Substrate specificity of the SecB chaperone
Knoblauch, N.T.M.; Rudiger, S.; Schonfeld, H.J.; Driessen, A.J.M.; Schneider-Mergener, J.; Bukau, B.
Published in: The Journal of Biological Chemistry
DOI: 10.1074/jbc.274.48.34219

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date: 1999

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Knoblauch, N. T. M., Rudiger, S., Schonfeld, H. J., Driessen, A. J. M., Schneider-Mergener, J., & Bukau, B. (1999). Substrate specificity of the SecB chaperone. The Journal of Biological Chemistry, 274(48), 34219 - 34225. https://doi.org/10.1074/jbc.274.48.34219

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment.

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Download date: 10-05-2022
The bacterial chaperone SecB assists translocation of proteins across the inner membrane. The mechanism by which it differentiates between secretory and cytosolic proteins is poorly understood. To identify its binding motif, we screened 2688 peptides covering sequences of 23 proteins for SecB binding. The motif is ~9 residues long and is enriched in aromatic and basic residues, whereas acidic residues are disfavored. Its identification allows the prediction of binding regions within protein sequences with up to 87% accuracy. SecB-binding regions occur statistically every 20–30 residues. The occurrence and affinity of binding regions are similar in SecB-dependent and -independent secretory proteins and in cytosolic proteins, and SecB lacks specificity toward signal sequences. SecB cannot thus differentiate between secretory and non-secretory proteins via its binding specificity. This conclusion is supported by the finding that SecB binds denatured luciferase, thereby allowing subsequent refolding by the DnaK system. SecB may rather be a general chaperone whose involvement in translocation is mediated by interactions of SecB and signal sequences of SecB-bound preproteins with the translocation apparatus.

SecB is a bacterial chaperone that assists translocation of precursor proteins across the cytoplasmic membrane (1–4). It associates with newly synthesized precursors, either late during or shortly after translation, and thereby maintains them in a translocation-competent state (5–10). SecB of Escherichia coli is furthermore known to interact with the SecA subunit of the translocation apparatus (5–10). SecB of E. coli associates with newly synthesized precursors, either late during or shortly after translation, and thereby maintains them in a translocation-competent state. This paper is available online at http://www.jbc.org

Nicola T. M. Knollauch†, Stefan Rüdiger‡, Hans-Joachim Schönfeld§, Arnold J. M. Driessen∥, Jens Schneider-Mergener∥∥, and Bernd Bukau**

From the  †Institut für Biochemie und Molekularbiologie, Universität Freiburg, Hermann-Herder-Straße 7, D-79104 Freiburg, Germany,  §F. Hoffmann-La Roche Ltd., Pharmaceutical Research-Infectious Diseases, CH-4070 Basel, Switzerland, the  ∥Department of Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Kerklaan 30, 9751 NN Haren, The Netherlands, and the  ∥∥Institut für Medizinische Immunologie, Universitätshospital Charité, Humboldt Universität zu Berlin, Schumannstraße 20-21, D-10098 Berlin, Germany

*This work was supported by grants from the Deutsche Forschungsgemeinschaft, Bundesministerium für Forschung und Technologie, and the Fonds der Chemischen Industrie (to A. J. M. D.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

**To whom correspondence should be addressed. Tel.: 49-761-203-5222/2221; Fax: 49-761-203-5257; E-mail: bukau@sun2.ruf.uni-freiburg.de.

† The abbreviations used are: MBP, maltose-binding protein; GBP, galactose-binding protein.

