FEBS Letters 498 (2001) 52–56 FEBS 24919

Complementation of bacterial SecE by a chloroplastic homologue

Linda Fröderberg[a], Thomas Röhl[b], Klaas-Jan van Wijk[b], Jan-Willem L. de Gier[a,*]

[a] *Department of Biochemistry and Biophysics, Arrhenius Laboratories, Stockholm University, S-106 91 Stockholm, Sweden*
[b] *Department of Plant Biology, Cornell University, Tower Road, Ithaca, NY 14853-5908, USA*

Received 6 April 2001; accepted 4 May 2001

First published online 17 May 2001

Edited by Felix Wieland

Abstract The SecE protein is an essential component of the SecAYE-translocase, which mediates protein translocation across the cytoplasmic membrane in bacteria. In the thylakoid membranes of chloroplasts, a protein homologous to SecE, chloroplastic (cp) SecE, has been identified. However, the functional role of cpSecE has not been established experimentally. In this report we show that cpSecE in cells depleted for bacterial SecE (i) supports growth, (ii) stabilizes, just like bacterial SecE, the Sec-translocase core component SecY, and (iii) supports Sec-dependent protein translocation. This indicates that cpSecE can functionally replace bacterial SecE in vivo, and strongly suggests that the thylakoid membrane contains a SecAYE-like translocase with functional and structural similarities to the bacterial complex. This study further underscores the evolutionary link between chloroplasts and bacteria.

Key words: Chloroplast; Protein translocation; Sec-translocase; *Escherichia coli*

1. Introduction

Most secretory proteins in bacteria are translocated across the cytoplasmic membrane via the Sec-translocase [1]. Genetic and biochemical studies have shown that the core of the bacterial Sec-translocase consists of the integral membrane protein components SecY and SecE, and the peripheral subunit SecA. SecY and SecE form a complex, with multiple SecY–SecE complexes forming a protein conducting channel. The peripheral SecA protein drives proteins in an ATP-dependent manner through the SecY/E channel across the cytoplasmic membrane [2,3].

Evidence is accumulating that the targeting of chloroplastic proteins to the thylakoid membrane in plants shows analogy to bacterial secretion [4,5]. Chloroplastic (cp) SecA and cpSecY are the chloroplastic homologues of the bacterial Sec-translocase components SecA and SecY, respectively [6,7]. Studies employing transposon tagged disruption mutants in maize, as well as in vitro import assays, have shown that both cpSecA and cpSecY are involved in the translocation of proteins across the thylakoid membrane [8–11]. Recently, also a chloroplastic homologue of bacterial SecE, designated cpSecE, has been identified. CpSecE could be purified with cpSecY in a 180 kDa complex, suggesting a functional interaction [8]. However, no experimental evidence for the function of cpSecE has yet been presented.

In *Escherichia coli*, SecE depletion strongly impairs Sec-dependent protein secretion, and, as a consequence, is detrimental to the cell [12]. *E. coli* SecE contains three transmembrane segments, and it has been shown that function of *E. coli* SecE is confined to the C-terminal transmembrane region of the protein (Fig. 1) [13]. SecE in *Thermotoga maritima* and *Bacillus subtilis* has only one transmembrane domain, which resembles the C-terminal region of *E. coli* SecE (Fig. 1). Both SecE molecules can substitute *E. coli* SecE in vivo [14,15]. The C-terminal region of cpSecE is homologous to these bacterial SecE proteins (Fig. 1) [8]. In this report we show that cpSecE can functionally complement SecE in *E. coli*. This indicates that cpSecE functions in protein translocation, and it strongly suggests that the thylakoid membrane contains a cpSecAYE-translocase with similar protein translocating properties as the *E. coli* Sec-translocase.

2. Materials and methods

2.1. Strains and culture conditions

The *E. coli* SecE depletion strain CM124 [12] was used in the complementation studies. Cells were cultivated in Luria Bertani (LB) medium at 37°C, unless otherwise stated. Where appropriate, medium was supplemented with ampicillin (final concentration 100 μg/ml), kanamycin (final concentration 25 μg/ml), arabinose (final concentration 0.2%) and isopropyl-1-thio-β-D-galactopyranoside (IPTG) (final concentration 1 mM).

