Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 355 (2007) 143–148
BBRC
www.elsevier.com/locate/ybbrc

Experimental confirmation of a key role for non-optimal codons in protein export

Yaramah M. Zalucki, Michael P. Jennings *

School of Molecular & Microbial Sciences, University of Queensland, St. Lucia, Qld 4072, Australia

Received 22 January 2007
Available online 31 January 2007

Abstract

Non-optimal codons are defined by low usage and low abundance of corresponding tRNA, and have an established role in translational pausing to allow the correct folding of proteins. Our previous work reported a striking abundance of non-optimal codons in the signal sequences of secretory proteins exported via the *sec*-dependent pathway in *Escherichia coli*. In the current study the signal sequence of maltose-binding protein (MBP) was altered so that non-optimal codons were substituted with the most optimal codon from their synonymous codon family. The expression of MBP from the optimized allele (*malE*-opt) was significantly less than wild-type *malE*. Expression of MBP from *malE*-opt was partially restored in a range of cytoplasmic and periplasmic protease deficient strains, confirming that reduced expression of MBP in *malE*-opt was due to its preferential degradation by cytoplasmic and periplasmic proteases. These data confirm a novel role for non-optimal codon usage in secretion by slowing the rate of translation across the N-terminal signal sequence to facilitate proper folding of the secreted protein.
© 2007 Elsevier Inc. All rights reserved.

Keywords: Codon usage; Protein export; *Escherichia coli*; Signal sequence

The movement of proteins across the bacterial inner membrane requires proteins to be in a translocationally competent state. This is an unfolded or loosely folded state, achieved prior to the final conformation of the protein [1]. A feature of all proteins exported via the general secretory pathway (*sec*) system is a signal peptide at the N-terminus of the protein. Export of proteins via the *sec* pathway can be achieved either post- or co-translationally; the choice of post- or co-translational export is determined by the relative hydrophobicity of the signal peptide [2,3]. For proteins exported co-translationally, signal-recognition particle (SRP) binds nascent peptide to facilitate translocation to the inner membrane and the export apparatus. In the case of post-translational export, molecular chaperones SecB [4,5] and DnaK [6] bind the pre-protein to maintain an export competent state. In addition, one of the proposed roles for the signal peptide is to slow the folding of the pre-protein to maintain it in the competent state [7–9]. Hence, a general feature of *sec*-dependent proteins is that there are mechanisms in the cytoplasm to slow the folding of the pre-protein to maintain an export competent state.

Non-optimal codons correlate with translational pause sites [10], and consecutive runs of non-optimal codons do reduce the rate of translation [11]. These pause sites have an established role in the correct folding of protein domains [12,13]. It has been observed that there is a higher abundance of non-optimal codons in the signal sequence of secretory proteins relative to both their N-terminus and non-secreted proteins of *Escherichia coli* [14,15]. This same phenomenon has been recently reported in a Gram-positive bacterium *Streptomyces coelicolor* [16]. It has been proposed that the high proportion of non-optimal codons would slow the translational rate, to allow efficient interaction of the pre-protein with the export machinery [14]. To date this hypothesis, and experimental confirmation of a key role for non-optimal codons in protein export has not been tested. In this study, the SecB dependent [4], periplasmically located maltose-binding protein (MBP)

* Corresponding author.
E-mail address: jennings@uq.edu.au (M.P. Jennings).

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.01.126

was used as a model system to investigate the role of signal sequence codon usage in protein export.

Materials and methods

Bacterial strains and growth conditions. A list of all strains used in this study is given in Table 1. Cells were grown at 37 °C in rich Luria–Bertani medium supplemented with the appropriate antibiotic in the following final concentrations: ampicillin 100 μg/ml; streptomycin 10 μg/ml; and kanamycin 100 μg/ml. Complementation of *malE* mutant strains was done in M9 minimal media with 0.4% (v/v) maltose (Sigma–Aldrich) as the sole carbon source [17]. Maltose fermentation test was as described previously [18].

DNA manipulation and protein sequencing. Sequencing was performed on PCR products by using QiaQuick gel extraction kit (Qiagen, Valencia, CA) and Big-Dye (Perkin-Elmer) sequencing kits. Data were analyzed by using Sequencher (version 1.0.3). Protein sequencing was done by Edman degradation (Applied Biosystem's Procise 492cLC).

