*

mutants. (A) A mini-Tn5-Kan transposon was hopped into the chromosome of BW25113 $\Delta ascA$ >500,000 times independently. The transposon insertion sites were determined using Illumina sequencing and compared to insertion sites in a library of transposon insertion mutants in the parent strain (22). Depicted is the region of the chromosome corresponding to nucleotides 3,774,300 to 3,789,300, which contains the *secB* gene (highlighted in orange). Transposon insertion sites in BW25113 (above) and BW25113 $\Delta ascA$ (below) are indicated by vertical lines. (B) MG1655 (an ancestor of BW25113), MG1655 $\Delta ascA$, MG1655 $\Delta secB$ and MG1655 $\Delta secB \Delta ascA$ were diluted into LB and grown at 21°C for 4 hours. Cells were stained with DAPI and imaged using fluorescence microscopy.

Figure 3.

Figure 3. AscA behaves like a molecular chaperone *in vivo* and *in vitro*. (A) A fusion protein between SUMO from *Saccharomyces cerevisiae* and AscA (SUMO-AscA), AscAΔMBD (SUMO-AscAΔMBD) or SUMO alone was purified from lysates of BL21(DE3) by virtue of an N-terminal Strep(II) tag. The co-purifying proteins were then resolved by SDS-PAGE and identified by mass spectrometry (LC-MS/MS). (B) 200 nM porcine citrate synthase (CS) was incubated at 50°C for 30 minutes in the indicated concentration of hen egg lysozyme, AscA or SecB (tetrameric). The amount of aggregation in the sample was then determined using light scattering at 320 nm.

Figure 4.

Figure 4. The effect of AscA on Sec-dependent protein translocation *in vivo* is dependent on SecB. (A) *E. coli* MM18 (which produces a MalE-LacZ reporter fusion protein), MM18 $\Delta ascA$ and MM18 $\Delta secB$ were transformed with the plasmid vector pTrc99a or pTrc99a containing IPTG-inducible copy of *ascA* (pAscA). Cells grown on LB containing 10 μ M IPTG, and the defective translocation of MalE-LacZ was determined by measuring the β -galactosidase activities of the strains. (B) BW25113 and BW25113 $\Delta secB$ containing pTrc99a or pAscA were grown on LB containing 2% maltose. The cell lysates were then resolved by SDS-PAGE and analysed by western blotting using anti-sera against MalE, OmpF and thioredoxin-1. The running positions of precursor-length MalE and OmpF (p), which contains the N-terminal signal sequence, and processed mature-length MalE and OmpF (m), which lack their signal sequences, are indicated.

Figure 5.

Figure 5. Diagrammatic model of putative role of AscA in Sec-dependent protein

translocation. AscA binds to ribosomes, presumably to recognise nascent substrate proteins as they emerge from the ribosome. AscA binds to newly synthesised substrate proteins in the cytoplasm. AscA then targets the substrate protein for Sec-dependent protein translocation by recruiting SecB. SecB then delivers the substrate protein for translocation by binding to SecA.

SUPPLEMENT

1

SecA	GERKVG	GRNDP	CP	CGS	SGKKY	KQCH	HGRLQ	901
YecA	AEEKP	GRNDP	CP	CGS	SGKKF	KQCC	CLH	221
		▲▲			▲	▲▲	▲▲▲	

Supplemental figure S1. Sequence comparison of the SecA and YecA MBDs. Sequence alignment of the C-terminal MBDs of SecA (above) and YecA (below) from *E. coli* MG1655. The amino acid number of the final amino amino acid is indicated. Amino acids involved in coordinating the bound metal are bolded. Identical amino acids are highlighted in black. Amino acids involved in binding to SecB in the x-ray crystal structure from *Haemophilus influenzae* (1OZB; (20)) are indicated with a carrot below.

Supplemental figure S2. Effect of AscA concentration on cosedimentation with the ribosome. 1 μ M vacant 70S ribosomes were incubated with the indicated concentration of AscA, and the binding reaction was layered on top of a 30% sucrose cushion. Ribosomes were then pelleted through the sucrose cushion by ultracentrifugation, the ribosomal pellets were resuspended buffer and the concentrations of the ribosomes in the resulting were normalised using absorbance at 260nm. The samples were then resolved by SDS-PAGE and analysed by western blotting using antiserum against AscA. Purified AscA at a concentration with a ratio of ~3.2:1 compared to ribosomes was included as a loading control.

Supplemental figure S3. Effect of growth conditions on expression of AscA. The steady state levels of AscA in BW25113 were determined by western blotting. 10 µl of cell lysate normalised to OD₆₀₀ 10 in 1X Laemmli buffer were resolved by SDS-PAGE and analysed by western blotting against AscA. Each experiment was repeated at least three independent times to ensure that differences between samples were not the result of differences in loading. (A) BW25113 containing plasmid pTrc99a (an empty vector containing an ampicillin resistance marker) was grown in the presence (+) or absence (-) of 200 µg/ml ampicillin. (B) BW25113 containing pTrc99a was grown in LB with ampicillin at 21°C (lane 4), 30°C (lane 5), 37°C (lane 6) or 42°C (lane 7). BW25113 ΔascA containing pAscA (lane 1) or pTrc99a (lane 2) were included as controls. (C) BW25113 containing pTrc99a was grown in LB (lane 1), LB containing 1 mM EDTA (lane 2) or LB containing 2 µM FeCl₃.

Supplemental figure S4. Deletion of the *ascA* gene enhances the cold sensitivity of a $\Delta secB$ mutant. MG1655, DRH959 ($\Delta secB::Kan^R$), DRH974 ($\Delta ascA$), DRH975 ($\Delta ascA \Delta secB::Kan^R$) were streaked onto LB plates and incubated overnight at 37°C or 48 hours at 21°C.

Supplemental figure S5. AscA overexpression suppresses the cold-sensitive growth defect caused by a $\Delta secB$ mutation. BW25113 or BW25113 $\Delta secB$ containing pTrc99a or pAscA were streaked on LB plates containing 10 μ M IPTG and grown overnight at 37°C or for 48 hours at 21°C.

Supplemental figure S6. SUMO-AscA copurifies with proteins with a range of molecular weights. Strep(II)₃-tagged SUMO from *Saccharomyces cerevisiae* (Strep-SUMO) or a SUMO-AscA fusion protein (Strep-SUMO-AscA) was produced in BL21(DE3), purified by affinity chromatography against the Strep tag and analysed by SDS-PAGE and Coomassie staining. A large number of co-purifying proteins with a range of molecular weights, which copurified with Strep-SUMO-AscA but not Strep-SUMO, were detectable by Coomassie staining.

Supplemental figure S7. Expression of AscA suppresses the lethality of Male-LacZ

induction. MM18 transformed with pTrc99a or pAscA was streaked onto M63 minimal plates containing 0.5% glycerol, 25 μ M IPTG, 200 μ g/ml ampicillin and x-gal in the absence (left) or presence (right) of 0.2% maltose to induce high-level expression of Male-LacZ, which is toxic.

Supplemental table S1. Genes unambiguously identified by TraDIS as essential for viability in BW25113 *ΔyecA*.

