Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 2540–2544, April 1989
Biochemistry

Ubiquitin fusion augments the yield of cloned gene products in *Escherichia coli*

(expression system/metallothionein/guanine nucleotide-binding protein/ubiquitin-N^{α}-protein hydrolase)

TAUSEEF R. BUTT*†, SOBHANADITYA JONNALAGADDA*‡, BRETT P. MONIA*§, EDMUND J. STERNBERG*, JONATHAN A. MARSH*, JEFFREY M. STADEL*, DAVID J. ECKER*, AND STANLEY T. CROOKE*§

*Smith Kline & French Laboratories, Department of Molecular Pharmacology, P.O. Box 1539, King of Prussia, PA 19406; and §Department of Pharmacology, School of Medicine, University of Pennsylvania, Philadelphia, PA 19101

Communicated by Irwin A. Rose, December 12, 1988

ABSTRACT **Despite the availability of efficient transcription and translation signals, some heterologous gene products are not adequately expressed when introduced into prokaryotes and eukaryotes. An expression system has been established in *Escherichia coli* to increase the yield of cloned gene products, where the C terminus of ubiquitin was fused to the N terminus of unstable or poorly expressed proteins. Fusion of ubiquitin to yeast metallothionein or to the α subunit of the adenylate cyclase-stimulatory GTP-binding protein increased the yield from undetectable to 20% of the total cellular protein. A ubiquitin-N^{α}-protein hydrolase has been partially purified from rabbit reticulocytes; this enzyme faithfully cleaves the junction peptide bond between the C-terminal Gly-76 of ubiquitin and the fusion protein. The increased yield of cloned gene products is very likely due to increased stability and/or more efficient translation of the fusion proteins. Possible mechanisms for the augmentation of ubiquitin fusion-protein expression in prokaryotes and eukaryotes are discussed.**

Our understanding of protein structure and function has been greatly advanced by the ability to express cloned gene products in prokaryotes and eukaryotes. Therapeutic and several other applications of proteins are also attributed to the efficient expression of cloned genes in various hosts. A problem commonly encountered in attempts to express cloned genes in heterologous hosts is the inefficient expression of proteins. Poor expression of proteins can be due to many factors, including inefficient transcription or translation or rapid breakdown of the mRNA or protein by the host. Several systems for heterologous gene expression use strong promoters and faithful transcription initiation and translation signals to ensure efficient and regulated expression of gene products (1, 2). However, the level of heterologous gene products under identical transcriptional and translational signals can vary from 0% to 40% of the total cellular protein (refs. 1–3 and this study). In addition, several proteins are expressed at high levels but are biologically inactive due to inappropriate folding or posttranslation modification(s) (refs. 4 and 5 and references therein).

β-Galactosidase fusion proteins have been constructed to stabilize the gene products in prokaryotes (6). The disadvantage of this approach is that the fusion gene products cannot be cleaved to yield authentic proteins. Expression systems have also been developed where the λ cII gene product, a highly expressed protein in *Escherichia coli*, is followed by a tetrapeptide inserted at the junction with the protein to be expressed. This peptide encodes a cleavage site for the blood coagulation factor X_a (7). Desired proteins are expressed as cII–junction peptide fusion proteins. The purified fusion protein is cleaved with factor X_a to release the pure protein (7). The disadvantages of this system are that desired N termini cannot be constructed and often the protein is cleaved at multiple sites by factor X_a.

Ubiquitin, a 76-amino acid polypeptide, is the most conserved protein known among eukaryotes (8). Ubiquitin and the ubiquitin pathway are not present in prokaryotes. In eukaryotes, ubiquitin exists as a free molecule or with its C terminus covalently linked to the ε-amino groups of lysine in other proteins (8). In addition, natural ubiquitin fusion-protein genes have been cloned and sequenced from eukaryotes in which the N-terminal end of the protein is ubiquitin followed by a C-terminal extension of 52–80 amino acids (9–11). Ubiquitin–β-galactosidase (12) and ubiquitin–metallothionein (UB–MT) (13) fusion proteins and human ubiquitin C-terminal extension proteins (HUBCEPs) (10) have been expressed in yeast, and the ubiquitin moiety was rapidly processed except when the first amino acid of the extension protein was proline.