Construction of malE-wt and malE-opt. The *malE*-wt allele was the *malE::lacZα* gene on the expression vector pMALp2e (New England Biolabs). The *malE*-opt allele was generated by PCR mutagenesis. An 111 bp primer P1-opt (Table 2) was designed spanning the MBP signal sequence incorporating all non-optimal to optimal changes (Fig. 1A). A 1.6-kb fragment was amplified using primers P1-opt and M13F-40 (Table 2) and digested with *Nde*I and *Pst*I. The vector pMALp2e (New England Biolabs) was digested with *Nde*I and *Pst*I and the fragment ligated and transformed into DH5α via electroporation. Clones were confirmed by sequencing the whole *malE* gene.

Expression analysis of malE-wt and malE-opt. A 3-mL starter culture was grown in LB ampicillin overnight then subcultured 1:100 into 4 mL LB ampicillin. After 2 h growth, cells were induced with 0.03 mM IPTG and grown for another 2 h. An optical density at 600 nm was measured (OD_{600nm}) and culture split, with 1 mL pelleted for Western analysis and 3 mL used for a miniprep and RNA preparation. For Western analysis, the pellet was resuspended in sample buffer, boiled for 10 min and a loading equivalent of 10^8 cells were loaded per sample on an 4–12% SDS–PAGE. Expression of MBP was detected using anti-MBP rabbit polyclonal antibody (NEB, Cat no. E8030 S) at 1:10, 000 dilution and detected using anti-rabbit IgG conjugated with alkaline phosphatase.

RNA extraction and semi-quantitative reverse transcriptase (SQRT). RNA was extracted from both *malE*-wt and *malE*-opt using the Rneasy Midiprep kit (Qiagen). The RNA was converted to cDNA as per manufacturer's instructions (TaqMan, Applied Biosystems International). The SQRT PCR contained 0.01 μM of the primers specific for 16 S transcript and 0.04 μM of primers specific for *malE::lacZα* transcript (primers listed in Table 2).

Calculation of translational time. The theoretical translational time of the 26 codons of the signal sequence was calculated according to previous published data [19]. The published translational times depended on two different conditions: the supply of tRNA (t_{sup}) and the codon demand (t_{dem}). As all strains were grown in LB, it was assumed that all tRNA species would be charged, so the supply of tRNA, t_{sup}, would not be limiting. For the purposes of this study it is assumed that t_{dem} times are the most appropriate.

Results

MBP expression is significantly reduced when malE signal sequence codon usage is optimized

The characteristics of MBP secretion have been extensively studied as a dominant model system in *E. coli* (for a review see [20]). In the pMALp2e expression system, wild-type MBP is fused with LacZα peptide (*malE::lacZα*). This provided a suitable system to express and study MBP. Analysis of the 26 amino acid MBP signal sequence revealed seven non-optimal codons as defined by Burns

Table 1
Strains

Strain	Relevant genotype	Parent	CGSC no; (reference)
DH5α	*F-(80dlacZ M15) (lacZYA-argF) U169 hsdR17(r-m+) recA1 endA1 relA1 deoR*		Lab stock
MC4100	*F-araD139 A(argF-lac)U169 rpsL150 deoCI relA1 thiA ptsF25 flbB5301*		6152
TST1	*malE::Tn10*	MC4100	6137
TL225	*malT^c-1 ΔmalE444*	MC4100	6961
KS272	*F-lacX74 galE galK thi rpsL (strA) phoA*		G. Georgiou [33]
KS474	*degP*	KS272	G. Georgiou [34]
SF100	*ompT*	KS272	G. Georgiou [34]
SF110	*degP ompT*	KS272	G. Georgiou [34]
SG20252	*Δlon-100*	MC4100	6830; [35]
SG21165	*Δlon-510 ΔclpA500*	MC4100	S. Gottesman [36]
KY2039	*ΔhslVU::Tc*	MC4100	M. Kanemori [37]
KY2263	*ΔclpPX–lon::Cm*	MC4100	M. Kanemori [37]
KY2266	*ΔclpPX–lon::Cm ΔhslVU::Tc*	MC4100	M. Kanemori [37]