	Gene	Gene product	Cellular process
	Cell envelope biogenesis		
	<i>yajC</i>	Sec translocon accessory complex subunit YajC	Sec-dependent protein translocation
	<i>secB</i>	SecB chaperone	Sec-dependent protein translocation
	<i>ymgC</i>	protein YmgC	Biofilm formation
	<i>yhhH</i>	PF15631 family protein YhhH	Biofilm formation
	<i>wzb</i>	protein-tyrosine phosphatase	Cell wall biogenesis
	<i>glmS</i>	L-glutamine—D-fructose-6-phosphate aminotransferase	Cell wall biogenesis
	<i>ydeQ</i>	putative fimbrial adhesin protein YdeQ	Fimbrial biogenesis
	<i>ydeT</i>	fimbrial usher domain-containing protein YdeT	Fimbrial biogenesis
	<i>yehC</i>	putative fimbrial chaperone YehC	Fimbrial biogenesis
	<i>yehD</i>	putative fimbrial protein YehD	Fimbrial biogenesis
	<i>fliE</i>	flagellar basal-body protein FliE	Flagellar biogenesis
	<i>hlyE</i>	hemolysin E	hemolysin
	<i>fepC</i>	ferric enterobactin ABC transporter ATP binding subunit	iron import
	<i>fepG</i>	ferric enterobactin ABC transporter membrane subunit FepG	iron import
	<i>fepB</i>	ferric enterobactin ABC transporter periplasmic binding protein	iron import

SUPPLEMENT

9

	<i>yciM</i>	lipopolysaccharide assembly protein B	LPS biogenesis
	<i>ynbB</i>	putative CDP-diglyceride synthase	LPS biogenesis
	<i>ubiF</i>	2-octaprenyl-3-methyl-6-methoxy-1,4-benzoquinol hydroxylase	quinone synthesis
	<i>yqeH</i>	putative LuxR family transcriptional regulator YqeH	remnant of type III secretion system
	<i>yqeJ</i>	protein YqeJ	remnant of type III secretion system
	<i>yqeL</i>	uncharacterized protein YqeL	remnant of type III secretion system
	<i>yqeK</i>	protein YqeK	remnant of type III secretion system
	<i>ygeF</i>	protein YgeF	remnant of type III secretion system
	<i>ygeH</i>	putative transcriptional regulator YgeH	remnant of type III secretion system
	<i>ygeI</i>	protein YgeI	remnant of type III secretion system
	<i>pbl</i>	transglycosylase domain-containing protein Pbl	remnant of type III secretion system
	<i>ygeK</i>	putative DNA-binding transcriptional regulator YgeK	remnant of type III secretion system
	<i>ygeN</i>	putative type III secretion system protein YgeN	remnant of type III secretion system
	<i>ygeO</i>	putative uncharacterized protein YgeO	remnant of type III secretion system
	<i>ygeG</i>	TPR repeat-containing putative chaperone YgeG	remnant of type III secretion

SUPPLEMENT

10

			system
	<i>yoaF</i>	DUF333 domain-containing lipoprotein YoaF	Unknown
	<i>ycfZ</i>	putative inner membrane protein	Unknown
Protein biogenesis			
	<i>dnaK</i>	chaperone protein DnaK	Molecular chaperone
	<i>ppiB</i>	peptidyl-prolyl <i>cis-trans</i> isomerase B	Molecular chaperone
	<i>spy</i>	ATP-independent periplasmic chaperone	Molecular chaperone
	<i>iscX</i>	accessory iron-sulfur cluster assembly protein IscX	Iron-sulfur cluster assembly
	<i>fdx</i>	ferredoxin	Iron-sulfur cluster assembly
	<i>hscA</i>	iron-sulfur cluster biosynthesis chaperone HscA	Iron-sulfur cluster assembly
	<i>hscB</i>	[Fe-S] cluster biosynthesis co-chaperone HscB	Iron-sulfur cluster assembly
	<i>iscU</i>	scaffold protein for iron-sulfur cluster assembly	Iron-sulfur cluster assembly
	<i>iscA</i>	iron-sulfur cluster insertion protein IscA	Iron-sulfur cluster assembly
	<i>ydiT</i>	ferredoxin-like protein YdiT	Iron-sulfur cluster assembly (possible)
	<i>elaD</i>	protease ElaD	Protease
Protein synthesis			
	<i>efp</i>	protein chain elongation factor EF-P	Elongation factor
	<i>rpmF</i>	50S ribosomal subunit protein L32	Ribosomal protein (non-essential)
	<i>rimL</i>	ribosomal-protein-L12-serine <i>N</i> -acetyltransferase	Ribosomal protein modification
	<i>rsmH</i>	16S rRNA m ⁴ C1402 methyltransferase	rRNA biogenesis
	<i>nusB</i>	transcription antitermination protein NusB	rRNA biogenesis
	<i>rne</i>	ribonuclease E	rRNA biogenesis
	<i>mmmA</i>	tRNA-specific 2-thiouridylase	tRNA biogenesis
DNA replication/Cell division			
	<i>ftsX</i>	cell division protein FtsX	Divisome (non-essential)

SUPPLEMENT

11

			component)
	<i>ftsE</i>	cell division protein FtsE	Divisome (non-essential component)
	<i>ftsK</i>	cell division DNA translocase FtsK	Divisome/chromosome segregation (non-essential region)
	<i>ruvB</i>	Holliday junction branch migration complex subunit RuvB	Chromosome segregation
	<i>ruvA</i>	Holliday junction branch migration complex subunit RuvA	Chromosome segregation
	<i>seqA</i>	negative modulator of initiation of replication	Replication initiation
	<i>hda</i>	inhibitor of reinitiation of DNA replication	Replication initiation
	<i>dnaQ</i>	DNA polymerase III subunit ϵ	DNA holopolymerase (non-essential component)
	<i>holC</i>	DNA polymerase III subunit χ	DNA holopolymerase (non-essential component)
	<i>holD</i>	DNA polymerase III subunit ψ	DNA holopolymerase (non-essential component)
	<i>cmk</i>	cytidylate kinase	dNTP synthesis
	<i>hns</i>	DNA-binding transcriptional dual regulator H-NS	nucleoid structure
Stress responses (non-cell envelope)			
	<i>ariR</i>	regulator of acid resistance, influenced by indole	Acid stress
	<i>sapB</i>	putrescine ABC exporter membrane subunit SapB	Acid stress
	<i>sapC</i>	putrescine ABC exporter membrane protein SapC	Acid stress
	<i>ydeO</i>	DNA-binding transcriptional dual regulator YdeO	Acid stress
	<i>degS</i>	serine endoprotease	Cell envelope stress
	<i>bhsA</i>	DUF1471 domain-containing multiple stress resistance outer membrane protein	Cell envelope stress

SUPPLEMENT

12

	<i>ydeM</i>	putative anaerobic sulfatase maturation enzyme YdeM	Oxidative stress
	<i>grxD</i>	glutaredoxin 4	Oxidative stress
	<i>yjdK</i>	antitoxin of the GhoTS toxin-antitoxin system	Toxin-antitoxin system
	<i>ycdT</i>	probable diguanylate cyclase DgcT	Affects motility
	<i>yciE</i>	DUF892 domain-containing protein YciE	Osmotic stress
	<i>yciF</i>	DUF892 domain-containing protein YciF	Osmotic stress
	<i>yciG</i>	stress-induced bacterial acidophilic repeat motifs-containing protein YciG	Multiple stresses (including osmotic)
	<i>yeaI</i>	putative c-di-GMP binding protein CdgI	Biofilm formation
Central metabolism			
	<i>trpA</i>	tryptophan synthase subunit α	Amino acid synthesis
	<i>ydiB</i>	shikimate dehydrogenase / quinate dehydrogenase	Amino acid synthesis
	<i>aroK</i>	shikimate kinase 1	Amino acid synthesis
	<i>dapF</i>	diaminopimelate epimerase	Amino acid synthesis
	<i>cysE</i>	serine acetyl transferase	Amino acid synthesis
	<i>pdxJ</i>	pyridoxine 5'-phosphate synthase	Pyridoxine synthesis
	<i>sucA</i>	2-oxoglutarate decarboxylase, thiamine-requiring	TCA cycle
	<i>sucB</i>	dihydrolipoyltranssuccinylase	TCA cycle
	<i>tpiA</i>	triose-phosphate isomerase	Glycolysis
	<i>nudB</i>	dihydroneopterin triphosphate diphosphatase	Tetrahydrofolate synthesis
Prophages			
	<i>ymfD</i>	putative SAM-dependent methyltransferase	e14 prophage
	<i>ymfE</i>	uncharacterized protein YmfE	e14 prophage
	<i>lit</i>	cell death peptidase Lit	e14 prophage
	<i>intE</i>	putative integrase	e14 prophage

