Co-translational Folding of an Eukaryotic Multidomain Protein in a Prokaryotic Translation System*

Continuous monitoring of the enzymatic activity of newly synthesized firefly luciferase in *Escherichia coli* cell-free translation system was performed to record folding kinetics of this multidomain eukaryotic protein in the prokaryotic cytosol. Whereas in *vitro* refolding of denatured luciferase in prokaryotic cytosol occurred with a low yield of active enzyme and took about an hour, the enzyme acquired its native structure immediately upon release from the ribosome, as seen from the immediate halt of active luciferase accumulation upon blocking of translation with inhibitors. The nascent luciferase was also capable of acquiring the active conformation prior to release from the ribosome, when its C terminus was extended with a polypeptide segment. Specific enzymatic activity of the firefly luciferase was found to be equally high irrespective of whether this protein was synthesized in eukaryotic or prokaryotic translation systems. The data presented demonstrate the fundamental ability of prokaryotic cytosol to support effective co-translational protein folding in general and co-translational folding of multidomain proteins in particular.

The co-translational mechanism of protein folding implies that the N-terminal part of a growing polypeptide starts its folding as soon as it has been synthesized, prior to the completion of entire polypeptide chain by the ribosome. A number of experimental findings have led to the conclusion that many proteins can attain their native structure co-translationally. The most direct evidence in favor of co-translational folding was the demonstration that proteins can display their enzymatic activity immediately upon the release from the ribosome, with no delay for posttranslational folding (1). The generation of protease-resistant domains typical for mature proteins (2–4) and the formation of the correct S-S bridges (5–7) were demonstrated for several nascent polypeptides. It was shown in some cases that a growing polypeptide was able to interact with free subunits of this multimeric protein, forming ribosome-associated oligomers (8–10). Several proteins were shown to bind their specific ligands or cofactors while bound to the ribosome as growing polypeptides (5, 11–13), thus suggesting their binding-competent conformation. Finally, it was demonstrated that under certain conditions, proteins could attain their enzymatic activity while still attached to the ribosome (14–16). Recently, a hypothesis has been put forward according to which a shift from posttranslational to co-translational folding mechanisms has occurred during evolution (3). It was hypothesized that the ability to support co-translational folding either has been lost (or reduced) by prokaryotes or has been acquired by eukaryotes. In the present study, the question has been raised of whether a multidomain eukaryotic protein is capable of co-translational folding in a prokaryotic translation system (on prokaryotic ribosomes). In order to approach the problem, we examined the kinetics of folding of a multidomain protein, *Photinus pyralis* luciferase, during its synthesis in a bacterial cell-free translation system. The activity of newly synthesized luciferase can be recorded continuously, which allows us to detect the active (i.e. properly folded) molecules as soon as they are formed in the cytosol (1). The same experimental strategy as was used earlier (1, 17) was applied in this study to measure the delay between the arrest of the synthesis and the cessation of the enzyme activity accumulation. This approach allows to estimate the time interval in which a full-length polypeptide released from the ribosome attains its native three-dimensional structure. We have found that the arrest of luciferase synthesis causes immediate cessation of active enzyme accumulation, thus suggesting that the enzyme acquires its native structure immediately upon release from the ribosome, without any delay for posttranslational folding. This result indicates the co-translational mode of luciferase folding in the prokaryotic cytosol, just the same as reported for this protein when it was synthesized in a eukaryotic translation system (1, 2).

EXPERIMENTAL PROCEDURES

**Plasmid Construction**—The plasmid pT7 luc(NTPII) was constructed by cloning the luciferase-NPT II fusion sequence from the plasmid pT7 luc(NTPII) (16) into the NdeI site of the plasmid vector pT7-7 (18). The sequence was first amplified by polymerase chain reaction with 5'-GCCCATCACCATCACCATCACCATCATATGGAAGACGCCAAAAAC-3' (upstream) and 5'-CTTCATATGCCCATACCCAGAGTCCCG-3' (downstream) primers (NdeI sites are underlined). The polymerase chain reaction product was subjected to limited digestion with NdeI, the resultant large fragment retaining its internal NdeI site (residing exactly between luciferase and NPT II sequences) was inserted into pT7-7, and orientation of the insert was determined by restriction analysis.