and outer membrane lipoprotein) of SecB (6, 12–14). SecB-independent secretory proteins may utilize other cytosolic chaperones for translocation, including GroEL (15–17) and the DnaK system (14, 18), but may possibly utilize SecB as well. SecB is a homotetramer composed of 17-kDa subunits (19–22) and whose interaction with substrates is independent of ATP (1, 23). The principles governing substrate selection by SecB are controversial. Based on their finding that, in vitro, SecB has 100-fold higher affinity for denatured signal sequence containing MBP precursor than for the corresponding mature protein, Watanabe and Blobel (19, 24) proposed that SecB recognizes the signal sequence directly. On the other hand, signal sequences were dispensable for association of SecB with other substrates, including MBP, both in vitro (25, 26) and in vivo (6, 27), although they might contribute to SecB binding, e.g. in the case of LamB (28). Randall and co-workers (1, 23, 29) postulated a kinetic partitioning model, according to which SecB does not bind specifically to the signal sequence, but to various segments of the precursor polypeptide. The role of the signal sequence in this model is to reduce the folding rate of newly synthesized precursors to allow association with SecB. The refolding rate of unfolded MBP is indeed faster in the absence than in the presence of the signal sequence (30, 31), and SecB is unable to associate with refolding mature MBP unless a mutation in mature MBP is introduced that slows down the folding rate without affecting the thermostability of the folded protein. This kinetic partitioning model has been challenged by stopped-flow kinetic measurements of SecB interactions with denatured bovine pancreatic trypsin inhibitor (BPTI) and barnase (32, 33). Association of SecB with these substrates is nearly diffusion-limited and therefore orders of magnitude faster than folding of newly synthesized polypeptides with or without signal sequences. Since the folding rates of newly synthesized proteins are in any case slower than the SecB association rate, they cannot therefore account for the ability of SecB to discriminate between cytosolic and exported proteins as required for the kinetic partitioning model. Recognition of high affinity binding sites in the precursor polypeptide might thus be a more important determinant for SecB substrate specificity than the folding rate of the substrate (32).

The sequence motif that is recognized by SecB is poorly understood. In the case of MBP (34), GBP (35), and OppA (36), the sequence motif that is recognized by SecB is poorly understood. In the case of MBP (34), GBP (35), and OppA (36), SecB binds to multiple fragments covering a large fraction of the precursor polypeptide. The motif is long and is enriched in aromatic and basic residues, whereas acidic residues are disfavored. Its identification allows the prediction of binding regions within protein sequences with up to 87% accuracy. SecB-binding regions occur statistically every 20–30 residues. The occurrence and affinity of binding regions are similar in SecB-dependent and -independent secretory proteins and in cytosolic proteins, and SecB lacks specificity toward signal sequences. SecB cannot thus differentiate between secretory and non-secretory proteins via its binding specificity. This conclusion is supported by the finding that SecB binds denatured luciferase, thereby allowing subsequent refolding by the DnaK system. SecB may rather be a general chaperone whose involvement in translocation is mediated by interactions of SecB and signal sequences of SecB-bound preproteins with the translocation apparatus.
folding of non-secretory proteins. These approaches allowed us to elucidate principles of action of this chaperone.

**EXPERIMENTAL PROCEDURES**

**Purification of SecB**—The secB gene was amplified by polymerase chain reaction using chromosomal DNA as template and cloned downstream of the isopropyl-β-D-thiogalactopyranoside-regulatable promoter of the pREP4 expression plasmid. Overexpression of secB was induced by addition of isopropyl-β-D-thiogalactopyranoside to the cell culture grown in double-concentrated Luria broth medium. About 30 g of wet bacterial cells obtained from 5 liters of fermentation broth was resuspended on ice in 140 ml of 50 mM Tris-HCl (pH 7.4) containing 10 mM benzenemido hydrochloride, 5 mM e-aminoacproic acid, 5 mM EGTA, 1 mM MgCl₂, 1 mM phenylmethylsulfonyl fluoride; 0.1 mM o-phenanthroline, 0.1 mM 3,4-dichloroisoocoumarin, 50 mM E6, 10 mM leupeptin, and 100 units/ml aprotinin. After supplementation with 1.4 mg of DNase (10 μg/ml) and 14 mg of lysozyme (100 μg/ml), cells were sonified for 10 min (Branson sonifier, medium rod, 50% cycle, 20 watts). After opening of cells by sonication, insoluble material was removed by centrifugation. SecB was precipitated from the supernatant by addition of ammonium sulfate up to 35% relative saturation, resolubilized in 50 mM piperazine-HCl (pH 5.0) (piperazine buffer), and then extensively dialyzed against the same buffer. After filtration through a 0.45-μm pore-sized filter, the solution was applied to a Q-Sepharose Fast Flow column (2.6 x 15 cm; Amersham Pharmacia Biotech, Uppsala, Sweden) equilibrated with piperazine buffer. After washing, bound material was eluted by a salt gradient. Fractions containing SecB as analyzed by SDS-polyacrylamide gel electrophoresis were pooled; adjusted to pH 7.4; and then applied to a Sepharyl S-300 HR column (2.6 x 100 cm) equilibrated with 10 mM sodium phosphate (pH 7.4) and 140 mM NaCl (–100 mg of SecB load/run). Fractions containing purified SecB were pooled and stored frozen at –80 °C. About 100 mg of homogeneous SecB was obtained from 1 liter of fermentation broth. Ion spray mass spectroscopy, N-terminal sequencing, and amino acid analysis verified the authenticity of the purified protein. Recombinant SecB started with serine at the amino terminus and was not blocked, in contradiction to what was reported earlier (20, 38). Purified SecB was protected against proteinase K cleavage by reduced and what was reported earlier (20, 38). Purified SecB was protected against proteinase K cleavage by reduced and [36] 16.5 and 43.5; class 2, medium affinity, [36] 4.5; and class 4, no affinity, [36].