SUPPLEMENT

13

<i>xisE</i>	putative excisionase	e14 prophage
<i>ymfI</i>	uncharacterized protein YmfI	e14 prophage
<i>ymfJ</i>	uncharacterized protein YmfJ	e14 prophage
<i>croE</i>	putative DNA-binding transcriptional regulator YmfT	e14 prophage
<i>ymfL</i>	uncharacterized protein YmfL	e14 prophage
<i>ymfM</i>	uncharacterized protein YmfM	e14 prophage
<i>oweE</i>	phage replication protein	e14 prophage
<i>beeE</i>	protein BeeE	e14 prophage
<i>jayE</i>	putative protein JayE	e14 prophage
<i>ymfQ</i>	DUF2313 domain-containing protein YmfQ	e14 prophage
<i>stfP</i>	protein StfP	e14 prophage
<i>tfaP</i>	putative tail fiber assembly protein TfaP	e14 prophage
<i>tfaE</i>	putative tail fiber assembly protein TfaE	e14 prophage
<i>stfE</i>	putative side tail fiber protein fragment	e14 prophage
<i>pinE</i>	site-specific DNA recombinase	e14 prophage
<i>mcrA</i>	5-methylcytosine-specific restriction enzyme McrA	e14 prophage
<i>aaaE</i>	phage terminase	e14 prophage; pseudogene
<i>ydaT</i>	protein YdaT	Rac prophage
<i>ydaW</i>	putative uncharacterized protein YdaW	Rac prophage
<i>kilR</i>	inhibitor of FtsZ	Rac prophage
<i>ynfN</i>	protein YnfN	Qin prophage
<i>cspI</i>	cold shock protein CspI	Qin prophage
<i>rzpQ</i>	DUF2514 domain-containing protein RzpQ	Qin prophage
<i>rzoQ</i>	putative lipoprotein RzoQ	Qin prophage
<i>gnsB</i>	protein GnsB	Qin prophage

SUPPLEMENT

14

Function unknown			
<i>ykgH</i>	uncharacterized protein YkgH		Unknown
<i>rclB</i>	DUF1471 domain-containing protein RclB		Unknown
<i>ybbC</i>	PF15631 family protein YbbC		Unknown
<i>ybbD</i>	putative uncharacterized protein YbbD		Unknown
<i>ybfB</i>	uncharacterized protein YbfB		Unknown
<i>ybfC</i>	uncharacterized protein YbfC		Unknown
<i>ybfQ</i>	inactive transposase YbfQ		Unknown
<i>yccE</i>	uncharacterized protein YccE		Unknown
<i>yceQ</i>	DUF2655 domain-containing protein YceQ		Unknown
<i>ycgH</i>	putative transposase InsZ		Unknown
<i>yncI</i>	putative transposase YncI		Unknown
<i>yddM</i>	DNA-binding transcriptional regulator YddM		Unknown
<i>ydiM</i>	putative exporter YdiM		Unknown
<i>ydiN</i>	putative transporter YdiN		Unknown
<i>ydjH</i>	putative sugar kinase YdjH		Unknown
<i>yecJ</i>	DUF2766 domain-containing protein YecJ		Unknown
<i>yfbN</i>	uncharacterized protein YfbN		Unknown
<i>yfdF</i>	protein YfdF		Unknown
<i>yhiL</i>	putative uncharacterized protein YhiL		Unknown
<i>yibV</i>	PF15596 family protein YibV		Unknown
<i>yjbL</i>	uncharacterized protein YjbL		Unknown
<i>yjbM</i>	uncharacterized protein YjbM		Unknown
<i>ytfI</i>	protein YtfI		Unknown
<i>yjhB</i>	putative sialic acid transporter		Unknown
<i>yjiC</i>	uncharacterized protein YjiC		Unknown

Supplemental table S2. *E. coli* protein identified by LC-MS/MS that copurify with SUMO-AscA.

UniProt Accession	Protein	Score	Coverage	Unique peptides	Length	Protein synthesis
P0A9P6	ATP-dependent RNA helicase DeaD OS=Escherichia coli (strain K12) GN=deaD PE=1 SV=2 - [DEAD_ECOLI]	416.84	66.61	36	629	N
P10408	Protein translocase subunit SecA OS=Escherichia coli (strain K12) GN=secA PE=1 SV=2 - [SECA_ECOLI]	276.93	57.94	48	901	N
P0A705	Translation initiation factor IF-2 OS=Escherichia coli (strain K12) GN=infB PE=1 SV=1 - [IF2_ECOLI]	162.07	40.00	35	890	Y
P0AG67	30S ribosomal protein S1 OS=Escherichia coli (strain K12) GN=rpsA PE=1 SV=1 - [RS1_ECOLI]	160.62	47.22	29	557	Y
P0AFG8	Pyruvate dehydrogenase E1 component OS=Escherichia coli (strain K12) GN=aceE PE=1 SV=2 - [ODP1_ECOLI]	157.53	53.78	41	887	N
P00957	Alanine--tRNA ligase OS=Escherichia coli (strain K12)	117.11	53.08	36	876	Y

SUPPLEMENT

16

	GN=alaS PE=1 SV=2 - [SYA_ECOLI]					
P0A7S9	30S ribosomal protein S13 OS=Escherichia coli (strain K12) GN=rpsM PE=1 SV=2 - [RS13_ECOLI]	113.16	68.64	10	118	Y
P0AD05	Uncharacterized protein YecA OS=Escherichia coli (strain K12) GN=yecA PE=4 SV=1 - [YECA_ECOLI]	110.05	56.56	8	221	N
P0A7W1	30S ribosomal protein S5 OS=Escherichia coli (strain K12) GN=rpsE PE=1 SV=2 - [RS5_ECOLI]	109.15	76.65	11	167	Y
P36683	Aconitate hydratase 2 OS=Escherichia coli (strain K12) GN=acnB PE=1 SV=3 - [ACON2_ECOLI]	103.53	47.05	31	865	N
P0AG55	50S ribosomal protein L6 OS=Escherichia coli (strain K12) GN=rplF PE=1 SV=2 - [RL6_ECOLI]	102.47	58.19	12	177	Y
P0A7V0	30S ribosomal protein S2 OS=Escherichia coli (strain K12) GN=rpsB PE=1 SV=2 - [RS2_ECOLI]	90.91	75.10	16	241	Y
P62399	50S ribosomal protein L5	80.15	60.34	13	179	Y

SUPPLEMENT

17

	OS=Escherichia coli (strain K12) GN=rplE PE=1 SV=2 - [RL5_ECOLI]					
P0AFG3	2-oxoglutarate dehydrogenase E1 component OS=Escherichia coli (strain K12) GN=sucA PE=1 SV=1 - [ODO1_ECOLI]	79.81	37.41	26	933	N
P61175	50S ribosomal protein L22 OS=Escherichia coli (strain K12) GN=rplV PE=1 SV=1 - [RL22_ECOLI]	79.67	63.64	12	110	Y
P0A7L0	50S ribosomal protein L1 OS=Escherichia coli (strain K12) GN=rplA PE=1 SV=2 - [RL1_ECOLI]	77.26	65.38	15	234	Y
P0A7V8	30S ribosomal protein S4 OS=Escherichia coli (strain K12) GN=rpsD PE=1 SV=2 - [RS4_ECOLI]	75.38	45.15	11	206	Y
P0AES6	DNA gyrase subunit B OS=Escherichia coli (strain K12) GN=gyrB PE=1 SV=2 - [GYRB_ECOLI]	75.17	36.94	23	804	N
P33602	NADH-quinone oxidoreductase subunit G OS=Escherichia coli (strain K12) GN=nuoG PE=1 SV=4 - [NUOG_ECOLI]	70.34	33.81	24	908	N