Table 2
Primers

Name	Sequence 5′–3′
P1-opt	ACCATAGCATATGAAAATCAAAACGGGTGCACGCATCCTGGCACTGTC CGCACTGACGACGATGATGTTTTCCGCCTCGGCTCTGGCCAAAATC
M13F-40	GTTTTCCCAGTCACGAC
P2e1	GTAATCGGTGTCTGCATTCATGTG
P2e3	CGGCAAGTACGACATTAAAGAC
16SF	ACGGAGGGTGCGAGCGTTAATC
16SR	CTGCCTTCGCCTTCGGTATTCCT

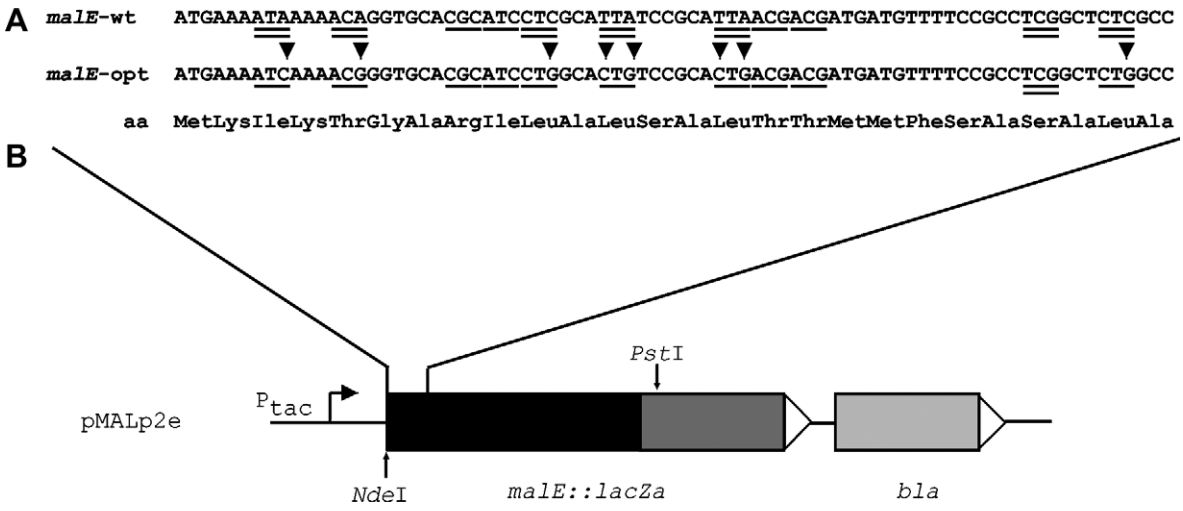

Fig. 1. Schematic of cloning strategy. (A) The nucleotide sequence of the MBP signal sequence on the parental plasmid pMALp2e is shown as *malE*-wt. The nucleotide changes made by PCR mutagenesis to create *malE*-opt are shown by block arrows. Single underline indicates optimal codons, double underline shows non-optimal codons and codons where there was no optimal/non-optimal choice are not underlined. (B) Schematic of the cloning of *malE*-opt into the *Nde*I and *Pst*I sites of pMAlp2e.

and Beacham [15], four optimal codons and a further 15 codons where there were no non-optimal/optimal codons in the synonymous codon family (Fig. 1A). Six of the non-optimal codons were changed to their most optimal synonymous codon by PCR mutagenesis to make the *malE*-opt allele (see Materials and methods). The serine codon, TCG, was not changed, as the serine codon family does not exhibit a preference for non-optimal codons in the signal sequence relative to the 5′ mature sequence of the secretory gene [14]. The final *malE*-opt and *malE*-wt alleles contain 10/26 and 4/26 codons defined as optimal, respectively (Fig. 1A). The theoretical translational times across the signal sequence was calculated according to Solomovici [19]. The theoretical translation time of the *malE*-opt allele signal sequence (1530 ms) is approximately 40% faster compared to the *malE*-wt allele (2539 ms).