**Substrate Specificity of SecB**—To elucidate the substrate specificity of SecB, we investigated the degree of specialization of SecB for secretory proteins. We tested in vitro whether SecB has the capability to assist folding of non-secretory proteins using firefly luciferase as substrate. Luciferase is cytosolic when produced in *E. coli* and requires the activity of the DnaK chaperone system for efficient refolding after thermal or chemical denaturation (40, 45–47). Guanidinium-denatured luciferase did not refold spontaneously or in the presence of bovine serum albumin (Fig. 1). SecB, even when added at a 20-fold molar excess of the SecB tetramer over luciferase, did not affect the rate of spontaneous refolding. In contrast, when DnaK and its co-chaperones DnaJ and GrpE (at a 10:2:5-fold molar excess over luciferase) were present in refolding buffer at time 0 when luciferase was added, luciferase refolded with a high rate to >80% of the native control. The simultaneous presence of SecB at an even higher concentration (25-fold molar excess over luciferase) did not affect the rate and yield of luciferase refolding, indicating that SecB does not interfere with the chaperone activity of the DnaK system. However, at this high concentration (25-fold molar excess over luciferase), SecB was capable of preventing aggregation of denatured luciferase for at least 1 h as measured by light scattering (data not shown).

Although it is a high concentration, it is still below the physiologic concentration of SecB (~13 μM tetramer).2 To test whether SecB can cooperate with the DnaK system in luciferase refolding, we performed order-of-addition experiments (Fig. 4).
1) in which the DnaK system was added 20 min after addition of luciferase to the refolding mixture. In the absence of SecB, this late addition reduced the refolding yield to ~25% of the maximal value due to aggregation of luciferase. Increasing concentrations of SecB present at time 0 steadily increased the rate and yield of luciferase refolding by the DnaK system (added at 20 min), with the highest refolding yield obtained at a 50-fold molar excess of SecB tetramer over luciferase (Fig. 1). The refolding yields were higher when DnaK was added already at 10 min after addition of luciferase (data not shown), indicating that SecB holds luciferase in a folding-competent state for only a limited time. These experiments indicate that SecB can act as a “holder” chaperone that prevents aggregation and that cooperates with DnaK in refolding of a non-native cytosolic protein.

Screening of Peptide Scans for Binding to SecB—To determine the binding motif within protein sequences recognized by SecB, we screened cellulose-bound peptide scans (48) representing the complete sequences of 23 proteins for SecB binding. The peptide scans were composed of 13-mer peptides that overlap by 10 residues and therefore present all potential linear binding sites for SecB. A subset contains secretory proteins represented in the library as precursors with signal sequences. They are either prokaryotic proteins translocated in a SecB-dependent (GBP, LamB, MBP, OmpA, and OmpF) or -independent (alkaline phosphatase, β-lactamase, outer membrane lipoprotein, and ribose-binding protein) manner or eukaryotic proteins translocated into the endoplasmic reticulum (bovine pancreatic trypsin inhibitor (BPTI) and prepro-o-factor) or mitochondria (citrate synthase, cytochrome b2, ATP synthase β-chain (F1β), and ATP synthase protein 9 (Su9)). For comparison, a subset of non-secretory proteins was screened (dihydrofolate reductase, PtsZ, luciferase, SecA, σ22, L2, λCI, and AO). They were incubated with SecB to equilibrium, followed by electrotransfer and immunodetection of the chaperone. Fig. 2 shows selected peptide scans.