SUPPLEMENT

18

P0A7V3	30S ribosomal protein S3 OS=Escherichia coli (strain K12) GN=rpsC PE=1 SV=2 - [RS3_ECOLI]	68.84	62.66	13	233	Y
P02359	30S ribosomal protein S7 OS=Escherichia coli (strain K12) GN=rpsG PE=1 SV=3 - [RS7_ECOLI]	66.84	61.45	13	179	Y
P21499	Ribonuclease R OS=Escherichia coli (strain K12) GN=rnr PE=1 SV=2 - [RNR_ECOLI]	66.20	38.25	27	813	N
P0A7R1	50S ribosomal protein L9 OS=Escherichia coli (strain K12) GN=rplI PE=1 SV=1 - [RL9_ECOLI]	66.06	73.15	13	149	Y
P05055	Polyribonucleotide nucleotidyltransferase OS=Escherichia coli (strain K12) GN=pnp PE=1 SV=3 - [PNP_ECOLI]	60.98	37.97	20	711	N
P60624	50S ribosomal protein L24 OS=Escherichia coli (strain K12) GN=rplX PE=1 SV=2 - [RL24_ECOLI]	57.23	56.73	8	104	Y
P07118	Valine--tRNA ligase OS=Escherichia coli (strain K12) GN=valS PE=1 SV=2 -	52.85	27.02	21	951	Y

SUPPLEMENT

19

	[SYV_ECOLI]					
P15877	Quinoprotein glucose dehydrogenase OS=Escherichia coli (strain K12) GN=gcd PE=1 SV=3 - [DHG_ECOLI]	52.53	34.67	21	796	N
P09373	Formate acetyltransferase 1 OS=Escherichia coli (strain K12) GN=pflB PE=1 SV=2 - [PFLB_ECOLI]	51.73	33.95	21	760	N
P07395	Phenylalanine--tRNA ligase beta subunit OS=Escherichia coli (strain K12) GN=pheT PE=1 SV=2 - [SYFB_ECOLI]	50.49	36.35	20	795	N
P0A6Y8	Chaperone protein DnaK OS=Escherichia coli (strain K12) GN=dnaK PE=1 SV=2 - [DNAK_ECOLI]	49.89	33.70	17	638	N
P0ABH9	ATP-dependent Clp protease ATP- binding subunit ClpA OS=Escherichia coli (strain K12) GN=clpA PE=1 SV=1 - [CLPA_ECOLI]	49.89	30.87	21	758	N
P21513	Ribonuclease E OS=Escherichia coli (strain K12) GN=rne PE=1 SV=6 - [RNE_ECOLI]	49.42	27.90	20	1061	N
P00490	Maltodextrin phosphorylase OS=Escherichia coli (strain K12)	49.22	26.73	18	797	N

SUPPLEMENT

20

	GN=malP PE=1 SV=7 - [PHSM_ECOLI]					
P0A6M8	Elongation factor G OS=Escherichia coli (strain K12) GN=fusA PE=1 SV=2 - [EFG_ECOLI]	48.26	35.51	17	704	Y
P0A7R9	30S ribosomal protein S11 OS=Escherichia coli (strain K12) GN=rpsK PE=1 SV=2 - [RS11_ECOLI]	47.99	67.44	8	129	Y
P02931	Outer membrane protein F OS=Escherichia coli (strain K12) GN=ompF PE=1 SV=1 - [OMPF_ECOLI]	46.87	37.85	11	362	N
P02413	50S ribosomal protein L15 OS=Escherichia coli (strain K12) GN=rplO PE=1 SV=1 - [RL15_ECOLI]	44.13	52.78	8	144	Y
P0A9Q7	Aldehyde-alcohol dehydrogenase OS=Escherichia coli (strain K12) GN=adhE PE=1 SV=2 - [ADHE_ECOLI]	43.44	27.50	17	891	N
P0ABB0	ATP synthase subunit alpha OS=Escherichia coli (strain K12) GN=atpA PE=1 SV=1 - [ATPA_ECOLI]	42.72	37.62	15	513	N
P0AES4	DNA gyrase subunit A OS=Escherichia coli (strain K12)	42.60	25.03	20	875	N

SUPPLEMENT

21

	GN=gyrA PE=1 SV=1 - [GYRA_ECOLI]					
P0A6F5	60 kDa chaperonin OS=Escherichia coli (strain K12) GN=groL PE=1 SV=2 - [CH60_ECOLI]	42.05	34.31	17	548	N
P0AA10	50S ribosomal protein L13 OS=Escherichia coli (strain K12) GN=rplM PE=1 SV=1 - [RL13_ECOLI]	40.71	71.83	10	142	Y
P0A850	Trigger factor OS=Escherichia coli (strain K12) GN=tig PE=1 SV=1 - [TIG_ECOLI]	40.19	49.07	19	432	Y
P0A9P0	Dihydrolipoyl dehydrogenase OS=Escherichia coli (strain K12) GN=lpdA PE=1 SV=2 - [DLDH_ECOLI]	40.07	35.23	12	474	N
P60422	50S ribosomal protein L2 OS=Escherichia coli (strain K12) GN=rplB PE=1 SV=2 - [RL2_ECOLI]	38.62	40.29	9	273	Y
P60723	50S ribosomal protein L4 OS=Escherichia coli (strain K12) GN=rplD PE=1 SV=1 - [RL4_ECOLI]	38.55	44.28	7	201	Y
P0A707	Translation initiation factor IF-3 OS=Escherichia coli (strain K12) GN=infC PE=1 SV=1 -	38.29	43.89	6	180	Y

SUPPLEMENT

22

	[IF3_ECOLI]					
P0AE08	Alkyl hydroperoxide reductase subunit C OS=Escherichia coli (strain K12) GN=ahpC PE=1 SV=2 - [AHPC_ECOLI]	37.70	58.29	10	187	N
P00864	Phosphoenolpyruvate carboxylase OS=Escherichia coli (strain K12) GN=ppc PE=1 SV=1 - [CAPP_ECOLI]	37.57	22.08	21	883	N
P0A9M8	Phosphate acetyltransferase OS=Escherichia coli (strain K12) GN=pta PE=1 SV=2 - [PTA_ECOLI]	37.12	27.87	15	714	N
P00956	Isoleucine--tRNA ligase OS=Escherichia coli (strain K12) GN=ileS PE=1 SV=5 - [SYI_ECOLI]	36.36	27.40	22	938	Y
P06612	DNA topoisomerase 1 OS=Escherichia coli (strain K12) GN=topA PE=1 SV=2 - [TOP1_ECOLI]	36.02	26.59	22	865	N
P07813	Leucine--tRNA ligase OS=Escherichia coli (strain K12) GN=leuS PE=1 SV=2 - [SYL_ECOLI]	32.89	23.95	18	860	Y
P06959	Dihydrolipoyllysine-residue acetyltransferase component of	31.56	24.92	15	630	N