2.2. Construction of the cpSecE expression vector pEH1cpSecE

The *Arabidopsis thaliana* gene encoding cpSecE (without the genetic information encoding its transit peptide) was amplified by PCR from plasmid pQE30cpSecE [8] using the upstream primer: 5′-CGCGCCATGGCGACGAGTAATCTGAGGAAATC-3′ (*Nco*I site underlined) and the downstream primer: 5′-CGCGCAAGCTTTCAGCTGAAGAAGTCTTGAAC-3′ (*Hin*dIII site underlined). This removed the first threonine of the mature cpSecE protein and converted the third amino acid from a threonine into an alanine (Fig. 1). The PCR fragment was digested with *Nco*I and *Hin*dIII and cloned into the *Nco*I–*Hin*dIII site of expression vector pEH1 [16], yielding the cpSecE expression vector pEH1cpSecE. The nucleotide sequence of the PCR fragment was verified by DNA sequencing. All DNA techniques were essentially performed as described by Sambrook et al. [17].

2.3. Antibody production and immunoblot analysis

For production of cpSecE antiserum, precursor cpSecE (cpSecE with its transit peptide) was overexpressed in inclusion bodies in *E. coli*, essentially as described previously [8]. Inclusion bodies were iso-

*Corresponding author. Fax: (46)-8-153679.
E-mail: degier@dbb.su.se

Abbreviations: cp, chloroplastic; IPTG, isopropyl-1-thio-β-D-galactopyranoside; SDS–PAGE, sodium dodecylsulfate–polyacrylamide gel electrophoresis; OmpA, outer membrane protein A

PII: S0014-5793(01)02494-2

lated as described by Paulsen et al. [18], and injected into rabbits to raise a polyclonal antiserum. CpSecE inclusion bodies and thylakoid membranes were used to test the antiserum. Antisera to bacterial SecE and SecY were raised using synthetic peptides (SecY-peptide: N-CSQYESALKKANLKGYGR-C, SecE-peptide: N-KGKATVA-FAREARTEVRKC-C). Antisera were raised by AgriSera AB, Umeå, Sweden. The antiserum to bacterial SecA was a gift from Arnold Driessen.

To monitor cpSecE, SecE, SecY and SecA levels in *E. coli*, CM124 cells with the cpSecE expression vector pEH1cpSecE were cultivated under different SecE expression/depletion regimes. After 8 h, cells were harvested and subsequently solubilized in Laemmli solubilization buffer. 0.2 OD_{600} units of cells were separated by sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS–PAGE) [19]. Proteins were subsequently transferred from the polyacrylamide gel to a polyvinylidene fluoride membrane, according to the guidelines of the manufacturer (Millipore). Membranes were decorated with cpSecE, SecE, SecY and SecA antisera. Proteins were visualized with secondary antibodies using either the alkaline phosphatase system (according to the instructions of the manufacturer, Sigma) or enhanced chemiluminescence (according to the instructions of the manufacturer, Amersham Pharmacia).

2.4. Assay for protein secretion

Cells were grown in M9 minimal medium for 8 h, essentially as described previously [20]. Aliquots were pulse-labeled for 30 s with 100 μCi of [^{35}S]methionine and precipitated in 10% TCA. TCA pellets were processed and immunoprecipitated with antiserum raised against outer membrane protein A (OmpA) as described previously [20].

2.5. Miscellaneous

Protein concentrations were determined using the BCA protein assay kit from Pierce. Chlorophyll concentrations of thylakoids were spectroscopically determined in 80% acetone [21].

3. Results

3.1. CpSecE can sustain growth in E. coli cells depleted for bacterial SecE

To test SecE complementation in *E. coli*, we used the SecE depletion strain CM124, in which the gene encoding SecE is under control of an arabinose inducible promoter [12]. The genetic information encoding the mature form of cpSecE (cpSecE) was cloned in the expression vector pEH1 (see Section 2 and Fig. 1). In the cpSecE expression vector, the gene encoding cpSecE is under control of an IPTG inducible promoter [16]. Both promoter systems are tightly regulated and use different inducers, which is naturally essential to test complementation. CM124 cells with the cpSecE expression vector and CM124 cells with the empty expression vector were cultivated under different SecE expression/depletion regimes (Fig. 2). In the absence of inducers, growth of cells with either the cpSecE expression vector or the empty expression vector ceased rapidly, as expected. In the presence of arabinose alone, both cells with the cpSecE expression vector and cells with the empty expression vector grew as wild-type *E. coli*, as expected. In the presence of only IPTG, cells with the control vector did not grow. Remarkably, cells with the cpSecE expression vector grew in the presence of only IPTG, albeit not as efficient as cells grown in the presence of only arabinose. These results strongly suggest that cpSecE is expressed in *E. coli*, and can sustain growth in cells depleted for bacterial SecE.