To investigate the effect of codon optimisation on the levels of MBP expression, the constructs were transferred into a *malE* deficient strain TST1 (Table 1), so that endogenous MBP production would not interfere with the Western analysis. Significantly less MBP is produced from *malE*-opt (MBP-opt) compared to *malE*-wt (MBP-wt) alleles (Fig.2A). To quantitate the difference, the MBP-wt sample was diluted until the MBP band intensity equalled that of MBP-opt. The results of these dilutions indicated that there was approximately 20-fold less mature MBP-opt produced from the *malE*-opt allele. The difference in expression was not due to plasmid copy number or RNA transcript levels as these were found to be equivalent in both *malE*-wt and *malE*-opt (Fig. 2B and C). The MBP produced from the both *malE*-wt and *malE*-opt alleles is exported to the periplasm and is functional, as they both complemented the *malE* mutant in TL225 (Table 1) and were scored Mal+ on maltose-tetrazolium indicator agar.

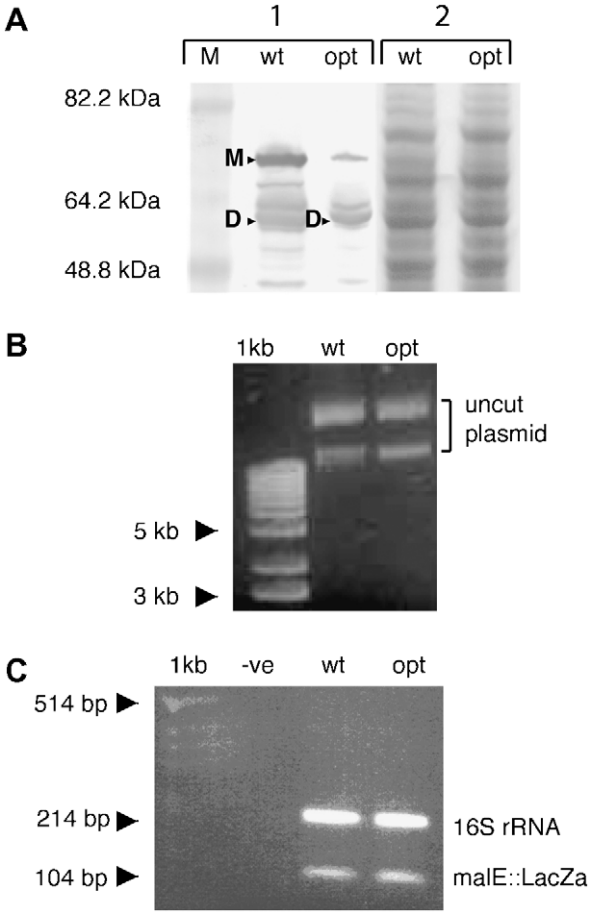

Fig. 2. Expression in a *malE*⁻ strain TST1. (A) 1, The expression of MPB-wt and MBP-opt as determined by Western blot. 2, Corresponding Coomassie strained gel. Mature MBP (M). Degradation product (B). (B) Plasmid minipreps from cultures grown for Western blot in (A). (C) RNA transcript levels from cultures grown for Western blot in (A) are identical as shown by SQRT PCR. Key: wt, MBP-wt; opt, MBP-opt; −ve , no template control.

In both the *malE*-opt and *malE*-wt samples, immuno-reactive, lower molecular weight material can be seen in the Western blot. A previous study has also reported lower molecular weight MBP by immunoprecipitation in a strain expressing wild type MBP from a plasmid [21]. N-terminal sequencing of a band within the material was identical to mature MBP, confirming that the material was degraded MBP derived at least partly from the periplasm, rather than a cross-reacting protein unrelated to MBP. These data are consistent with proteolytic cleavage of MBP as the source of the immunoreactive material.

Expression in strains deficient in periplasmic proteases

To determine which periplasmic proteases were responsible for the degradation of MBP-opt in Western analysis, the plasmids were introduced into strains deficient in proteases OmpT and DegP (Table 1). Endogenous MBP production from these *malE*$^{+}$ strains could be distinguished from MBP expression from the plasmid constructs as the MBP::LacZα fusion runs approximately 15 kDa higher on a Western blot. Strains deficient in the proteases DegP and OmpT gave an increase in expression of MBP-opt relative to the parent strain (Fig. 3A). Although MBP-opt expression increased in this multiple periplasmic protease deficient background, it did not return to MBP-wt levels indicating the possibility that cytoplasmic proteases could also be involved in its degradation.