SUPPLEMENT

23

	pyruvate dehydrogenase complex OS=Escherichia coli (strain K12) GN=aceF PE=1 SV=3 - [ODP2_ECOLI]					
P0AFG6	Dihydrolipoyllysine-residue succinyltransferase component of 2- oxoglutarate dehydrogenase complex OS=Escherichia coli (strain K12) GN=sucB PE=1 SV=2 - [ODO2_ECOLI]	31.49	29.88	11	405	N
P0A7K6	50S ribosomal protein L19 OS=Escherichia coli (strain K12) GN=rplS PE=1 SV=2 - [RL19_ECOLI]	31.31	66.09	7	115	Y
P60438	50S ribosomal protein L3 OS=Escherichia coli (strain K12) GN=rplC PE=1 SV=1 - [RL3_ECOLI]	29.85	45.93	8	209	Y
P0ADY3	50S ribosomal protein L14 OS=Escherichia coli (strain K12) GN=rplN PE=1 SV=1 - [RL14_ECOLI]	29.46	43.90	5	123	Y
P0A7W7	30S ribosomal protein S8 OS=Escherichia coli (strain K12) GN=rpsH PE=1 SV=2 - [RS8_ECOLI]	28.30	57.69	8	130	Y
P0AG59	30S ribosomal protein S14	27.88	39.60	5	101	Y

SUPPLEMENT

24

	OS=Escherichia coli (strain K12) GN=rpsN PE=1 SV=2 - [RS14_ECOLI]					
P0A7L3	50S ribosomal protein L20 OS=Escherichia coli (strain K12) GN=rplT PE=1 SV=2 - [RL20_ECOLI]	27.27	46.61	9	118	Y
P63284	Chaperone protein ClpB OS=Escherichia coli (strain K12) GN=clpB PE=1 SV=1 - [CLPB_ECOLI]	26.49	23.92	18	857	N
P23538	Phosphoenolpyruvate synthase OS=Escherichia coli (strain K12) GN=ppsA PE=1 SV=5 - [PPSA_ECOLI]	25.94	20.08	13	792	N
P13029	Catalase-peroxidase OS=Escherichia coli (strain K12) GN=katG PE=1 SV=2 - [KATG_ECOLI]	25.30	18.04	13	726	N
P68919	50S ribosomal protein L25 OS=Escherichia coli (strain K12) GN=rplY PE=1 SV=1 - [RL25_ECOLI]	24.16	52.13	6	94	Y
P0A8T7	DNA-directed RNA polymerase subunit beta' OS=Escherichia coli (strain K12) GN=rpoC PE=1 SV=1 - [RPOC_ECOLI]	23.10	9.38	11	1407	N
P0ADY7	50S ribosomal protein L16	22.59	44.85	5	136	Y

SUPPLEMENT

25

	OS=Escherichia coli (strain K12) GN=rplP PE=1 SV=1 - [RL16_ECOLI]					
P0A6T5	GTP cyclohydrolase 1 OS=Escherichia coli (strain K12) GN=folE PE=1 SV=2 - [GCH1_ECOLI]	22.50	49.55	9	222	N
P0A7X3	30S ribosomal protein S9 OS=Escherichia coli (strain K12) GN=rpsI PE=1 SV=2 - [RS9_ECOLI]	22.32	50.00	7	130	Y
P0A910	Outer membrane protein A OS=Escherichia coli (strain K12) GN=ompA PE=1 SV=1 - [OMPA_ECOLI]	22.20	32.95	8	346	N
P0AG44	50S ribosomal protein L17 OS=Escherichia coli (strain K12) GN=rplQ PE=1 SV=1 - [RL17_ECOLI]	21.86	33.07	5	127	Y
P0A7R5	30S ribosomal protein S10 OS=Escherichia coli (strain K12) GN=rpsJ PE=1 SV=1 - [RS10_ECOLI]	21.07	52.43	7	103	Y
P0A7J3	50S ribosomal protein L10 OS=Escherichia coli (strain K12) GN=rplJ PE=1 SV=2 - [RL10_ECOLI]	21.04	44.85	6	165	Y

SUPPLEMENT

26

P23909	DNA mismatch repair protein MutS OS=Escherichia coli (strain K12) GN=mutS PE=1 SV=1 - [MUTS_ECOLI]	20.94	13.48	10	853	N
P33195	Glycine dehydrogenase (decarboxylating) OS=Escherichia coli (strain K12) GN=gcvP PE=1 SV=3 - [GCSP_ECOLI]	20.91	9.30	7	957	N
P60240	RNA polymerase-associated protein RapA OS=Escherichia coli (strain K12) GN=rapA PE=1 SV=2 - [RAPA_ECOLI]	20.21	12.71	11	968	N
P0ABB4	ATP synthase subunit beta OS=Escherichia coli (strain K12) GN=atpD PE=1 SV=2 - [ATPB_ECOLI]	18.72	18.48	6	460	N
P32132	GTP-binding protein TypA/BipA OS=Escherichia coli (strain K12) GN=typA PE=1 SV=2 - [TYPA_ECOLI]	18.46	19.77	10	607	Y
P0A7U3	30S ribosomal protein S19 OS=Escherichia coli (strain K12) GN=rpsS PE=1 SV=2 - [RS19_ECOLI]	18.34	56.52	5	92	Y
P0A9L3	FKBP-type 22 kDa peptidyl-prolyl cis-trans isomerase OS=Escherichia coli (strain K12) GN=fklB PE=1	18.09	35.44	6	206	N

SUPPLEMENT

27

	SV=2 - [FKBB_ECOLI]					
P0A7T7	30S ribosomal protein S18 OS=Escherichia coli (strain K12) GN=rpsR PE=1 SV=2 - [RS18_ECOLI]	18.09	50.67	4	75	Y
P69441	Adenylate kinase OS=Escherichia coli (strain K12) GN=adk PE=1 SV=1 - [KAD_ECOLI]	17.76	44.39	8	214	N
P23865	Tail-specific protease OS=Escherichia coli (strain K12) GN=prc PE=1 SV=2 - [PRC_ECOLI]	17.29	16.72	10	682	N
P31224	Multidrug efflux pump subunit AcrB OS=Escherichia coli (strain K12) GN=acrB PE=1 SV=1 - [ACRB_ECOLI]	17.25	9.72	8	1049	N
P04825	Aminopeptidase N OS=Escherichia coli (strain K12) GN=pepN PE=1 SV=2 - [AMPN_ECOLI]	16.91	10.00	9	870	N
P46837	Protein YhgF OS=Escherichia coli (strain K12) GN=yhgF PE=1 SV=3 - [YHGF_ECOLI]	16.42	15.14	11	773	N
P0AG63	30S ribosomal protein S17 OS=Escherichia coli (strain K12) GN=rpsQ PE=1 SV=2 - [RS17_ECOLI]	16.29	32.14	2	84	Y
P60785	Elongation factor 4 OS=Escherichia	16.19	14.36	8	599	Y

SUPPLEMENT

28

	coli (strain K12) GN=lepA PE=1 SV=1 - [LEPA_ECOLI]					
P68679	30S ribosomal protein S21 OS=Escherichia coli (strain K12) GN=rpsU PE=1 SV=2 - [RS21_ECOLI]	15.44	33.80	6	71	Y
P0A7S3	30S ribosomal protein S12 OS=Escherichia coli (strain K12) GN=rpsL PE=1 SV=2 - [RS12_ECOLI]	15.39	25.00	5	124	Y
P0AFF6	Transcription termination/antitermination protein NusA OS=Escherichia coli (strain K12) GN=nusA PE=1 SV=1 - [NUSA_ECOLI]	15.34	17.78	8	495	N
P0C018	50S ribosomal protein L18 OS=Escherichia coli (strain K12) GN=rplR PE=1 SV=1 - [RL18_ECOLI]	15.32	45.30	4	117	Y
P0A7N4	50S ribosomal protein L32 OS=Escherichia coli (strain K12) GN=rpmF PE=1 SV=2 - [RL32_ECOLI]	15.22	43.86	2	57	Y
P45577	RNA chaperone ProQ OS=Escherichia coli (strain K12) GN=proQ PE=1 SV=2 - [PROQ_ECOLI]	14.76	32.33	6	232	N