The plasmid pT7 luc was constructed in the following way: the *SalI-EcoRI* fragment of pT7 luc(NPT II), containing the sequence of C-terminal part of luciferase fused with NPT II, was replaced with the *SalI-EcoRI* fragment of pGEM-luc (Promega) that comprised only the C-terminal part of luciferase gene. Thus, the final construct (pT7 luc) contained the complete luciferase gene followed by its natural stop codon.

**Preparation of mRNA—** *In vitro* transcription with T7 RNA polymerase (19) was used to prepare mRNA. Full-length luciferase mRNA with stop codon was transcribed from pT7 luc linearized with XhoI; mRNA encoding the enzyme extended by 59 amino acid residues was transcribed from pT7 luc(NPT II) digested with PstI. DNA template for synthesis of mRNA encoding full-length luciferase without a stop codon was prepared by polymerase chain reaction amplification of pT7 luc (NPT II) with 5'-TAATACGACACTATAGGG-3' (upstream) and 5'-TACAATTTGAGCTTCGCC-3' (downstream) primers.

Transcripts were purified by conventional phenol-chloroform treat-
ment and precipitation with 3 m LiCl. Purity and size of mRNAs synthesized were controlled by 5% PAGE in 7 m urea.

In Vitro Translation—All variants of translations were carried out at 25 °C. Concentrations of mRNAs in the reactions varied from 40 to 120 μg/ml.

Translation in 8% Escherichia coli S30 extract (20) was carried out in the presence of 2 mM ATP, 1.6 mM GTP, 0.1 mg/ml leucovorin (Serva), 2 mM phosphoenolpyruvate (Fluka), 0.05 mg/ml pyruvate kinase (Roche Molecular Biochemicals), 5% PEG 6000 (Merck), 80 μg each amino acid, and 0.72 mg/ml bulk E. coli tRNA in Buffer A (8.5 mM Mg(OAc)₂, 125 mM KOAc, 80 mM Tris acetate, pH 8.2, 0.2 mM EDTA, and 4 mM DTT).

The reactions also contained 0.1 mM luciferin (Sigma) and 0.2 mM coenzyme A (Roche Molecular Biochemicals). Radioactive amino acids ([14C]leucine (305 mCi/mmol, Amersham Pharmacia Biotech) or [35S]methionine (>1000 Ci/mmol, Amersham Pharmacia Biotech)) were added to translation systems instead of unlabeled ones when indicated.

Translation in 30% wheat germ extract (21) was performed in the presence of 1.3 mM ATP, 0.25 mM GTP, 16 mM creatine phosphate (Roche Molecular Biochemicals), 0.05 mg/ml creatine kinase (Roche Molecular Biochemicals), and 80 μg each amino acid in buffer containing 2.25 mM Mg(OAc)₂, 106 mM KOAc, 20 mM HEPES-KOH, pH 7.5, 0.1 mM EDTA, 0.25 mM spermidine (Fluka), and 3 mM DTT. A 70% nuclease-treated reticulocyte lysate (Promega) was used for luciferase synthesis in the presence of 60 μg each amino acid and 20 mM [14C]leucine.

Blocking of translation was performed by addition of RNase A or thiostrepton, both in Buffer A. The samples of individual chaperones of the GroEL and the DnaK families were kindly provided by Roche Diagnostics GmbH.

In Vitro Refolding—P. pyrolysis luciferase (Roche Molecular Biochemicals) dissolved in buffer (0.5 M HEPES-KOH, pH 7.5) was denatured by mixing with 49 volumes of 8 M urea in Buffer A. Refolding was initiated by dilution of a 0.5-μl aliquot of the solution containing 20 ng of denatured enzyme with 200 μl of prokaryotic or eukaryotic translation system devoid of mRNA but containing luciferase substrates. The time course of refolding was continuously recorded by recovery of light-emitting activity in a luminometer (1).

Specific Activity Determination—Synthesis of luciferase was performed at 25 °C in wheat germ extract, rabbit reticulocyte lysate, and E. coli S30 extract in parallel. All reactions contained 20 μg [14C]leucine (305 mCi/mmol, Amersham Pharmacia Biotech) and luciferase mRNA with stop codon. The same mRNA concentrations (40 and 120 μg/ml; no mRNA in control experiments) were used for all three cell-free extracts. After 1 h of incubation, the enzymatic activity of luciferase was determined in 0.5-μl aliquots of the cell-free lysates. Other aliquots from each of the translation reactions were analyzed by 12% SDS-PAGE. The protein bands of full-length enzyme were cut from the gel and solubilized in 0.15 ml of 30% hydrogen peroxide at 60 °C in the presence of 2% ammonia and then mixed with 10 ml of Supersolve X scintillation mixture (Koch-Light) for radioactivity measurements.