Expression of MPB-opt in cytoplasmic protease mutants.

To investigate which cytoplasmic proteases were involved in the degradation of MBP-opt, a panel of strains deficient in a number of single, double and triple proteases were examined (Table 1). The expression of MBP-opt did not increase in any single protease mutant (*lon, hslVU*) or the double mutant (*lon clpA*) (data not shown). Expression of MBP-opt was increased in a triple protease mutant (*lon clpPX hslVU*) relative to expression in the parent strain (Fig. 3B). This increase of MBP-opt expression was greater than in the multiple periplasmic protease deficient strain, suggesting that more degradation was occurring in the cytoplasm than in the periplasm. These results suggest a role for the ATP dependant heat-shock cytoplasmic proteases in the preferential degradation of MBP-opt compared to MBP-wt. Expression of MBP-wt in all protease deficient strains was unchanged relative to the wt parent strain.

Fig. 3. Analysis of expression of MBP-wt and MBP-opt in protease mutants. The gene names above indicate which proteases are deficient in the strains. (A) Western blot demonstrating expression in periplasmic proteases deficient strain relative to the parent strain. (B) Western blot showing expression in multiple cytoplasmic protease strain relative to the parent strain. Black line indicates 82.2 kDa marker. Key: wt, MBP-wt; opt, MBP-opt.

Discussion

In this study, we test the hypothesis that the high proportion of non-optimal codons present in the signal sequence may play a key role in protein export using the MBP model system. In each case a change was made from a non-optimal to the most optimal codon from the synonymous codon family (where a choice existed). The amino acid sequence of the signal sequence remained unchanged. A striking difference in expression between *malE*-opt and *malE*-wt was observed that could not be explained by plasmid copy number or RNA. The reduction in expression was unexpected, as the literature suggests that, in general optimizing codon usage should increase expression [22].

The key difference between the *malE*-wt and *malE*-opt alleles is that the translation rate is increased across the signal sequence of *malE*-opt. Previous studies have shown that the MBP signal peptide does interact with the newly translated protein to slow its folding in the wild-type situation [7–9], suggesting that differential interaction of MBP-wt and MBP-opt signal peptide with the newly translated protein may provide a mechanism to explain the MBP-opt misfolding phenotype. However, at the point in time when the signal peptide is available to interact with the adjacent newly translated protein, i.e. emergence of the adjacent MBP region from the ribosome tunnel, both *malE*-opt and *malE*-wt transcripts are translated at the same rate as the transcripts are identical from codon 26 onwards. The key initial difference in translation rate occurs when the signal peptide is still well inside the ribosome tunnel. Interaction between the signal peptide and the adjacent polypeptide in the ribosome tunnel seems very unlikely given the restricted space available, 100 Å long by 10–20 Å in diameter, which is equivalent to a linear chain of approximately 30 amino acids, or 45 amino acids as an α-helix [23]. These dimensions are thought to preclude formation of all tertiary structures and some secondary structures [24]. However, due to their compact nature, α-helices can form in both eukaryotic [25] and *E. coli* [26] ribosome tunnels. Therefore, the increased translational rate across the signal sequence must alter a time-critical early event in MBP-opt. This event could be the misfolding

of the α-helical signal peptide, which may require translational pausing to fold efficiently. Emergence of a misfolded or incompletely folded MBP-opt signal peptide may effect the kinetics of the interaction between the signal peptide and nascent MBP, causing a conformation that is recognised by the cell as misfolded. Supporting this conclusion is the fact that earlier studies on MBP lacking a signal peptide (MBPΔ2–26) was found to be stably folded in the cytoplasm as SecB does not bind this mutant MBP [21,27]. Yet when the translation time of the first 26 codons of this MBP mutant is calculated, it is faster than *malE*-opt (1258 ms versus 1530 ms): the increased translation rate at the N-terminus, relative to *malE*-wt, has no effect on the folding of this protein as MBPΔ2–26 lacks the hydrophobic signal peptide. This implies that a slower translational rate is required for hydrophobic signal sequences, to prevent misfolding and preferential degradation of the exported protein.