SUPPLEMENT

29

P0A7J7	50S ribosomal protein L11 OS=Escherichia coli (strain K12) GN=rplK PE=1 SV=2 - [RL11_ECOLI]	14.43	38.03	5	142	Y
P02358	30S ribosomal protein S6 OS=Escherichia coli (strain K12) GN=rpsF PE=1 SV=1 - [RS6_ECOLI]	14.23	23.70	4	135	Y
P0ADZ4	30S ribosomal protein S15 OS=Escherichia coli (strain K12) GN=rpsO PE=1 SV=2 - [RS15_ECOLI]	14.20	24.72	5	89	Y
P00452	Ribonucleoside-diphosphate reductase 1 subunit alpha OS=Escherichia coli (strain K12) GN=nrdA PE=1 SV=2 - [RIR1_ECOLI]	13.42	18.27	12	761	N
P62707	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase OS=Escherichia coli (strain K12) GN=gpmA PE=1 SV=2 - [GPMA_ECOLI]	13.03	22.80	5	250	N
P0AFG0	Transcription termination/antitermination protein NusG OS=Escherichia coli (strain K12) GN=nusG PE=1 SV=2 - [NUSG_ECOLI]	12.82	34.25	5	181	N

SUPPLEMENT

30

P23836	Transcriptional regulatory protein PhoP OS=Escherichia coli (strain K12) GN=phoP PE=1 SV=1 - [PHOP_ECOLI]	12.20	27.35	5	223	N
P00968	Carbamoyl-phosphate synthase large chain OS=Escherichia coli (strain K12) GN=carB PE=1 SV=2 - [CARB_ECOLI]	11.71	8.57	9	1073	N
P0A6P1	Elongation factor Ts OS=Escherichia coli (strain K12) GN=tsf PE=1 SV=2 - [EFTS_ECOLI]	11.33	21.91	5	283	Y
P0A7X6	Ribosome maturation factor RimM OS=Escherichia coli (strain K12) GN=rmmM PE=1 SV=1 - [RIMM_ECOLI]	10.94	31.32	4	182	Y
P0A9D8	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase OS=Escherichia coli (strain K12) GN=dapD PE=1 SV=1 - [DAPD_ECOLI]	10.86	17.88	4	274	N
P0AG90	Protein translocase subunit SecD OS=Escherichia coli (strain K12) GN=secD PE=1 SV=1 - [SECD_ECOLI]	10.54	9.11	4	615	N
P28635	D-methionine-binding lipoprotein MetQ OS=Escherichia coli (strain	10.39	16.61	3	271	N

SUPPLEMENT

31

	K12) GN=metQ PE=1 SV=2 - [METQ_ECOLI]					
P0ACJ8	cAMP-activated global transcriptional regulator CRP OS=Escherichia coli (strain K12) GN=crp PE=1 SV=1 - [CRP_ECOLI]	9.95	20.48	4	210	N
P0AG48	50S ribosomal protein L21 OS=Escherichia coli (strain K12) GN=rplU PE=1 SV=1 - [RL21_ECOLI]	9.56	18.45	2	103	Y
P0A6F9	10 kDa chaperonin OS=Escherichia coli (strain K12) GN=groS PE=1 SV=1 - [CH10_ECOLI]	9.49	52.58	4	97	N
P76558	NADP-dependent malic enzyme OS=Escherichia coli (strain K12) GN=maeB PE=1 SV=1 - [MAO2_ECOLI]	9.47	7.38	6	759	N
P0A7L8	50S ribosomal protein L27 OS=Escherichia coli (strain K12) GN=rpmA PE=1 SV=2 - [RL27_ECOLI]	9.44	37.65	3	85	Y
P0A6W5	Transcription elongation factor GreA OS=Escherichia coli (strain K12) GN=greA PE=1 SV=1 - [GRE_A_ECOLI]	9.24	35.44	5	158	N
P07014	Succinate dehydrogenase iron-sulfur	8.99	19.75	4	238	N

SUPPLEMENT

32

	subunit OS=Escherichia coli (strain K12) GN=sdhB PE=1 SV=1 - [SDHB_ECOLI]					
P0CE47	Elongation factor Tu 1 OS=Escherichia coli (strain K12) GN=tufA PE=1 SV=1 - [EFTU1_ECOLI]	8.97	16.75	5	394	Y
P0A717	Ribose-phosphate pyrophosphokinase OS=Escherichia coli (strain K12) GN=prs PE=1 SV=2 - [KPRS_ECOLI]	8.93	17.14	4	315	N
P30843	Transcriptional regulatory protein BasR OS=Escherichia coli (strain K12) GN=basR PE=1 SV=3 - [BASR_ECOLI]	8.93	14.41	3	222	N
P0ACA3	Stringent starvation protein A OS=Escherichia coli (strain K12) GN=sspA PE=1 SV=2 - [SSPA_ECOLI]	8.85	26.89	5	212	N
P09372	Protein GrpE OS=Escherichia coli (strain K12) GN=grpE PE=1 SV=1 - [GRPE_ECOLI]	8.81	19.29	3	197	N
P0AEK4	Enoyl-[acyl-carrier-protein] reductase [NADH] FabI OS=Escherichia coli (strain K12) GN=fabI PE=1 SV=2 - [FABI_ECOLI]	8.60	24.81	6	262	N

SUPPLEMENT

33

P0A7G2	Ribosome-binding factor A OS=Escherichia coli (strain K12) GN=rbfA PE=1 SV=2 - [RBFA_ECOLI]	8.15	35.34	4	133	Y
P0AC41	Succinate dehydrogenase flavoprotein subunit OS=Escherichia coli (strain K12) GN=sdhA PE=1 SV=1 - [SDHA_ECOLI]	8.08	5.61	3	588	N
P33599	NADH-quinone oxidoreductase subunit C/D OS=Escherichia coli (strain K12) GN=nuoC PE=1 SV=3 - [NUOCD_ECOLI]	8.07	14.93	8	596	N
P00961	Glycine--tRNA ligase beta subunit OS=Escherichia coli (strain K12) GN=glyS PE=1 SV=4 - [SYGB_ECOLI]	7.99	6.39	4	689	Y
P0A8F0	Uracil phosphoribosyltransferase OS=Escherichia coli (strain K12) GN=upp PE=1 SV=1 - [UPP_ECOLI]	7.95	23.56	4	208	N
P0A917	Outer membrane protein X OS=Escherichia coli (strain K12) GN=ompX PE=1 SV=1 - [OMPX_ECOLI]	7.95	25.73	3	171	N
P69783	Glucose-specific phosphotransferase enzyme IIA component OS=Escherichia coli (strain K12)	7.93	26.63	3	169	N

SUPPLEMENT

34

	GN=crr PE=1 SV=2 - [PTGA_ECOLI]					
P0AEK2	3-oxoacyl-[acyl-carrier-protein] reductase FabG OS=Escherichia coli (strain K12) GN=fabG PE=1 SV=1 - [FABG_ECOLI]	7.80	15.16	4	244	N
P0A8V2	DNA-directed RNA polymerase subunit beta OS=Escherichia coli (strain K12) GN=rpoB PE=1 SV=1 - [RPOB_ECOLI]	7.64	4.69	7	1342	N
P0AAI3	ATP-dependent zinc metalloprotease FtsH OS=Escherichia coli (strain K12) GN=ftsH PE=1 SV=1 - [FTSH_ECOLI]	7.51	6.52	3	644	N
P77611	Electron transport complex subunit Rsx C OS=Escherichia coli (strain K12) GN=rsxC PE=1 SV=1 - [RSXC_ECOLI]	7.44	11.22	5	740	N
P0ABD8	Biotin carboxyl carrier protein of acetyl-CoA carboxylase OS=Escherichia coli (strain K12) GN=accB PE=1 SV=1 - [BCCP_ECOLI]	7.42	23.72	3	156	N
P0AGE0	Single-stranded DNA-binding protein OS=Escherichia coli (strain K12) GN=ssb PE=1 SV=2 - [SSB_ECOLI]	6.95	13.48	2	178	N