We have observed that the fusion of genes to ubiquitin sequences greatly increased their yield (up to 20% of total cell protein) in *E. coli*. Expression of UB–MT and UB–$G_s\alpha$ [where $G_s\alpha$ is the α subunit of the GTP-binding stimulatory protein of adenylate cyclase (14)] were studied in *E. coli*. The unfused proteins were extremely unstable or not translated efficiently, whereas fusion with ubiquitin stabilized the proteins (and perhaps enhanced their translation), thus increasing their yield. We have also characterized and partially purified an enzyme, ubiquitin–N^{α}-protein hydrolase (UαPH), from rabbit reticulocytes that faithfully cleaves these ubiquitin fusion proteins to release ubiquitin and the authentic protein. The utility of this system to increase the yield of unstable or poorly expressed proteins in *E. coli* is discussed.

MATERIALS AND METHODS

Construction of Ubiquitin Fusion-Protein Expression Vectors. Ubiquitin genes were chemically synthesized in this laboratory (15) and expressed under control of the λ P_L promoter (1) in *E. coli* strain AR58 carrying the cI857 temperature-sensitive mutation. The construction of the UB–MT fusion gene has been described (13). The first eight amino acids of yeast MT are not necessary for metal binding and are removed during MT biosynthesis in yeast (16). The processed yeast MT contains glutamine at the N terminus (16). The

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: MT, metallothionein; UB–MT, ubiquitin–MT fusion protein; $G_s\alpha$, α subunit of GTP-binding protein that stimulates adenylate cyclase; UB–$G_s\alpha$, ubiquitin–$G_s\alpha$ fusion protein; HUBCEP, human ubiquitin C-terminal extension protein; UαPH, ubiquitin–N^{α}-protein hydrolase.

†To whom reprint requests and all correspondence should be addressed.
‡Present address: Department of Biochemistry, Osmania University, Hyderabad, India 500007.

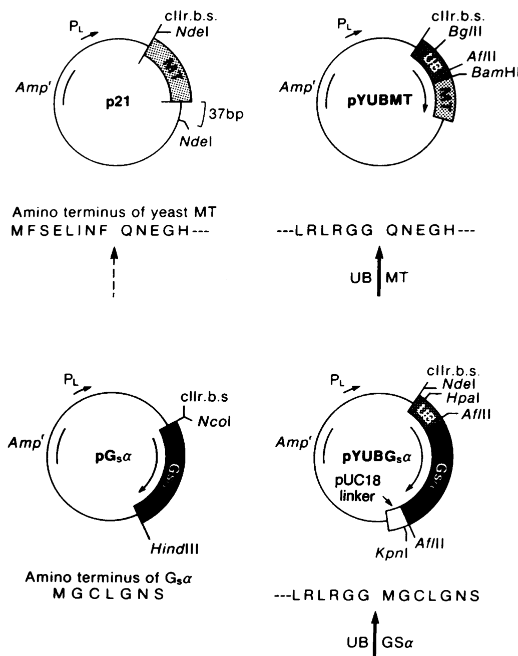

FIG. 1. Expression vectors. p21 and p$G_s\alpha$ are *E. coli* vectors that express the complete yeast MT gene (17) and rat $G_s\alpha$ gene (14). pYUBMT is an *E. coli* expression vector in which the ubiquitin C-terminal glycine codon is fused to the N-terminal glutamine codon of mature yeast MT. pYUB$G_s\alpha$ is an *E. coli* expression vector in which the C-terminal glycine codon of ubiquitin is fused to the N-terminal methionine codon of the $G_s\alpha$ protein. The N-terminal sequence of yeast MT and of rat $G_s\alpha$ and the amino acid sequence of the ubiquitin–protein junctions are given in one-letter code below the diagrams. The dashed arrow refers to the cleavage site of MT in yeast (16). Solid arrows refer to the cleavage sites of the rabbit reticulocyte UαPH. *Amp*^r, ampicillin-resistance gene used for selection of *E. coli* transformants; r.b.s., ribosome binding site; bp, base pairs. For further details, see *Materials and Methods.*