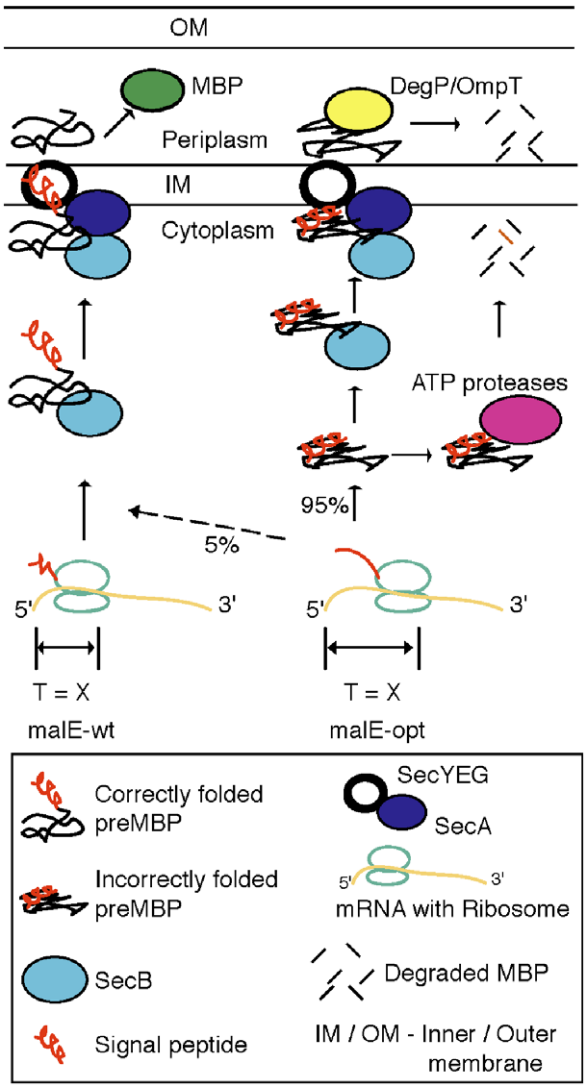

Fig. 4. Model for the different fates of MBP produced from the two constructs (see Discussion).

In Fig. 4 we propose a model to explain the different fates of MBP produced from the two alleles. For the *malE*-wt allele we would expect the export of MBP-wt to take place along the normal pathway. For the *malE*-opt allele there are three possible fates. Approximately 5% of the MBP produced follows the normal pathway and is exported into the periplasm. We demonstrated that this is active as a maltose dependent growth phenotype can be observed when grown on maltose minimal media. The other 95% has two possible fates. The majority is degraded in the cytoplasm, most likely by heat shock ATP dependant proteases. The rest is exported into the periplasm, facilitated by SecB. Once in the periplasm, misfolded MBP-opt is degraded by periplasmic proteases the DegP and OmpT. The recovery of *malE*-opt expression in these protease deficient strains rules out the possibility that RNA secondary structure effects, introduced by the codon changes, played any role in the reduction of expression of *malE*-opt.

The increased expression of *malE*-opt in the periplasmic protease deficient strain is indicative of degradation occurring in the periplasm after export. This data is consistent with the misfolded form of MBP being exported to the periplasm. It has been reported that some tertiary structures of ProOmpA can be translocated through the SecYEG channel [28]. For MBP, SecB is reported to bind to MBP after the first third of the protein is translated and maintains it in a loosely folded state [4]. Hence some tertiary structure information from the cytoplasm could be translated across to the periplasm, whereby a misfolded moiety of MBP-opt could be degraded in the periplasm.

Non-optimal codons have been ascribed various roles aside from encoding amino acids. It has been shown that that translational pause sites corresponding to non-optimal codons occur at the boundaries of protein domains, to allow them to fold correctly [12,13]. Non-optimal codons are selected against in highly expressed genes [29], and hence are more common in regulatory or lowly expressed genes. This has been hypothesised as either a mechanism for regulating translation of regulatory genes [30,31] or due to the lack of selection against their presence [32]. A high proportion of non-optimal codons have been observed in *E. coli* signal sequences [14,15]. The role these codons may play in protein export have previously been hypothesised to allow efficient interaction of the pre-protein with the export machinery [14]. This study confirms the hypothesis that the established role for non-optimal codons in translational pausing has evolved to play a key and novel role in protein export, by introducing a translational pause to facilitate folding of the secretory protein.