SUPPLEMENT

35

P0A8F4	Uridine kinase OS=Escherichia coli (strain K12) GN=udk PE=3 SV=1 - [URK_ECOLI]	6.82	15.02	3	213	N
P0AEZ3	Septum site-determining protein MinD OS=Escherichia coli (strain K12) GN=minD PE=1 SV=2 - [MIND_ECOLI]	6.80	12.96	3	270	N
P0ACJ0	Leucine-responsive regulatory protein OS=Escherichia coli (strain K12) GN=lrp PE=1 SV=2 - [LRP_ECOLI]	6.65	37.80	7	164	N
P0A805	Ribosome-recycling factor OS=Escherichia coli (strain K12) GN=frr PE=1 SV=1 - [RRF_ECOLI]	6.44	13.51	2	185	Y
P0A7E5	CTP synthase OS=Escherichia coli (strain K12) GN=pyrG PE=1 SV=2 - [PYRG_ECOLI]	6.42	6.42	3	545	N
P0A9Q1	Aerobic respiration control protein ArcA OS=Escherichia coli (strain K12) GN=arcA PE=1 SV=1 - [ARCA_ECOLI]	6.29	15.13	4	238	N
P0A6H5	ATP-dependent protease ATPase subunit HslU OS=Escherichia coli (strain K12) GN=hslU PE=1 SV=1 - [HSLU_ECOLI]	6.24	8.58	4	443	N
P0A6P5	GTPase Der OS=Escherichia coli	6.17	10.82	5	490	N

SUPPLEMENT

36

	(strain K12) GN=der PE=1 SV=1 - [DER_ECOLI]					
P25665	5- methyltetrahydropteroyltriglutamate- -homocysteine methyltransferase OS=Escherichia coli (strain K12) GN=metE PE=1 SV=6 - [METE_ECOLI]	6.14	4.52	3	753	N
P0A9A9	Ferric uptake regulation protein OS=Escherichia coli (strain K12) GN=fur PE=1 SV=1 - [FUR_ECOLI]	6.06	27.03	3	148	N
P61714	6,7-dimethyl-8-ribityllumazine synthase OS=Escherichia coli (strain K12) GN=ribE PE=1 SV=1 - [RISB_ECOLI]	5.98	19.23	2	156	N
P0A6N4	Elongation factor P OS=Escherichia coli (strain K12) GN=efp PE=1 SV=2 - [EFP_ECOLI]	5.91	17.02	2	188	Y
P0ABA0	ATP synthase subunit b OS=Escherichia coli (strain K12) GN=atpF PE=1 SV=1 - [ATPF_ECOLI]	5.91	21.15	3	156	N
P0A9Y6	Cold shock-like protein CspC OS=Escherichia coli (strain K12) GN=cspC PE=1 SV=2 - [CSPC_ECOLI]	5.89	42.03	2	69	N

SUPPLEMENT

37

P0A722	Acyl-[acyl-carrier-protein]--UDP-N-acetylglucosamine O-acyltransferase OS=Escherichia coli (strain K12) GN=lpxA PE=1 SV=1 - [LPXA_ECOLI]	5.68	13.36	3	262	N
P0AGD3	Superoxide dismutase [Fe] OS=Escherichia coli (strain K12) GN=sodB PE=1 SV=2 - [SODF_ECOLI]	5.63	17.62	3	193	N
P0AFC7	NADH-quinone oxidoreductase subunit B OS=Escherichia coli (strain K12) GN=nuoB PE=1 SV=1 - [NUOB_ECOLI]	5.46	9.55	2	220	N
P0AE91	Protein CreA OS=Escherichia coli (strain K12) GN=creA PE=3 SV=1 - [CREA_ECOLI]	5.46	19.75	3	157	N
P0A887	Ubiquinone/menaquinone biosynthesis C-methyltransferase UbiE OS=Escherichia coli (strain K12) GN=ubiE PE=1 SV=1 - [UBIE_ECOLI]	5.38	9.96	2	251	N
P0A780	N utilization substance protein B OS=Escherichia coli (strain K12) GN=nusB PE=1 SV=1 - [NUSB_ECOLI]	5.34	18.71	3	139	N
P16659	Proline--tRNA ligase OS=Escherichia coli (strain K12)	5.32	4.02	2	572	Y

SUPPLEMENT

38

	GN=proS PE=1 SV=4 - [SYP_ECOLI]					
P0AGE9	Succinyl-CoA ligase [ADP-forming] subunit alpha OS=Escherichia coli (strain K12) GN=sucD PE=1 SV=2 - [SUCD_ECOLI]	5.25	15.22	4	289	N
P69222	Translation initiation factor IF-1 OS=Escherichia coli (strain K12) GN=infA PE=1 SV=2 - [IF1_ECOLI]	5.15	47.22	3	72	Y
P13035	Aerobic glycerol-3-phosphate dehydrogenase OS=Escherichia coli (strain K12) GN=glpD PE=1 SV=3 - [GLPD_ECOLI]	5.10	12.57	5	501	N
P33363	Periplasmic beta-glucosidase OS=Escherichia coli (strain K12) GN=bglX PE=1 SV=2 - [BGLX_ECOLI]	5.08	5.23	3	765	N
P00579	RNA polymerase sigma factor RpoD OS=Escherichia coli (strain K12) GN=rpoD PE=1 SV=2 - [RPOD_ECOLI]	5.08	5.55	3	613	N
P0A6Q3	3-hydroxydecanoyl-[acyl-carrier- protein] dehydratase OS=Escherichia coli (strain K12) GN=fabA PE=1 SV=2 - [FABA_ECOLI]	5.05	18.60	3	172	N

SUPPLEMENT

39

P63020	Fe/S biogenesis protein NfuA OS=Escherichia coli (strain K12) GN=nfuA PE=1 SV=1 - [NFUA_ECOLI]	5.01	17.28	3	191	N
P27294	Protein InaA OS=Escherichia coli (strain K12) GN=inaA PE=1 SV=3 - [INAA_ECOLI]	5.01	11.57	2	216	N
P0A9V1	Lipopolysaccharide export system ATP-binding protein LptB OS=Escherichia coli (strain K12) GN=lptB PE=1 SV=2 - [LPTB_ECOLI]	4.88	11.62	2	241	N
P0ABK5	Cysteine synthase A OS=Escherichia coli (strain K12) GN=cysK PE=1 SV=2 - [CYSK_ECOLI]	4.78	7.43	2	323	N
P27302	Transketolase 1 OS=Escherichia coli (strain K12) GN=tktA PE=1 SV=5 - [TKT1_ECOLI]	4.75	4.68	3	663	N
P0A998	Bacterial non-heme ferritin OS=Escherichia coli (strain K12) GN=ftnA PE=1 SV=1 - [FTNA_ECOLI]	4.74	13.94	2	165	N
P0ABP8	Purine nucleoside phosphorylase DeoD-type OS=Escherichia coli (strain K12) GN=deoD PE=1 SV=2 - [DEOD_ECOLI]	4.73	14.64	3	239	N