expression vector p21 (Fig. 1 and ref. 17) contains the complete yeast MT gene, with an N-terminal methionine codon, under control of the P_L promoter system (1). The expression vector pYUBMT (Fig. 1) contains the yeast ubiquitin C-terminal glycine codon fused to the N-terminal glutamine codon of the processed yeast MT. p$G_s\alpha$ is an expression vector that encodes rat $G_s\alpha$. The $G_s\alpha$ gene is contained in an *Nco* I–*Afl* II fragment of plasmid pGEM2G_s-αR that was cloned from a rat cDNA library (14). The *Nco* I–*Hin*dIII fragment of pGEM2$G_s\alpha$R was ligated to *Nco* I/*Hin*dIII-digested *E. coli* expression vector pMG27 (4) to construct p$G_s\alpha$, in which the $G_s\alpha$ gene is controlled by the λ P_L promoter (Fig. 1). To construct the UB–$G_s\alpha$ expression vector pYUB$G_s\alpha$, pGEM2$G_s\alpha$R was digested with *Xba* I and *Nco* I. An *Xba* I–*Nco* I synthetic DNA linker encoding the C terminus of ubiquitin was inserted in pGEM2$G_s\alpha$R to yield pGEM2SAL. A yeast ubiquitin expression vector, YEp52aa (13), was digested with *Afl* II and the *Afl* II–*Afl* II fragment from pGEM2GSAL was inserted into YEp52aa to yield UB–$G_s\alpha$ yeast expression vector YEp52a-$G_s\alpha$. The *Hpa* I–*Kpn* I fragment of YEp52a-$G_s\alpha$, encoding UB–$G_s\alpha$, was inserted into digested pNMHUB (15) to yield an *E. coli* expression vector, pYUB$G_s\alpha$, where the UB–$G_s\alpha$ gene is under control of P_L (Fig. 1).

Determination of Half-Lives of Proteins in *E. coli*. Protein-induction experiments were carried out in *E. coli* lysogens carrying a temperature-sensitive mutation in the phage λ *c*I gene (*c*I857), which allows P_L-directed transcription to be activated by temperature shift (1). Induction is accomplished simply by raising the temperature of the culture from 32°C to 42°C. Reducing the temperature to 32°C presumably renatures the cI repressor, and the transcription is stopped. The half-lives of unfused and ubiquitin-fused proteins were determined by pulse-labeling with [^{35}S]cysteine at 42°C and chasing with excess cysteine at 32°C as described (17). Alternatively, Western blots of unlabeled *E. coli* extracts were probed with antibodies against either ubiquitin or the appropriate protein. Autoradiographs of SDS/polyacrylamide gels or of Western blots were scanned in a Beckman DU8 spectrophotometer equipped with a gel-scanning device. To correct for variations in sample loading, the radioactivity in the band of interest was normalized to that in a protein band that was constitutively expressed in each lane of the autoradiograph.

Purification of Ubiquitin Fusion Proteins. *E. coli*-expressed UB–MT was purified to homogeneity by the procedure described for *E. coli*-expressed ubiquitin (15), except that all buffers contained 1 mM dithiothreitol. The UB–MT peak was eluted from a fast-flow Mono Q (Pharmacia) column between 0.3 and 0.4 M KCl. The peak fractions were pooled, reapplied to the Mono Q column, and eluted with a 0–0.6 M KCl gradient. Essentially homogeneous fractions of UB–MT were stored at 2 mg/ml at −70°C. *E. coli*-expressed HUBCEP52 was purified as described (10).