Luciferase Activity Assay—Activity was measured in 50-μl aliquots of sucrose gradient fractions or in 2-μl aliquots of translation reactions diluted with 48 μl of buffer containing 20 mM HEPES-KOH, pH 7.5, 2 mM Mg(OAc)₂, 100 mM KOAc, 0.1 mM EDTA, 2 mM DTT, and 1 mg/ml of bovine serum albumin (Koch-Light). The sample was placed in a luminometer cell at 25 °C, and a luciferase-catalyzed reaction was initiated by injection of 50 μl of a solution containing 20 mM HEPES-KOH, pH 7.5, 10 mM Mg(OAc)₂, 40 mM EDTA, 0.2% Triton X-100 (Merck), 2 mM ATP, 0.2 mM luciferin, and 0.4 mM coenzyme A.

RESULTS

Formation of Active Firefly Luciferase in Bacterial Cell-free Translation System—Continuous monitoring of the enzymatic activity of luciferase in the course of translation was performed by placing the translation reaction into a luminometer cell at 25 °C (1). Luciferin and ATP, the substrates of luciferase, were present in the mixture from the beginning of incubation, allowing the newly synthesized enzyme to display its activity. The light emitted as a result of luciferase-catalyzed reaction was recorded to measure the amount of enzymatically active luciferase throughout the experiment. The translation was initiated by addition of luciferase mRNA. The kinetics curve of active luciferase accumulation is shown in Fig. 1a. It can be seen that the detectable activity appeared after 8 min of incubation, was clearly visible by 10 min, rapidly rose over the next 20 min, and reached a plateau by 30–40 min.

Aliquots from the same incubation mixture were analyzed by 15% SDS-PAGE with subsequent autoradiography. It is seen in Fig. 1b that the full-length luciferase can be detected as an intensive band in the gel by 10 min of incubation. Thus, the emergence of the light-emitting activity correlates with the appearance of the full-length polypeptide. The correlation indicates that there is no significant lag between the formation of the complete luciferase chain and the development of luciferase activity and thus that the protein has acquired its native conformation mainly during translation.

Immediate Cessation of Active Luciferase Accumulation upon Blocking Translation—In the next series of experiments, the translation was stopped by injection of inhibitors to the reaction mixture at some point of luciferase accumulation curve. In such a case, a further increase in enzymatic activity would reflect the folding of the protein that has already been released from the ribosome (1, 17). As is evident from Fig. 2, a and b, the addition of such inhibitors as RNase A or thiostrepton to the translation mixtures resulted in abrupt cessation of the increase in luciferase activity. The addition of a buffer instead of the inhibitors resulted in only a slight decrease of the slope of the activity accumulation curve, reflecting the dilution of the mixture (Fig. 2c).

The immediate halt of the activity accumulation upon addition of the translation inhibitors indicates that all luciferase molecules released from the ribosome already had the active conformation.

Similar experiments on translation inhibition by thiostrepton were carried out with reaction mixtures containing an excess of purified chaperones. The following three combina-

\[1\] The abbreviations used are: PAGE, polyacrylamide gel electrophoresis; DTT, dithiothreitol. 
Addition of buffer instead of an inhibitor.

Preparation of karyotic Cytosols—In the refolding experiments, a commercial chaperones in bacterial S30 extracts used. Cessation of luciferase activity accumulation upon arrest of translation. a, blocking of luciferase synthesis by injection of RNase A into translation system to final concentration of 0.8 mg/ml. b, blocking of luciferase synthesis by 20 μM thistrepton. c, control, with addition of buffer instead of an inhibitor.

Addition of the same amount of native enzyme to the translation system did not increase in enzymatic activity, suggesting completeness of folding. The half-time of the refolding in the prokaryotic cytosol is estimated to be about 20 min (Fig. 3b).

It can be clearly seen that the refolding of denatured luciferase in the prokaryotic cytosol is a slow and inefficient process, in contrast to the virtually instant acquisition of the properly folded conformation by the luciferase released from the ribosome. Taken together, the data on the refolding rates, the enzymatic activity appearance during translation, and the cessation of active enzyme accumulation upon the arrest of its synthesis indicate that luciferase starts its folding while it still resides on the translating prokaryotic ribosome and becomes folded when leaving the ribosome.