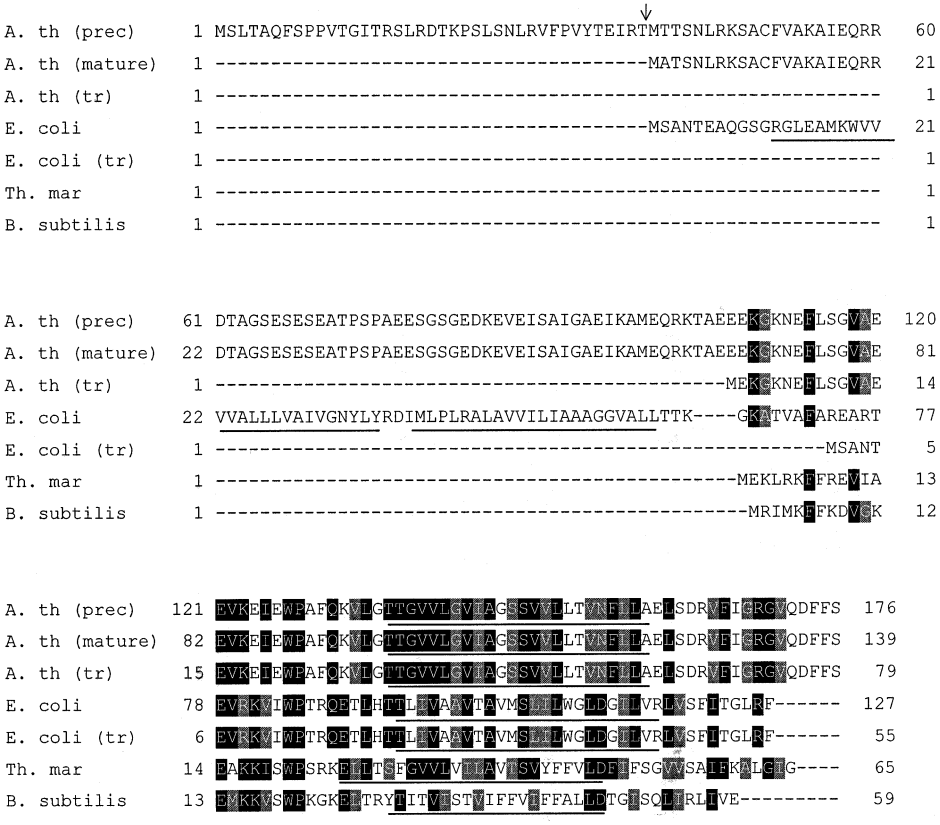

Fig. 1. Alignment of *A. thaliana* cpSecE with bacterial SecE sequences. Sequences of precursor *A. thaliana* cpSecE, mature *A. thaliana* cpSecE and a truncated form (tr) of *A. thaliana* cpSecE were aligned to *E. coli* SecE, a truncated form of *E. coli* SecE (E. coli (tr)) that can support growth in *E. coli* [13], *T. maritima* SecE and *B. subtilis* SecE. The mature form of cpSecE was used in this study for the expression and functional complementation in *E. coli*. The alignment was made using ClustalW1.8 and Boxshade (version 3.2.1). Predicted transmembrane domains are underlined. The putative cleavage site for the stromal processing peptidase is indicated by an arrow.