Acknowledgments

Thanks to Masaaki Kanemori, Susan Gottesman, George Georgiou, and the *E. coli* genetic stock centre for strains, Tim Riddles for tetrazolium assays, and Ifor Beacham for comments on the manuscript. M.P.J. lab is funded by NHMRC Program Grant 284214.

References

[1] A.P. Pugsley, The complete general secretory pathway in gram-negative bacteria, Microbiol. Rev. 57 (1993) 50–108.

[2] H.C. Lee, H.D. Bernstein, The targeting pathway of *Escherichia coli* presecretory and integral membrane proteins is specified by the hydrophobicity of the targeting signal, Proc. Natl. Acad. Sci. USA 98 (2001) 3471–3476.

[3] D. Huber, D. Boyd, Y. Xia, M.H. Olma, M. Gerstein, J. Beckwith, Use of thioredoxin as a reporter to identify a subset of *Escherichia coli* signal sequences that promote signal recognition particle-dependent translocation, J. Bacteriol. 187 (2005) 2983–2991.

[4] D.N. Collier, V.A. Bankaitis, J.B. Weiss, P.J. Bassford Jr., The antifolding activity of SecB promotes the export of the *E. coli* maltose-binding protein, Cell 53 (1988) 273–283.

[5] C.A. Kumamoto, J. Beckwith, Evidence for specificity at an early step in protein export in *Escherichia coli*, J. Bacteriol. 163 (1985) 267–274.

[6] G.J. Phillips, T.J. Silhavy, Heat-shock proteins DnaK and GroEL facilitate export of LacZ hybrid proteins in *E. coli*, Nature 344 (1990) 882–884.

[7] S. Park, G. Liu, T.B. Topping, W.H. Cover, L.L. Randall, Modulation of folding pathways of exported proteins by the leader sequence, Science 239 (1988) 1033–1035.

[8] K. Beena, J.B. Udgaonkar, R. Varadarajan, Effect of signal peptide on the stability and folding kinetics of maltose binding protein, Biochemistry 43 (2004) 3608–3619.

[9] J.R. Thom, L.L. Randall, Role of the leader peptide of maltose-binding protein in two steps of the export process, J. Bacteriol. 170 (1988) 5654–5661.

[10] S. Varenne, J. Buc, R. Lloubes, C. Lazdunski, Translation is a non-uniform process. Effect of tRNA availability on the rate of elongation of nascent polypeptide chains, J. Mol. Biol. 180 (1984) 549–576.

[11] M.A. Sorensen, C.G. Kurland, S. Pedersen, Codon usage determines translation rate in *Escherichia coli*, J. Mol. Biol. 207 (1989) 365–377.

[12] A.A. Komar, T. Lesnik, C. Reiss, Synonymous codon substitutions affect ribosome traffic and protein folding during in vitro translation, FEBS Lett. 462 (1999) 387–391.

[13] T.A. Thanaraj, P. Argos, Protein secondary structural types are differentially coded on messenger RNA, Protein Sci. 5 (1996) 1973–1983.

[14] P.M. Power, R.A. Jones, I.R. Beacham, C. Bucholtz, M.P. Jennings, Whole genome analysis reveals a high incidence of non-optimal codons in secretory signal sequences of *Escherichia coli*, Biochem. Biophys. Res. Commun. 322 (2004) 1038–1044.

[15] D.M. Burns, I.R. Beacham, Rare codons in *E. coli* and *S. typhimurium* signal sequences, FEBS Lett. 189 (1985) 318–324.

[16] Y.D. Li, Y.Q. Li, J.S. Chen, H.J. Dong, W.J. Guan, H. Zhou, Whole genome analysis of non-optimal codon usage in secretory signal sequences of *Streptomyces coelicolor*, Biosystems 85 (2006) 225–230.

[17] J.H. Miller, Experiments in Molecular Genetics, ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1972.