SUPPLEMENT

40

P24251	Sigma factor-binding protein Crl OS=Escherichia coli (strain K12) GN=crl PE=1 SV=5 - [CRL_ECOLI]	4.70	22.56	3	133	N
P08839	Phosphoenolpyruvate-protein phosphotransferase OS=Escherichia coli (strain K12) GN=ptsI PE=1 SV=1 - [PT1_ECOLI]	4.70	4.17	2	575	N
P0A912	Peptidoglycan-associated lipoprotein OS=Escherichia coli (strain K12) GN=pal PE=1 SV=1 - [PAL_ECOLI]	4.69	15.61	2	173	N
P0ACL7	Putative L-lactate dehydrogenase operon regulatory protein OS=Escherichia coli (strain K12) GN=lldR PE=2 SV=1 - [LLDR_ECOLI]	4.66	8.14	2	258	N
P0A862	Thiol peroxidase OS=Escherichia coli (strain K12) GN=tpx PE=1 SV=2 - [TPX_ECOLI]	4.57	13.10	2	168	N
P24182	Biotin carboxylase OS=Escherichia coli (strain K12) GN=accC PE=1 SV=2 - [ACCC_ECOLI]	4.55	9.13	4	449	N
P0A6Z3	Chaperone protein HtpG OS=Escherichia coli (strain K12) GN=htpG PE=1 SV=1 - [HTPG_ECOLI]	4.40	3.37	2	624	N

SUPPLEMENT

41

P0C0R7	Ribosomal RNA large subunit methyltransferase E OS=Escherichia coli (strain K12) GN=rlmE PE=1 SV=1 - [RLME_ECOLI]	4.40	11.48	2	209	N
P0A8W8	UPF0304 protein YfbU OS=Escherichia coli (strain K12) GN=yfbU PE=1 SV=1 - [YFBU_ECOLI]	4.39	26.22	4	164	N
P69828	Galactitol-specific phosphotransferase enzyme IIA component OS=Escherichia coli (strain K12) GN=gatA PE=3 SV=1 - [PTKA_ECOLI]	4.38	17.33	2	150	N
P0A8J8	ATP-dependent RNA helicase RhlB OS=Escherichia coli (strain K12) GN=rhlB PE=1 SV=2 - [RHLB_ECOLI]	4.38	10.93	3	421	N
P0A9K9	FKBP-type peptidyl-prolyl cis-trans isomerase SlyD OS=Escherichia coli (strain K12) GN=slyD PE=1 SV=1 - [SLYD_ECOLI]	4.37	11.73	2	196	N
P0C054	Small heat shock protein IbpA OS=Escherichia coli (strain K12) GN=ibpA PE=1 SV=1 - [IBPA_ECOLI]	4.35	18.98	3	137	N
P69503	Adenine phosphoribosyltransferase OS=Escherichia coli (strain K12)	4.34	12.57	2	183	N

SUPPLEMENT

42

	GN=apt PE=1 SV=1 - [APT_ECOLI]					
P0A6D7	Shikimate kinase 1 OS=Escherichia coli (strain K12) GN=aroK PE=1 SV=2 - [AROK_ECOLI]	4.34	12.72	2	173	N
P0A8I5	tRNA (guanine-N(7)-)-methyltransferase OS=Escherichia coli (strain K12) GN=trmB PE=1 SV=1 - [TRMB_ECOLI]	4.30	9.62	2	239	N
P0A9X9	Cold shock protein CspA OS=Escherichia coli (strain K12) GN=cspA PE=1 SV=2 - [CSPA_ECOLI]	4.28	31.43	2	70	N
P60546	Guanylate kinase OS=Escherichia coli (strain K12) GN=gmk PE=1 SV=1 - [KGUA_ECOLI]	4.26	12.08	2	207	N
P0AE52	Putative peroxiredoxin bcp OS=Escherichia coli (strain K12) GN=bcp PE=1 SV=1 - [BCP_ECOLI]	4.23	14.74	2	156	N
P37765	Ribosomal large subunit pseudouridine synthase B OS=Escherichia coli (strain K12) GN=rluB PE=1 SV=2 - [RLUB_ECOLI]	4.22	8.93	2	291	N
P0A7Z4	DNA-directed RNA polymerase subunit alpha OS=Escherichia coli	4.21	10.03	3	329	N

SUPPLEMENT

43

	(strain K12) GN=rpoA PE=1 SV=1 - [RPOA_ECOLI]					
NP17169	Glutamine--fructose-6-phosphate aminotransferase [isomerizing] OS=Escherichia coli (strain K12) GN=glmS PE=1 SV=4 - [GLMS_ECOLI]	4.19	3.45	1	609	N
P0A7G6	Protein RecA OS=Escherichia coli (strain K12) GN=recA PE=1 SV=2 - [RECA_ECOLI]	4.15	7.93	2	353	N
P0A9X4	Rod shape-determining protein MreB OS=Escherichia coli (strain K12) GN=mreB PE=1 SV=1 - [MREB_ECOLI]	4.12	6.34	2	347	N

Supplemental table S3. Strains and plasmids used in this study.

Strain	Genotype	Source
MG1655	<i>Escherichia coli</i> K-12 λ^- F ⁻	Lab stock
BW25113	F ⁻ / Δ <i>araD-araB567 lacZ4787Δ::<i>rrnB-3</i> λ^- <i>rph-1</i> Δ<i>rhaD-rhaB568</i> <i>hsdR514</i></i>	(23)
MC4100	F ⁻ / <i>araD139</i> Δ <i>lacU169 rpsL150 thi rbsR</i>	(49)
MM18	MC4100 ϕ (<i>malE-lacZ</i>)	(27)
BL21(DE3)	<i>ompT hsdS_B</i> (<i>r_B⁻</i> , <i>m_B⁻</i>) <i>gal dcm</i> λ (DE3)	Lab stock
DRH959	MG1655 Δ <i>secB</i> ::Kan	This study
DRH974	MG1655 Δ <i>ascA</i>	This study
DRH975	MG1655 Δ <i>ascA</i> Δ <i>secB</i> ::Kan	This study
JW3584	BW25113 Δ <i>secB</i> ::Kan	(23)
JW1896	BW25113 Δ <i>ascA</i> ::Kan	(23)
CC01	BW25113 Δ <i>ascA</i> (Kan ^S)	This study
EH01	BW25113 Δ <i>ascA</i> Δ <i>secB</i> ::Kan + pAscA	This study
DRH966	MM18 Δ <i>ascA</i> ::Kan	This study
DRH969	MM18 Δ <i>secB</i> ::Kan	This study
Plasmid	Description	Source
pTrc99a	High-copy number plasmid with IPTG-inducible promoter and ampicillin-resistance marker	Stratagene
pAscA	pTrc99a containing <i>ascA</i> gene inserted between <i>NcoI</i> and <i>BamHI</i> restriction sites	This study

SUPPLEMENT

45

pCA528	High-copy number plasmid containing T7-inducible copy of gene encoding a His ₆ -tagged SUMO protein from <i>S. cerevisiae</i> .	(47)
pCA597	High-copy number plasmid containing T7-inducible copy of gene encoding a Strep(II) ₃ -tagged SUMO protein from <i>S. cerevisiae</i> .	(47)
pDH585	pCA528 containing <i>secB</i> gene inserted between <i>BsaI</i> and <i>BamHI</i> restriction sites	This study
pDH963	pCA597 containing <i>ascA</i> gene inserted between <i>BsaI</i> and <i>BamHI</i> restriction sites	This study
pCS070	pCA528 containing <i>ascA</i> gene inserted between <i>BsaI</i> and <i>BamHI</i> restriction sites	This study
pCS071	pCA528 containing <i>ascAΔMBD</i> gene inserted between <i>BsaI</i> and <i>BamHI</i> restriction sites	This study
pCS163	pCA597 containing <i>ascAΔMBD</i> gene inserted between <i>BsaI</i> and <i>BamHI</i> restriction sites	This study