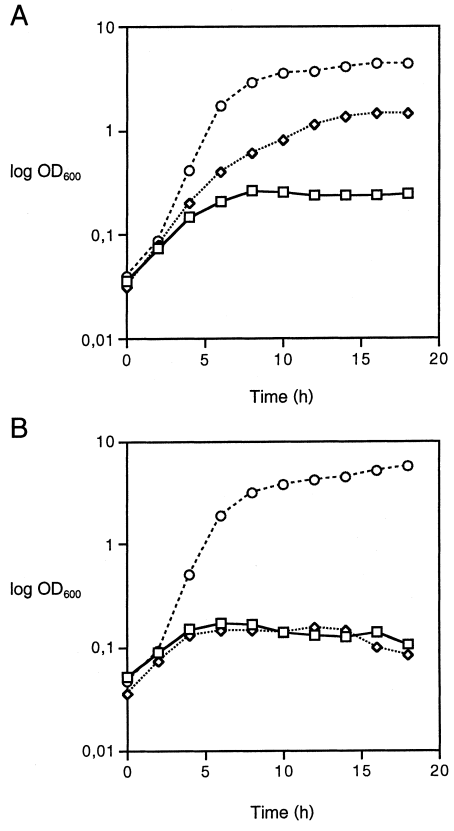

Fig. 2. CpSecE can support growth of *E. coli* cells depleted for bacterial SecE. CM124 cells with the cpSecE expression vector pEH1c-pSecE (A) and CM124 cells with the empty expression vector pEH1 (B) were cultivated under different SecE expression/depletion regimes. Over night cultures were cultivated in the presence of both arabinose and IPTG. Cells were washed twice with LB medium before they were 1:100 backdiluted in LB medium with only arabinose (○), medium with only IPTG (◇) and medium without any arabinose and IPTG (□). Arabinose leads to the induction of expression of bacterial SecE, and IPTG leads to the induction of expression of cpSecE. Growth was monitored by measuring the OD_{600} with a Shimadzu UV-1601 spectrophotometer.

CpSecE has a long stromal N-terminus that does not share any homology with any other known protein. Therefore, we tested whether this soluble domain was required for the complementation of for SecE depleted *E. coli* cells. Indeed, the truncated version (see Fig. 1) did complement, but very inefficiently (results not shown). This indicates that the soluble domain is needed for expression and/or stability. Therefore, we continued to use full length cpSecE in our complementation studies.

3.2. Detection of cpSecE, bacterial SecE, SecY, and SecA

To be able to detect cpSecE, we raised an antiserum to precursor cpSecE. The cpSecE antiserum reacted specifically with both precursor cpSecE in inclusion bodies and processed cpSecE in the thylakoid membrane, as shown by means of Western blotting (Fig. 3). The cpSecE antiserum was then used to monitor expression of cpSecE in CM124 cells harboring the cpSecE expression vector grown under different SecE expression/depletion regimes. As shown in Fig. 4A, cpSecE could only be detected in cells cultivated in the presence of IPTG, whereas bacterial SecE could only be detected in cells cultivated in the presence of arabinose (Fig. 4B). This indicates that in the absence of arabinose bacterial SecE is effectively depleted in CM124 cells [12,22], and that in the presence of IPTG cpSecE is expressed. Cell fractionation experiments indicated that cpSecE accumulated stably in the *E. coli* cytoplasmic membrane (results not shown).

The Sec-translocase components SecE and SecY form a complex. It has been shown that SecE stabilizes SecY; i.e. if SecY does not form a complex with SecE it is rapidly degraded by the FtsH protease [23]. Therefore, we also monitored the SecY levels in cells grown under different SecE expression/depletion regimes (Fig. 4C). In cells grown in the presence of arabinose SecY could be detected, whereas in cells depleted for bacterial SecE, no SecY could be detected. In cells depleted for bacterial SecE but complemented with cpSecE, considerable amounts of SecY could be detected. This shows that cpSecE can stabilize SecY, indicating a direct interaction of cpSecE with SecY.

Depletion of SecE has been shown to result in the upregulation of SecA expression, which is a common phenomenon in cells experiencing protein secretion defects [22]. If cpSecE truly can complement for SecE, upregulation of SecA should be suppressed. Therefore, we also monitored SecA levels in cells grown under the different SecE expression/depletion regimes (Fig. 4D). In cells cultivated in the presence of arabinose wild-type levels of SecA were present, whereas depletion of SecE led to very strong upregulation of SecA. However, in *E. coli* cells that we depleted for bacterial SecE and complemented with cpSecE, SecA levels were marginally higher than the wild-type SecA levels. This indicates that cells complemented with cpSecE experience only little protein secretion stress.