Preparation of UαPH. UαPH cleaves the peptide bond between the C terminus (Gly-76) of ubiquitin and the N terminus of the fused protein. To avoid any confusion in nomenclature of the ubiquitin-hydrolyzing enzymes, we have adopted the abbreviated names suggested by Rose (18). In previous papers (e.g., ref. 13), we referred to this enzyme as (αNH_2-ubiquitin) protein endopeptidase. The detailed properties and purification of UαPH will be published elsewhere. In brief, rabbit reticulocyte lysate was prepared (19), and the UαPH activity was localized in the 20–60% (saturation) ammonium sulfate precipitate of the lysate. The precipitate was dialyzed overnight in 50 mM Tris·HCl, pH 7.4/1 mM dithiothreitol. The retentate was applied to a column of ion-exchange fast-flow Q Sepharose (Pharmacia) and eluted with a linear gradient of 0–1 M KCl in 50 mM Tris·HCl, pH 7.4/1 mM dithiothreitol. Two peaks of UαPH activity were detected using purified UB–MT as a substrate (see below). Peak I was eluted at ≈0.2 M KCl and peak II at ≈0.5 M KCl. Fractions from peak II were routinely used to process ubiquitin fusion proteins.

UαPH enzymatic activity was assayed by two methods. (*i*) Purified UB–MT or HUBCEP52 and enzyme fractions were incubated, and the cleavage products were analyzed by SDS/PAGE. (*ii*) Alternatively, the precision of the UαPH cleavage was assayed by the production of stoichiometric quantities of ubiquitin by ubiquitin-activating enzyme (E1) assays (19, 20). The UαPH assays were carried out in 50 mM Tris·HCl, pH 8.0/1 mM dithiothreitol. Enzyme fractions (10 μg of protein) were incubated with substrate (3 μg of pure UB–MT) in a 20-μl reaction volume for 30 min at 37°C. The reaction was stopped either by adding 1% SDS buffer, for analysis by PAGE, or by heating at 70°C for 10 min to inactivate the enzymes, whereupon samples were taken for E1 enzymatic analysis of ubiquitin. Ubiquitin-mediated E1 activity was determined by $^{32}PP_i$/ATP exchange (19). Production of intact ubiquitin was also measured by the estimation of E1-activated ubiquitin–[^{3}H]AMP complex formation (the end-point assay, ref. 20).

RESULTS

E. coli containing MT and UB–MT expression vectors (p21 and pYUBMT; Fig. 1) were analyzed for the synthesis of proteins in the uninduced (U) and induced (I) state by pulse labeling with [^{35}S]cysteine and autoradiography. The MT and UB–MT bands were well resolved when analyzed by nondenaturing PAGE (Fig. 2*A*). On SDS/PAGE, the electropho-

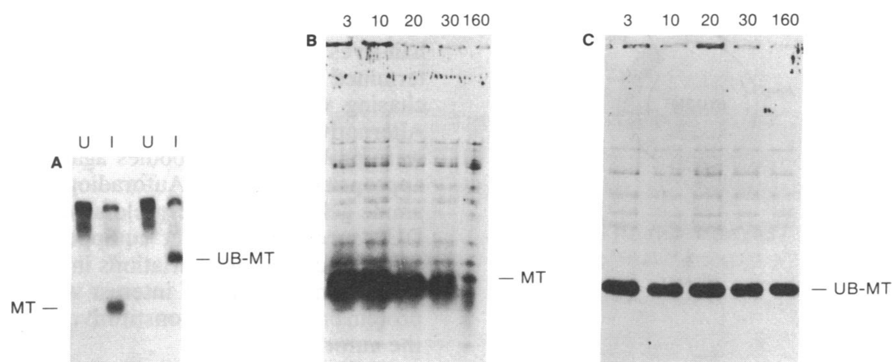

FIG. 2. Expression of yeast MT and UB–MT in *E. coli* and determination of the half-lives of the proteins. *E. coli* containing p21 (MT) or pYUBMT were induced for 10 min at 42°C and labeled with [^{35}S]cysteine for another 10 min. (*A*) Uninduced (U) and induced (I) cell extracts were subjected to nondenaturing 18% PAGE and autoradiographed. (*B*) After pulse-labeling, *E. coli* cells containing p21 (MT) were incubated at 32°C with 1 mM nonradioactive cysteine for 3, 10, 20, 30, or 160 mins, and samples were analyzed by denaturing SDS/18% PAGE and autoradiography. (*C*) *E. coli* cells containing pYUBMT were pulsed and chased as described in *B*.