SecB bound only to a subset of the peptides, indicating that it differentiates between amino acid side chains. It bound frequently to neighboring peptides in the scan, indicating that a SecB-binding site is shared by these peptides. SecB-binding peptides existed in all peptide scans tested, with no apparent clustering within the scans (e.g. at N or C termini). Sequence alignment of overlapping binding peptides allowed definition of SecB-binding regions (see below). These occurred frequently, every 20–30 residues within the protein sequences tested. This frequency was not affected by cellular and organellar origin, size, and oligomeric status of the proteins and did not differ between secretory and non-secretory and homologous and heterologous proteins. SecB thus has broad substrate-binding specificity at the peptide level, which by itself cannot differentiate between substrates and non-substrates.

The SecB mutants L75Q and E77K associate with precursor polypeptides, but are deficient in SecA interaction (39). We compared the specificity of these mutants with wild-type SecB for association with 76 peptides derived from the AC1 sequence and found identical binding patterns (data not shown). This suggests that the SecB peptide-binding site is different from the SecA recognition site.

Amino Acid Distribution within SecB-binding Peptides—The large data set allowed reliable statistical analysis of the substrate motif recognized by SecB. All screened peptides were grouped into four classes according to their affinity for SecB (high, medium, low, and no affinity) as determined by fluororimager quantification of the SecB signals (Fig. 3A).

The relative occurrence of the 20 amino acids in the peptide library is similar to what is found in natural proteins, except for a low representation of Cys (44) (data not shown). Substantial differences existed between the amino acid distribution of SecB-binding and non-binding peptides (Fig. 3B). Medium affinity SecB-binding peptides are enriched in basic residues (Arg and Lys). High affinity SecB binders are, in addition, up to 2-fold enriched in aromatic residues (Phe, Tyr, and Trp). Acidic residues (Asp and Glu) are strongly disfavored, and most other residues are slightly disfavored. Large hydrophobic residues (Ile, Leu, and Val) are not enriched.

Sequence Motif Recognized by SecB—94 regions with high affinity for SecB were aligned to identify the consensus binding motif. It consists of ~9 neighboring residues, as judged from
Downloaded from www.jbc.org on March 14, 2007

Substrate Specificity of SecB

For 2688 peptides representing 23 protein sequences, the relative amino acid occurrence was determined. A, normalized affinity of SecB for the peptides investigated. Peptides are ordered according to their SecB affinity (class 1, black; class 2, dark gray; class 3, light gray; and class 4, white). B, comparison of peptides of class 1 (high SecB affinity; black bars), classes 2 and 3 (medium and low SecB affinity; gray bars), and class 4 (no SecB affinity; white bars). The numbers for each amino acid are normalized to its occurrence in the whole peptide library (set as 100). The differences between class 1 and class 4 populations for Val, Gly, Cys, and Thr are not statistically significant in the χ² test (p > 0.05).

We determined the localization of identified SecB-binding regions within native protein structures. These findings indicate that the observed SecB-binding

DISCUSSION

We determined the principles governing substrate recognition by the SecB chaperone employing cellulose-bound peptide scans. This approach avoids solubility problems of hydrophobic peptides and allows screening of thousands of peptides, which permits identification of the binding motif and all potential linear binding sites within the tested protein sequences. It was successfully established to dissect the substrate specificity of E. coli DnaK chaperone (44, 51). This peptide-based approach is appropriate for SecB since peptides bind SecB with high affinity and compete with protein substrates for binding (37). Peptide studies were in fact the basis for establishment of the kinetic partitioning model for SecB substrate selection (1). We were concerned about the possibility that the binding of SecB to peptides does not reflect SecB-substrate interactions, but instead the high affinity interaction of SecB with the C terminus of SecA. The C terminus of SecA indeed contains a SecB-binding region, which, however, is not among the strongest SecB-binding regions identified in our screen. Furthermore, SecB mutants that have lost the ability to interact with SecA showed the same substrate-binding pattern as wild-type SecB. In addition, recent evidence demonstrates that the C terminus of SecA coordinates a zinc ion that is needed for high affinity binding of SecB (52), and zinc was not present in our experiments. These findings indicate that the observed SecB-binding
Substrate Specificity of SecB