3.3. CpSecE supports protein translocation in E. coli cells depleted for bacterial SecE

The growth and blotting experiments indicate that cpSecE can complement bacterial SecE. To study this complementation directly in a functional assay, the translocation of the OmpA was studied in CM124 cells with the cpSecE expression vector, and, as a control, in CM124 cells with the empty expression vector. Translocation of OmpA was monitored by studying the conversion of precursor OmpA (OmpA with a signal sequence) into mature OmpA (OmpA without a signal sequence) (Fig. 5). Previously, it has been shown that the translocation of OmpA across the inner membrane is medi-

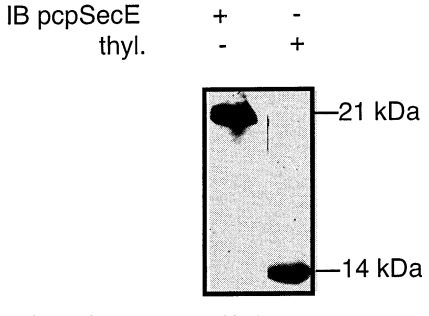

Fig. 3. Detection of cpSecE. Purified precursor cpSecE inclusion bodies (IB pcpSecE, 0.5 μg of protein) and thylakoid membranes (thyl., 12 μg of chlorophyll) were separated by means of SDS–PAGE and subsequently subjected to immunoblot analysis with anti-cpSecE antibodies. On the right the relative molecular masses of marker proteins are indicated.

ated by the SecAYE-translocase [12,24,25], and translocation of OmpA thus serves as a good assay to monitor functional complementation by cpSecE.

Cells were cultured for 8 h in minimal medium and subsequently labeled with [^{35}S]methionine for 30 s. In cells with either vector, cultivated in the absence of both arabinose and IPTG, translocation of OmpA was strongly hampered. A similar result was obtained in cells with the empty expression vector, cultivated in the presence of IPTG alone. However, in cells with the cpSecE expression vector, when cultivated in the presence of IPTG alone, translocation of OmpA proceeded as in wild-type cells. This indicates that cpSecE can support Sec-dependent protein translocation in cells depleted for bacterial SecE. All controls worked as expected. When cultivated in the presence of arabinose alone, translocation of OmpA was not affected in cells with either the cpSecE expression vector or the control vector, and processed OmpA was the dominant band (Fig. 5).

4. Discussion

We have used a complementation approach to study the function of the chloroplastic protein cpSecE, which shows a strong homology in its C-terminal region with the C-terminal region of *E. coli* SecE. When expressed in *E. coli* cells depleted for bacterial SecE, cpSecE can (i) support growth, (ii) stabilize the Sec-translocase component SecY, and (iii) support Sec-dependent protein translocation.

Bacterial SecE stabilizes SecY through a direct interaction [23]. The observation that SecY is stabilized by cpSecE indicates that cpSecE and SecY interact directly. Upon complementation of the SecE depletion mutant with cpSecE, translocation of the Sec-dependent protein OmpA across the cytoplasmic membrane is completely restored. Furthermore, SecA is only marginally upregulated upon complementation with cpSecE, indicating that the complemented cells experience only little protein secretion stress. These observations all point to a functional hybrid SecAYcpSecE-translocase.