retic mobility of MT was similar to that of UB–MT (Fig. 2 *B* and *C*). To determine the half-lives of UB–MT and MT, *E. coli* were induced by raising the temperature to 42°C and pulse-labeled with [^{35}S]cysteine as described (17). The label was "chased" by incubating the cells with 1 mM nonradioactive cysteine at 32°C, where the transcription is repressed. At 3, 10, 20, 30, and 160 min of the chase, samples (200 μl) of the cultures were removed and equal amounts of protein-incorporated radioactivity were analyzed by SDS/18% PAGE followed by autoradiography. The yeast MT was unstable in *E. coli* and was completely degraded by 160 min (Fig. 2*B*). Under these experimental conditions, the half-life of yeast MT was 20 min. In contrast, no degradation of UB–MT was detected, even after 160 min (Fig. 2*C*). MT is a random coil and in the absence of metal ions it moves as a diffuse band in SDS/polyacrylamide gels (Fig. 2*B*), whereas UB–MT migrates as a sharp band (Fig. 2*C*). We do not know whether the attachment of ubiquitin to MT affects the structure of MT. Two-dimensional NMR spectroscopy of UB–MT in solution showed no profound structural change in ubiquitin proton resonances (unpublished data). It is possible that the presence of an eight-amino acid sequence at the N terminus [which is cleaved in yeast after translation (16)] may destabilize the protein in *E. coli*.

To test whether N^{α}-ubiquitinated proteins are generally stable in *E. coli*, we determined the half-life of $G_s\alpha$, which is localized at the inner face of the plasma membrane of eukaryotic cells (14). Pulse–chase induction–repression experiments with *E. coli* expressing the $G_s\alpha$ plasmid (p$G_s\alpha$; Fig. 1) did not show the appearance of a 43-kDa $G_s\alpha$ protein band; however, a putative breakdown product was seen at ≈25 kDa in the induced lane, and this protein subsequently was degraded by 120 min during the chase (Fig. 3). In contrast, fusion of $G_s\alpha$ with ubiquitin (pYUB$G_s\alpha$; Fig. 1) led to induction of a prominent radioactive band at ≈52 kDa, and no significant degradation of this protein was observed during the first 60 min of the chase (Fig. 3). The half-life of UB–$G_s\alpha$ in this experiment was ≈90 min.

To verify that the radioactive 52-kDa band was in fact UB–$G_s\alpha$, the above induction–repression experiment was repeated in a rich, nonradioactive medium. Western blots were probed with polyclonal antibodies raised against rat $G_s\alpha$, and immunoreactive bands were detected by incubation with ^{125}I-labeled protein A followed by autoradiography. A distinct 52-kDa band that reacted with the $G_s\alpha$ antibodies was induced at 42°C in cells carrying pYUB$G_s\alpha$ (Fig. 4*A*). The $G_s\alpha$ antibodies showed nonspecific crossreaction with two *E. coli* proteins (marked by stars in Fig. 4 Western blots). The kinetics of UB–$G_s\alpha$ degradation determined from Western blots were similar to the results of the pulse–chase experiment described in Fig. 3. Analysis of protein samples from cells carrying p$G_s\alpha$ did not show an immunoreactive band at 43 kDa (Fig. 4*B*). These data suggest that $G_s\alpha$ was either translated at a lower rate or degraded soon after its translation. The $G_s\alpha$ degradation product seen in Fig. 3 suggested that the half-life of nonubiquitinated $G_s\alpha$ was less than a few seconds. After autoradiography the Western blots were stained with amido black (Fig. 4 *C* and *D*). Examination of Fig. 4*C* lanes U and i shows that a protein band (asterisk) was inducible and was stable until 60 min. The degradation kinetics of this band were analogous to those of the immunoreactive 52-kDa band seen in the Western blot (Fig. 4*A*). The stained blot for $G_s\alpha$ did not show any induction of a band at 43 kDa (Fig. 4*D*). These data suggest that $G_s\alpha$, a membrane