signals in our screen reflect true interactions of SecB with substrates via its substrate-binding site.

We considered that the binding of SecB to positively charged residues is unspecific, given that SecB is an acidic protein (20). If this were the case, one would expect two populations of SecB-binding peptides, positively charged peptides and peptides enriched in aromatic residues. There is, however, only one population of SecB-binding peptides since the number of positively charged residues is connected to the number of aromatic residues. Thus, peptides with 2 aromatic residues bind SecB only if there is an additional Arg or Lys, whereas peptides with 3 aromatic residues bind in the absence of Arg or Lys. In all cases, SecB binding can be inhibited by the presence of acidic residues within the peptides, providing a further indication that the recognition of basic and aromatic residues is a linked event. We can also rule out the possibility that recognition of aromatic residues is an unspecific hydrophobic interaction since Leu, Ile, and Val are not enriched in the binding peptides.

The characteristics of the SecB-binding regions identified in this study are in agreement with and extend earlier findings of studies investigating the affinity of SecB for fragments of protein substrates and selected peptides (34–37). Furthermore, our approach allows, for the first time, the identification of the SecB-binding motif and the prediction of SecB-binding regions within protein substrates. The motif consists of a continuous stretch of ~9 residues enriched in basic and aromatic residues, whereas acidic residues are strongly disfavored. In contrast, large hydrophobic aliphatic residues are not enriched. This indicates that SecB has binding pockets or surfaces that are specific for aromatic residues. The aromatic side chains of high affinity SecB-binding regions typically occur within core regions of folded proteins, as shown for MBP (Fig. 5). The nature of this substrate-binding motif allows SecB to bind preferentially to unfolded conformers of protein substrates and thus forms a basis for its function as a chaperone.

The SecB-binding motif shares overall similarity with the motif recognized by the DnaK chaperone in that both motifs comprise a hydrophobic patch in which negatively charged residues are disfavored (44, 53). Differences exist with respect to the length of this patch (~9 residues for SecB and 4–5 residues for DnaK), the positioning of basic residues (within this patch for SecB and outside for DnaK), and the nature of hydrophobic residues. Whereas SecB favors aromatic residues, DnaK favors large hydrophobic residues with a strong preference for leucine (44). Despite these differences, many binding sites for SecB and DnaK are shared. Thus, the luciferase sequence contains 13 high affinity binding sites for DnaK and nine for SecB, eight of which are common to both (if adjacent...
sites melted to a broad site, they were counted only once). SecB and DnaK therefore have the potential to interact with similar sets of proteins, although additional parameters will clearly also be of relevance, e.g. the association rates of SecB-substrate complexes and the kinetics of substrate folding. Similarities in the protein substrate spectra of SecB and DnaK have been established in vivo by demonstrating that the DnaK chaperone system can support export of SecB-dependent substrates in secB mutant cells (14). Furthermore, we show here that SecB shares with DnaK (40, 46) the ability to prevent aggregation of unfolded firefly luciferase.

Our identification of SecB-binding regions within protein sequences excludes that SecB is able to distinguish between secretory and non-secretory proteins on the basis of differences in binding sites. Of particular importance in this respect is the finding that there is no correlation between SecB dependence of transport and the ability of SecB to bind to signal sequences. Furthermore, the signal sequences of some SecB-dependent secretory proteins do not provide SecB-binding sites of detectable affinity. Our results support a model according to which SecB associates with several internal segments of the mature parts of precursor proteins (54). They also suggest that SecB does not act very early co-translationally since the signal sequences are not prime targets. Our data are instead consistent with the finding for MBP that SecB binds late co-translationally to the nascent polypeptide chain after it has reached a length of ~150 residues (55). An MBP fragment of this length contains six high affinity SecB-binding regions. Furthermore, the MBP segment comprising residues 151–186 that was identified to be required for SecB binding to MBP (6) contains a high affinity SecB-binding region, as found in this study.