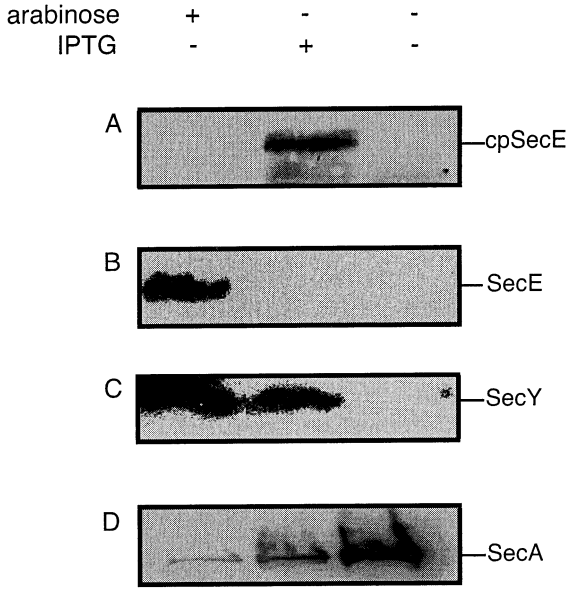

Fig. 4. Detection of cpSecE, bacterial SecE, SecY and SecA. CM124 cells with the cpSecE expression vector pEH1cpSecE cultivated under different SecE expression/deletion conditions. Arabinose leads to the induction of expression of bacterial SecE, and IPTG leads to the induction of expression of cpSecE. Cells (0.2 OD_{600} units) were separated by means of SDS–PAGE, and subsequently subjected to immunoblot analysis with anti-cpSecE (A), bacterial SecE (B), SecY (C), and SecA (D) antibodies.

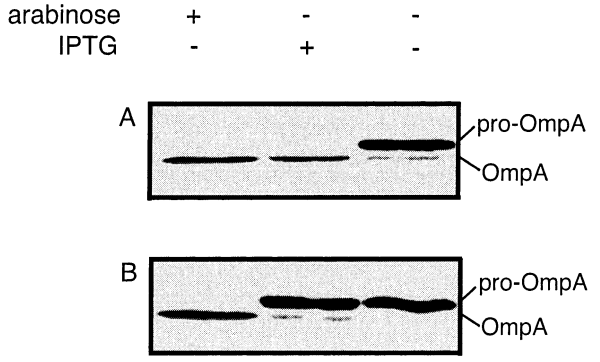

Fig. 5. CpSecE can support OmpA secretion in *E. coli* cells depleted for bacterial SecE. Autoradiograph of OmpA translocation experiment. The translocation of the Sec-dependent protein OmpA was monitored in CM124 cells with the cpSecE expression vector pEH1cpSecE (A) and CM124 cells with the empty expression vector pEH1 (B), cultivated under different bacterial and cpSecE expression/depletion regimes. Over night cultures were cultivated in M9 medium in the presence of both arabinose and IPTG and washed twice with M9 medium before they were backdiluted 1:50 in medium with only arabinose, medium with only IPTG or medium without any arabinose and IPTG. Arabinose leads to the induction of expression of bacterial SecE, and IPTG leads to the induction of expression of cpSecE. After 8 h cells were pulse-labeled with [^{35}S]methionine and processed as described under Section 2. Pro-OmpA is the precursor form of OmpA, and OmpA is the processed/secreted form of OmpA.

CpSecE has a large N-terminal region that does not share any homology with other known proteins. Complementation of the SecE depletion strain with a truncated version of cpSecE was very inefficient as compared to complementation with full length cpSecE. It is very likely that this soluble N-terminal domain is directly involved in the stabilization of cpSecE, since we could not detect any truncated cpSecE in *E. coli* cells, even when the truncated form of cpSecE was tagged with immunogenic epitopes. There is an interesting similarity with bacterial SecE, as it has recently been shown that also the non-essential N-terminal region consisting of two transmembrane domains of *E. coli* SecE is important for the stability of SecE [26]. Without the N-terminal domain *E. coli* SecE is still functional, but it is rapidly degraded by the FtsH protease. In the thylakoid membrane of chloroplasts there is a homologue of the bacterial FtsH protease, which has been shown to play a role in the degradation of thylakoidal membrane proteins [27]. Thus, it is very well conceivable that the N-terminal region of cpSecE stabilizes the protein.

Taken together, our cpSecE complementation study indicates that cpSecE has a similar function as bacterial SecE. It once more shows the strength of complementation to study the function of proteins that are not easily accessible in their native system. It had been shown earlier that cpSecA and cpSecY, the chloroplastic homologues of the bacterial Sec-translocase components SecA and SecY, are involved in the translocation of proteins across the thylakoid membrane in chloroplasts, and that cpSecE and cpSecY form a complex [8–11]. These observations, together with our complementation experiments, show that in chloroplasts there

is a cpSecAYE-translocase. Last but not least, our study underscores the evolutionary link between chloroplasts and bacteria.