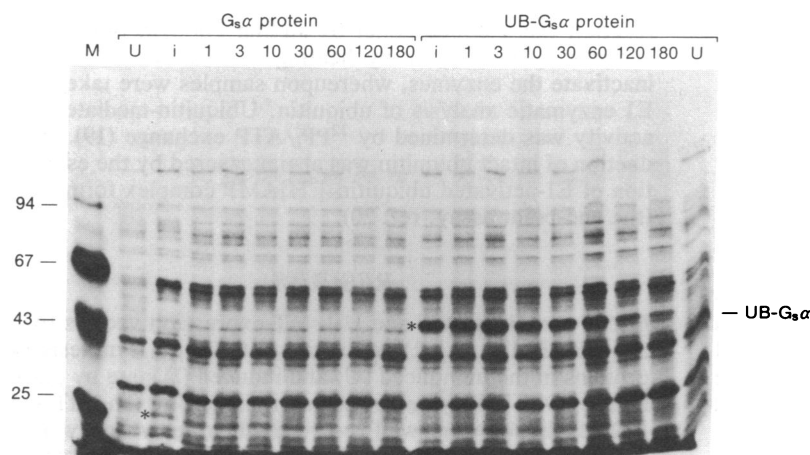

FIG. 3. Induction of $G_s\alpha$ and UB–$G_s\alpha$ in *E. coli* and determination of the half-lives of the proteins. Cells containing the appropriate plasmids were pulse-labeled with [^{35}S]cysteine for 15 min and chased with nonradioactive cysteine for 1–180 min at 32°C. Asterisk in $G_s\alpha$ lanes indicates degraded product of $G_s\alpha$, and asterisk in UB–$G_s\alpha$ lanes indicates the intact UB–$G_s\alpha$, protein band. U, uninduced; i, induced. Marker proteins are shown in lane M (sizes at left in kilodaltons).