Enzymatic Activity of the Ribosome-bound Luciferase—To check whether luciferase can attain its active conformation while bound to the ribosome as a peptidyl-tRNA, translation of luciferase mRNA devoid of a stop codon was applied in E. coli S30 extract. As it was shown earlier, full-length luciferase does not display its activity when bound to eukaryotic ribosomes until it has been released (1) or until the nascent polypeptide has been extended by at least 26 additional amino acid residues at its C terminus (14, 16). To synthesize such extended luciferase, plasmid pT7 luc(NTPII) was constructed containing a ribosome-binding site followed by sequence of a fusion protein consisting of luciferase and neomycin phosphotransferase (NPT II). The C terminus of luciferase was fused to the N terminus of NPT II directly, without a linker or stop codons, exactly as in the fusion protein used in Ref. 16. Two sorts of luciferase mRNA that differed in their 3′-termini were prepared with the use of pT7 luc(NTPII) plasmid: one contained only the luciferase sequence without a stop codon, and the other consisted of the same sequence but with an additional 59 triplet segment encoding polypeptide for the N terminus of NPT II. Both mRNAs were translated in E. coli S30 extract, and the translation mixtures were then fractionated by sucrose gradient centrifugation. Luciferase activity was measured in aliquots of the gradient fractions before and after addition of puromycin. As seen in Fig. 4, the full-length luciferase remained inactive while bound to the ribosome but acquired the active conformation after puromycin-induced release from the ribosome. In contrast, luciferase activity was detected on the 70 S ribosome bearing the fusion protein, in which luciferase was extended by 59 C-terminal amino acid residues. A large part of the activity was also associated with polysomes (Fig. 4b). The puromycin-induced release of the fusion enzyme did not result in a substantial increase in enzymatic activity, suggesting completeness of folding of the protein into its active conformation on ribosomes.

Refolding of Denatured Luciferase in Prokaryotic and Eukaryotic Cytosols—In the refolding experiments, a commercial preparation of P. pyralis luciferase showing a single protein band with a molecular weight of 62,000 when analyzed by SDS-PAGE (not shown) was used. Refolding of luciferase denatured with urea was performed by 400-fold dilution with translation mixture in the absence of mRNA. Thus, the refolding occurred at the same conditions and in the presence of the same components, except mRNA, as the folding during translation. The concentration of luciferase in the refolding experiments was also within the same range as in the cell-free translation system. The course of refolding proceeded in a luminometer cell was recorded by measuring the luciferase activity as a function of time. Samples of nondenatured active luciferase diluted with the corresponding translation mixtures were used as controls. The refolding of luciferase in wheat germ cytosol proceeds with the same efficiency and the same average half-time of 14 min (Fig. 3a) as was reported earlier (1). In contrast, the refolding in E. coli cytosol was inefficient (Fig. 3b), giving about 8% activity recovered after 1 h of incubation (a plateau level; data not shown).

The active enzyme recovery in the refolding tests did not depend upon duration of the protein incubation in denatured state (for time intervals ranging from 10 s to 10 min; data not shown). At the same time, the unfolding of luciferase seemed to complete within the 10 s incubation with urea, as the enzyme lost its activity completely during this incubation, and the kinetics of refolding was the same as that of luciferase denatured for longer time intervals. The result indicates that the...
Abrupt blocking of the synthesis of the extended luciferase by thiostrepton resulted in immediate cessation of activity accumulation (Fig. 5a). Thus, it can be concluded that the N-terminal part of the luciferase-NPT II fusion was folded into active conformation within the time interval required for the C-terminal extension to be synthesized. The formation of active ribosome-bound luciferase is equivalent to folding of N-terminal domain of a multidomain protein when the synthesis of a C-proximal domain is not yet completed. This result clearly suggests the co-translational mode of the multidomain protein folding in the bacterial translation system.