On the basis of our findings and the demonstrated fast kinetics of SecB association with substrates (32, 33), it is conceivable that SecB can interact with a large variety of folding proteins even when they are cytosolic proteins. Unfolded firefly luciferase was one such substrate, although a large molar excess of SecB was needed to prevent its aggregation (Fig. 1). This suggests that SecB is capable of binding to a large variety of substrates. Since SecB is the most abundant cytosolic chaperone besides DnaK, with a cellular concentration of SecB (~13 μM tetramer, half the concentration of DnaK)2 that is even above that used for the luciferase refolding experiment shown in Fig. 1, the high amount of SecB in the cell might also allow productive interaction with unfolded cytosolic polypeptides. It is unclear to what extent the cell takes advantage of this general chaperone function of SecB to prevent protein aggregation. A mechanistically important finding of our study is that the protein substrate, unfolded firefly luciferase, can dissociate rapidly from association with SecB and be transferred in non-native conformation to the DnaK chaperone system. This rapid dynamic equilibrium between bound and free states may allow the ligand to kinetically partition between folding (1) and re-binding to SecB or, only in the case of secretory proteins, to be transferred to the translocon. The dedicated role of SecB in protein translocation may thus result from events downstream of the SecB-substrate interaction, in particular the association of SecA with SecB and the interaction of the signal sequence with components of the translocon.

Acknowledgments—We thank P. Fekkes for the gift of SecB mutant proteins; E. Schaffitzel for unpublished results on SecB levels in E. coli; M. Stieger for overexpression of SecB; B. Wipf for fermentation; B. Poschi for technical assistance; and M. Müller, D. Dougan, and E. Deuerling for reviewing the manuscript.

![Substrate Specificity of SecB](image.png)

**FIG. 6. SecB association with signal sequences.** The SecB affinity of 13-mer peptides as determined by scanning the signal sequences of secretory E. coli proteins is indicated on the ordinate. The SecB affinity class of each peptide is indicated. The abscissa indicates the number of each peptide. The most C-terminal peptide with the highest number still contains at least 6 residues of the signal sequence; the three C-terminal peptides also therefore contain N-terminal residues of the mature protein. SecB affinity for signal sequence-derived peptides does not follow the classification into SecB-dependent and -independent proteins.

![Table: SecB Dependent and Independent E. coli Proteins](table.png)

| Protein | SecB Affinity |
|---------|--------------|
| MBP     | High         |
| OmpF    | Medium       |
| OmpA    | Medium       |
| LamB    | Low          |
| GBP     | No           |

| Protein | SecB Affinity |
|---------|--------------|
| AP      | High         |
| OML     | Medium       |
| β-Lactamase | Low   |
| RBP     | No           |