[18] T.J. Silhavy, M.L. Berman, L.W. Enquist, Experiments with Gene Fusions, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1984.

[19] J. Solomovici, T. Lesnik, C. Reiss, Does *Escherichia coli* optimize the economics of the translation process? J. Theor. Biol. 185 (1997) 511–521.

[20] P.J. Bassford Jr., Export of the periplasmic maltose-binding protein of *Escherichia coli*, J. Bioenerg. Biomembr. 22 (1990) 401–439.

[21] J.B. Weiss, P.J. Bassford Jr., The folding properties of the *Escherichia coli* maltose-binding protein influence its interaction with SecB in vitro, J. Bacteriol. 172 (1990) 3023–3029.

[22] S.C. Makrides, Strategies for achieving high-level expression of genes in *Escherichia coli*, Microbiol. Rev. 60 (1996) 512–538.

[23] S.A. Etchells, F.U. Hartl, The dynamic tunnel, Nat. Struct. Mol. Biol. 11 (2004) 391–392.

[24] P. Nissen, J. Hansen, N. Ban, P.B. Moore, T.A. Steitz, The structural basis of ribosome activity in peptide bond synthesis, Science 289 (2000) 920–930.

[25] C.A. Woolhead, P.J. McCormick, A.E. Johnson, Nascent membrane and secretory proteins differ in FRET-detected folding far inside the ribosome and in their exposure to ribosomal proteins, Cell 116 (2004) 725–736.

[26] R.J. Gilbert, P. Fucini, S. Connell, S.D. Fuller, K.H. Nierhaus, C.V. Robinson, C.M. Dobson, D.I. Stuart, Three-dimensional structures of translating ribosomes by Cryo-EM, Mol. Cell 14 (2004) 57–66.

[27] J.B. Weiss, C.H. MacGregor, D.N. Collier, J.D. Fikes, P.H. Ray, P.J. Bassford Jr., Factors influencing the in vitro translocation of the *Escherichia coli* maltose-binding protein, J. Biol. Chem. 264 (1989) 3021–3027.

[28] S.H. Lecker, A.J. Driessen, W. Wickner, ProOmpA contains secondary and tertiary structure prior to translocation and is shielded from aggregation by association with SecB protein, EMBO J. 9 (1990) 2309–2314.

[29] M. Gouy, C. Gautier, Codon usage in bacteria: correlation with gene expressivity, Nucleic Acids Res. 10 (1982) 7055–7074.

[30] H. Grosjean, W. Fiers, Preferential codon usage in prokaryotic genes: the optimal codon-anticodon interaction energy and the selective codon usage in efficiently expressed genes, Gene 18 (1982) 199–209.

[31] W. Konigsberg, G.N. Godson, Evidence for use of rare codons in the dnaG gene and other regulatory genes of *Escherichia coli*, Proc. Natl. Acad. Sci. USA 80 (1983) 687–691.

[32] P.M. Sharp, W.H. Li, The rate of synonymous substitution in enterobacterial genes is inversely related to codon usage bias, Mol. Biol. Evol. 4 (1987) 222–230.

[33] K.L. Strauch, J. Beckwith, An *Escherichia coli* mutation preventing degradation of abnormal periplasmic proteins, Proc. Natl. Acad. Sci. USA 85 (1988) 1576–1580.

[34] F. Baneyx, G. Georgiou, In vivo degradation of secreted fusion proteins by the *Escherichia coli* outer membrane protease OmpT, J. Bacteriol. 172 (1990) 491–494.

[35] P. Trisler, S. Gottesman, lon transcriptional regulation of genes necessary for capsular polysaccharide synthesis in *Escherichia coli* K-12, J. Bacteriol. 160 (1984) 184–191.

[36] S. Gottesman, Minimizing proteolysis in *Escherichia coli*: genetic solutions, Methods Enzymol. 185 (1990) 119–129.

[37] M. Kanemori, K. Nishihara, H. Yanagi, T. Yura, Synergistic roles of HslVU and other ATP-dependent proteases in controlling in vivo turnover of sigma32 and abnormal proteins in *Escherichia coli*, J. Bacteriol. 179 (1997) 7219–7225.