Specific Activity of Luciferase Synthesized in Different Cell-free Translation Systems—In order to compare the efficiency of the co-translational formation of active luciferase in prokaryotic and eukaryotic extracts, the measurements of the specific activity of the luciferase synthesized in different cell-free translation systems were made. To this end, a series of translation experiments were performed with the luciferase mRNA in wheat germ extract, rabbit reticulocyte lysate, and E. coli S30 extract. The synthesis of luciferase proceeded in the presence of [14C]leucine. After 15 min of incubation, the translation reaction mixtures were fractionated by centrifugation in linear 4–20% sucrose gradient in buffer containing 10 mM Mg(OAc)$_2$, 100 mM KOAc, 20 mM HEPES-KOH, pH 7.5, 0.1 mM EDTA, and 2 mM DTT. Centrifugation was carried out for 2 h at 40,000 rpm in an SW41 rotor at 4 °C. Activity of luciferase in fractions is shown by dark gray bars. Light gray bars indicate the activity after addition of puromycin to final concentration of 0.5 mM. a, nascent luciferase without extension at the C terminus. b, nascent luciferase extended by 59 amino acid residues at the C terminus.

As seen in the Table I, all three translation systems are almost equally effective in providing the proper folding of the enzyme (although their productivity in terms of polypeptide yield was different). This suggests that the co-translational folding of luciferase in the prokaryotic cell-free translation system is as efficient as that in the eukaryotic cytosol.

**DISCUSSION**

The main result of the experiments reported here is the demonstration of co-translational mode of folding of a multidomain eukaryotic protein in the prokaryotic cytosol. The most significant argument against co-translational folding of proteins in prokaryotes (3) is the higher speed of bacterial translation relative to eukaryotic one. It could be thought that folding events would not be able to keep pace with the fast polymerization of polypeptide chain, leaving only the post-translational way for the chain to be folded. Whereas the synthesis of the full-length firefly luciferase in eukaryotic cytosol on the gel, radioactivity counts in the protein bands of full-length enzyme were measured upon solubilization of the gel pieces with hydrogen peroxide. Thus, both the amount of full-length luciferase and its enzymatic activity were determined in aliquots of translation systems, allowing us to calculate specific activities of the enzyme synthesized.

**TABLE I**

| Origin of cell-free system | mRNA added | [14C]Leu in FL protein | Activity in aliquot | Specific activity |
|---------------------------|------------|------------------------|-------------------|-----------------|
| E. coli                   | 40 µg/ml   | 455 cpm                | 816 AU            | 1023 AU/µg      |
| Rabbit reticulocyte       | 40 µg/ml   | 523 cpm                | 807 AU            | 880 AU/µg      |
| Wheat germ                | 40 µg/ml   | 598 cpm                | 855 AU            | 817 AU/µg      |

Co-translational Folding in Bacterial Translation System
takes from 15 to 18 min (1, 2), the enzyme appears in prokaryotic cell-free translation system in 8 min. Thus, the synthesis of enzymatically active luciferase in prokaryotic cytosol occurs much earlier than the half-time of in vitro refolding of denatured enzyme under identical conditions. The low yield of active luciferase in the refolding process when it takes place in prokaryotic cytosol also suggests a co-translational, rather than posttranslational, mechanism of the active enzyme formation.

There are a number of reports also suggesting the co-translational mechanism of folding in prokaryotes. The classical example is β-galactosidase, which forms enzymatically active tetramer with participation of one subunit bound to the prokaryotic ribosome (9, 10). The possibility was not excluded, however, that binding of free subunits could induce the native (or competent to binding) conformation of the nascent one. Later, the experiments on immunological activity of ribosome-bound growing β-galactosidase demonstrated binding of conformation-dependent anti-β-galactosidase antibodies to growing polypeptide chains well before the appearance of active enzyme, at very early times after induction of the enzyme synthesis in cells (22). The result proved that the growing chains of β-galactosidase begin to fold before their release from the ribosome, i.e. co-translationally.

The contribution of co-translational folding to the formation of β-subunit of bacterial luciferase in homologous translation system was clearly shown, although the formation of enzymatically active heterodimer was achieved posttranslationally (17).

Generation of proper S-S bridges in single-chain antibodies during the synthesis in a bacterial cell-free system (23), as well as their ability to display ligand- or antigen-binding features in the ribosome-bound state (24) also indicated the capacity for co-translational folding in prokaryotes, although the more direct kinetics studies and the demonstration of correct S-S bridges formation in nascent single-chain antibodies are necessary to prove co-translational mechanism of protein folding in bacteria. The acquisition of enzymatic activity by bovine rhodanese bound to the prokaryotic ribosome as a peptidyl-tRNA (15) is also in line with the above results.