**REFERENCES**

1. Randall, L. L., and Hardy, S. J. S. (1995) *Trends Biochem. Sci.* 20, 65–69
2. Bukau, B., Hesterkamp, T., and Luirink, J. (1996) *Trends Cell Biol.* 6, 480–486
3. Kumamoto, C. A. (1990) *J. Bioenerg. Biomembr.* 22, 337–351
4. Driessen, A. J. M. (1994) *J. Membr. Biol.* 142, 145–159
5. Randall, L. L., and Hardy, S. J. S. (1983) *J. Bacteriol.* 154, 463–469
6. Collier, D. N., Bankaitis, V. A., Weiss, J. B., and Bassford, P. J., Jr. (1988) *Cell* 53, 273–283
7. Hartl, F. U., Lecker, S., Schiel, E., Hendrick, J. P., and Wickner, W. (1990) *Cell* 63, 269–279
8. Ernst, F., Hofschoff, H. K., Thome-Kromer, B., Swidersky, U. E., Werner, P. K., and Muller, M. (1994) *J. Biol. Chem.* 269, 12840–12845
9. Hartl, F. U., Lecker, S., Schiel, E., Hendrick, J. P., and Wickner, W. (1990) *Cell* 63, 269–279
10. Kumamoto, C. A., and Prunecz, O. (1993) *J. Bacteriol.* 175, 2184–2188
11. Hofschoff, H. K., Drees, B., and Muller, M. (1994) *J. Biol. Chem.* 269, 12833–12839
12. Kumamoto, C. A., and Beckwith, J. (1983) *J. Bacteriol.* 154, 253–260
13. Kumamoto, C. A., and Beckwith, J. (1988) *J. Bacteriol.* 166, 267–274
14. Wild, J., Altman, E., Yura, T., and Gross, C. A. (1992) *Genes Dev.* 6, 1165–1172
15. Bochkareva, E. S., Lissin, N. M., and Girshovich, A. S. (1988) *Nature* 336, 254–257
16. Kusukawa, N., Yura, T., Ueguchi, C., Akiyama, Y., and Ito, K. (1989) *EMBO J.* **8**, 3517–3521
17. Laminet, A. A., Ziegelhoffer, T., Georgopoulos, C., and Plückthun, A. (1990) *EMBO J.* **9**, 2315–2319
18. Wild, J., Rossmeisl, P., Walter, W. A., and Gross, C. A. (1996) *J. Bacteriol.* **178**, 3608–3613
19. Watanabe, M., and Blobel, G. (1989) *Cell* **58**, 695–705
20. Weiss, J. B., Ray, P. H., and Bassford, P. J., Jr. (1988) *Proc. Natl. Acad. Sci. U. S. A.* **85**, 8978–8982
21. Schönfeld, H.-J., and Behlke, J. (1998) *Methods Enzymol.* **290**, 269–296
22. Smith, V. F., Schwartz, B. L., Randall, L. L., and Smith, R. D. (1996) *Protein Sci.* **5**, 488–494
23. Hardy, S. J. S., and Randall, L. L. (1991) *Science* **251**, 439–443
24. Watanabe, M., and Blobel, G. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92**, 10133–10136
25. Randall, L. L., Topping, T. B., Suria, D., and Hardy, S. J. S. (1998) *Protein Sci.* **7**, 1195–1200
26. Randall, L. L., Topping, T. B., and Hardy, S. J. S. (1990) *Science* **248**, 860–863
27. Gannon, P. M., Li, P., and Kumamoto, C. A. (1989) *J. Bacteriol.* **171**, 813–818
28. Altman, E., Emr, S. D., and Bukau, B. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90**, 4137–4144
29. Khisty, V., and Randall, L. L. (1995) *J. Bacteriol.* **177**, 3277–3282
30. Liu, G., Topping, T. B., and Randall, L. L. (1989) *Proc. Natl. Acad. Sci. U. S. A.* **86**, 2728–2732
31. McCarty, J. S., Ruüger, S., Schońfeld, H.-J., and Bukau, B. (1996) *Protein Sci.* **5**, 9213–9217
32. McCarty, J. S., Ruüger, S., Schońfeld, H.-J., and Bukau, B. (1996) *J. Mol. Biol.* **256**, 829–837
33. Fekkes, P. M., de Wit, J. G., Boorsma, A., Friesen, R., and Driessen, A. J. M. (1999) *Biochemistry* **38**, 5111–5116
34. Fekkes, M. P., de Wit, J. G., Boorsma, A., Friesen, R., and Driessen, A. J. M. (1999) *Biochemistry* **38**, 5111–5116
35. Rüdiger, S., Buchberger, A., and Bukau, B. (1997) *Nat. Struct. Biol.* **4**, 342–349
36. Randall, L. L., Hardy, S. J. S., Topping, T. C., Smith, V. F., Bruce, J. E., and Smith, R. D. (1998) *Proteins* **7**, 2384–2390
37. Randall, L. L., Traci, B., Hardy, S. J. S., Pavlov, M. Y., Freistroffer, D. V., and Ehrenberg, M. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94**, 802–807
38. Shabb, A. J., Rodseth, L. E., and Qiocho, F. A. (1993) *Biochemistry* **32**, 10553–10559