Acknowledgements: We gratefully thank Chen-ni Chin and Iwona Adamska for stimulating discussions and critically reading the manuscript. Neil Hoffman and Jenny Broome-Smith are thanked for plasmids. Arnold Driessen is thanked for the SecA antiserum. This work was supported by grants from the Swedish Natural Sciences Research Council, the Swedish Foundation for Strategic Research and the Carl Trygger Foundation to J.W.d.G. and grants from the Swedish Natural Sciences Research Council and the Carl Trygger Foundation to K.J.v.W.

References

[1] Manting, E.H. and Driessen, A.J. (2000) Mol. Microbiol. 37, 226–238.
[2] Meyer, T.H., Menetret, J.F., Breitling, R., Miller, K.R., Akey, C.W. and Rapoport, T.A. (1999) J. Mol. Biol. 285, 1789–1800.
[3] Manting, E.H., van Der Does, C., Remigy, H., Engel, A. and Driessen, A.J. (2000) EMBO J. 19, 852–861.
[4] Keegstra, K. and Cline, K. (1999) Plant Cell 11, 557–570.
[5] Dalbey, R.E. and Kuhn, A. (2000) Annu. Rev. Cell Dev. Biol. 16, 51–87.
[6] Yuan, J.G., Henry, R., McCaffery, M. and Cline, K. (1994) Science 266, 796–798.
[7] Laidler, V., Chaddock, A.M., Knott, T.G., Walker, D. and Robinson, C. (1995) J. Biol. Chem. 270, 17664–17667.
[8] Schuenemann, D., Amin, P., Hartmann, E. and Hoffman, N.E. (1999) J. Biol. Chem. 274, 12177–12182.
[9] Mori, H., Summer, E.J., Ma, X. and Cline, K. (1999) J. Cell Biol. 146, 45–56.
[10] Voelker, R. and Barkan, A. (1995) EMBO J. 14, 3905–3914.
[11] Haward, S.R., Napier, J.A. and Gray, J.C. (1997) Eur. J. Biochem. 248, 724–730.
[12] Traxler, B. and Murphy, C. (1996) J. Biol. Chem. 271, 12394–12400.
[13] Schatz, P.J., Bieker, K.L., Ottemann, K.M., Silhavy, T.J. and Beckwith, J. (1991) EMBO J. 10, 1749–1757.
[14] Jeong, S.M., Yoshikawa, H. and Takahashi, H. (1993) Mol. Microbiol. 10, 133–142.
[15] Murphy, C.K. and Beckwith, J. (1994) Proc. Natl. Acad. Sci. USA 91, 2557–2561.
[16] Hashemzadeh-Bonehi, L., Mehraein-Ghomi, F., Mitsopoulos, C., Jacob, J.P., Hennessey, E.S. and Broome-Smith, J.K. (1998) Mol. Microbiol. 30, 676–678.
[17] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Cold Spring Harbour Laboratory, Cold Spring Harbour, NY.
[18] Paulsen, H., Rümler, U. and Rüdiger, W. (1990) Planta 181, 204–211.
[19] Laemmli, U.K. (1970) Nature 227, 680–685.
[20] de Gier, J.W.L., Scotti, P.A., Sääf, A., Valent, Q.A., Kuhn, A., Luirink, J. and von Heijne, G. (1998) Proc. Natl. Acad. Sci. USA 95, 14646–14651.
[21] Porra, R.J., Thompson, W.A. and Kriedemann, P.E. (1989) Biochim. Biophys. Acta 975, 384–394.
[22] Yang, Y.B., Yu, N. and Tai, P.C. (1997) J. Biol. Chem. 272, 13660–13665.
[23] Akiyama, Y., Kihara, A., Tokuda, H. and Ito, K. (1996) J. Biol. Chem. 271, 31196–31201.
[24] Driessen, A.J.M. (1992) EMBO J. 11, 847–853.
[25] Joly, J.C. and Wickner, W. (1993) EMBO J. 12, 255–263.
[26] Nishiyama, K., Suzuki, H. and Tokuda, H. (2000) Biosci. Biotechnol. Biochem. 64, 2121–2127.
[27] Lindahl, M., Spetea, C., Hundal, T., Oppenheim, A.B., Adam, Z. and Andersson, B. (2000) Plant Cell 12, 419–431.