The results presented here are the first and the most direct evidence in favor of co-translational folding of a protein in a prokaryotic system, generally, and folding of a eukaryotic multi-domain protein on prokaryotic ribosomes, specifically. These findings cast doubt on the idea that the co-translational folding was acquired by eukaryotes during evolution (3). Instead, one can believe that the co-translational mode of folding is the most ancient and universal way of protein structure formation, whereas the posttranslational folding mechanisms involving chaperones arose as intracellular differentiation and transport systems were evolved.

Acknowledgments—We thank A. V. Finkelstein, A. Kommer, and D. E. Agafonov for helpful suggestions and comments; A. Kommer for his invaluable help in experiments with chaperones; and S. Tabor for kindly supplying the pT7-7 vector.

REFERENCES

1. Kolb, V. A., Makeyev, E. V., and Spirin, A. S. (1994) EMBO J. 13, 3631–3637
2. Frydman, J., Nimmegern, E., Ohtsuka, K., and Hartl, F. U. (1994) Nature 370, 111–117
3. Netzer, W. J., and Hartl, F. U. (1997) Nature 388, 343–349
4. Lin, L., DeMartino, G. N., and Greene, W. C. (1998) Cell 92, 819–828
5. Chen, W., Helenius, J., Braakman, I., and Helenius, A. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 6229–6233
6. Bergman, L. W., and Kuehl, W. M. (1979) J. Biol. Chem. 254, 8869–8876
7. Peters, T., and Davidson, L. K. (1982) J. Biol. Chem. 257, 8847–8853
8. Gilmore, R., Coffey, M. C., Leone, G., McLure, K., and Lee, P. W. K. (1996) EMBO J. 15, 2651–2658
9. Kiho, Y., and Rich, A. (1964) Proc. Natl. Acad. Sci. U. S. A. 51, 111–118
10. Zipser, D., and Perrin, D. (1963) Cold Spring Harbor Symp. Quant. Biol. 28, 523–537
11. Komar, A. A., Kommer, A., Krasheninnikov, I. A., and Spirin, A. S. (1997) J. Biol. Chem. 272, 10646–10651
12. Mullet, J. E., Klein, P. G., and Klein, R. R. (1996) Proc. Natl. Acad. Sci. U. S. A. 97, 4038–4042
13. Kim, J., Klein, P. G., and Mullet, J. E. (1991) J. Biol. Chem. 266, 14931–14938
14. Kolb, V. A., Makeyev, E. V., Kommer, A., and Spirin, A. S. (1995) Biochem. Cell Biol. 73, 1217–1220
15. Kudlicki, W., Chirgwin, J., Kramer, G., and Hardesty, B. (1995) Biochemistry 34, 14284–14287
16. Makeyev, E. V., Kolb, V. A., and Spirin, A. S. (1996) FEBS Lett. 378, 166–170
17. Fedorov, A. N., and Baldwin, T. O. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 1227–1231
18. Tabor, S., and Richardson, C. C. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 1074–1078
19. Gurevich, V. V., Pokrovskaya, I. D., Obukhova, T. A., and Zozulya, S. A. (1991) Anal. Biochem. 195, 207–213
20. Zubay, G. (1975) Annu. Rev. Genet. 9, 267–287
21. Roberts, B. E., and Paterson, B. M. (1973) Proc. Natl. Acad. Sci. U. S. A. 70, 2330–2334
22. Hamlin, J., and Zabin, I. (1972) Proc. Natl. Acad. Sci. U. S. A. 69, 412–416
23. Ryabova, L. A., Desplanque, D., Spirin, A. S., and Pluckthun, A. (1997) Nature Biotechnol. 15, 79–84
24. Hanes, J., and Pluckthun, A. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 4937–4942

Acknowledgments—We thank A. V. Finkelstein, A. Kommer, and D. E. Agafonov for helpful suggestions and comments; A. Kommer for his invaluable help in experiments with chaperones; and S. Tabor for kindly supplying the pT7-7 vector.
Co-translational Folding of an Eukaryotic Multidomain Protein in a Prokaryotic Translation System
Vyacheslav A. Kolb, Eugeny V. Makeyev and Alexander S. Spirin

J. Biol. Chem. 2000, 275:16597-16601.
doi: 10.1074/jbc.M002030200 originally published online March 20, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M002030200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 24 references, 13 of which can be accessed free at http://www.jbc.org/content/275/22/16597.full.html#ref-list-1