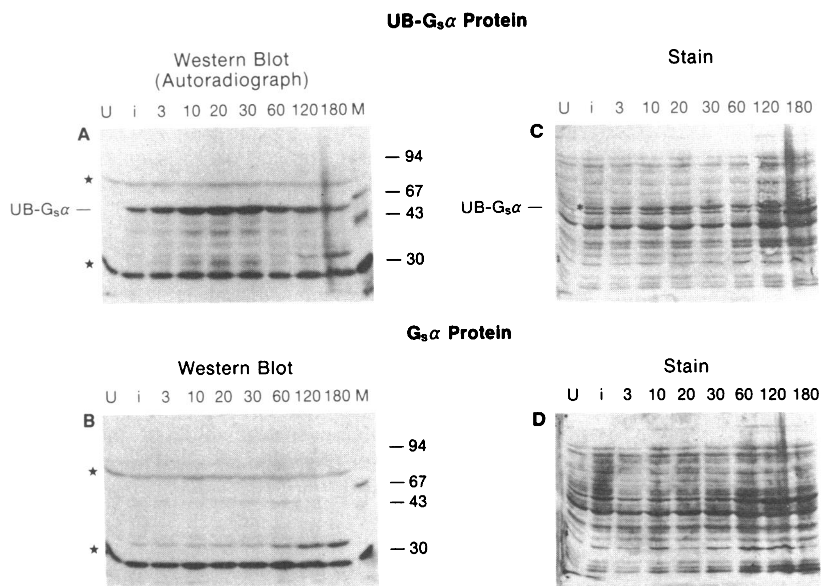

FIG. 4. Analysis of half-lives of $G_s\alpha$ and UB–$G_s\alpha$ by crossreaction with $G_s\alpha$ antibody. *E. coli* cells containing $G_s\alpha$ or pYUBG$_s\alpha$ were induced at 42°C in rich, nonradioactive medium for 15 min. After induction, cells were brought back to 32°C, aliquots were removed at 1–180 min, and equal amounts of protein were analyzed by Western blotting with polyclonal antibodies against $G_s\alpha$ protein. The blots were then incubated with ^{125}I-labeled protein A and autoradiographed (*A* and *B*). After autoradiography the same blots were stained with amido black (*C* and *D*). Stars in *A* and *B* indicate two bands that reacted with $G_s\alpha$ antibody nonspecifically, as they were present in uninduced cells. Asterisk in *C* identifies the inducible 52-kDa UB–$G_s\alpha$ protein band. U, uninduced; i, induced; M, markers.

protein of eukaryotes, was highly unstable in *E. coli*. The UB–$G_s\alpha$ fusion protein was highly stable in *E. coli*, and the gel scan data showed that it represented 20% of the total protein even 60 min after a 10-min induction period.

To confirm that the slow migration of the UB–MT and UB–$G_s\alpha$ proteins was due to ubiquitin sequences and did not reflect posttranslational modification of the proteins, an induction–repression experiment was done with *E. coli* carrying UB–$G_s\alpha$ or UB–MT expression vector, and Western blots were probed with affinity-purified antibodies against ubiquitin. Cells carrying the UB–$G_s\alpha$ expression vector showed induction of a major ubiquitin-crossreactive band that migrated at the molecular mass (52 kDa) expected for the fusion protein (Fig. 5*A*). Ubiquitin antibodies also reacted with a smaller *E. coli* protein that was also present in uninduced cells (Fig. 5*A*). The kinetics of UB–$G_s\alpha$ degradation were similar to those observed in previous experiments (Figs. 3 and 4). As before, UB–MT was found to be extremely stable in *E. coli* (Fig. 5*B*). After 1 hr of transcription repression at 32°C, a small fraction of UB–MT was cleaved to release free ubiquitin. This observation is consistent with

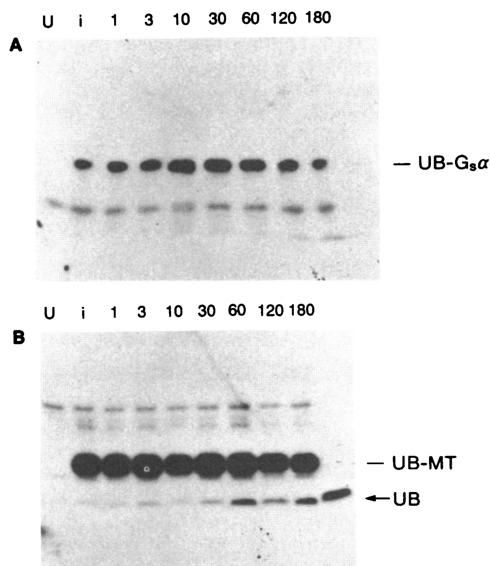

FIG. 5. Analysis of the half-lives of UB–MT and UB–$G_s\alpha$ protein by crossreaction with antibodies against ubiquitin. The blots were probed with affinity-purified, polyclonal ubiquitin antibodies (21) and autoradiographed. U, uninduced; i, induced.

our previous report (21) that *E. coli* contains a protease activity that, even though it is extremely inefficient, cleaves ubiquitin from N-terminally ubiquitinated proteins.

An equally important component of this technology is UαPH, which faithfully cleaves ubiquitin from the fusion proteins. UαPH was partially purified from rabbit reticulocyte lysates as described in *Materials and Methods*. UB–MT, HUBCEP52, and HUBCEP80 purified from *E. coli* were used as substrates to monitor purification. UαPH activity was determined either by a change in the mobility of the fusion protein on SDS/PAGE or by estimation of the intact, biologically active ubiquitin in ^{32}PP$_i$/ATP exchange assays carried out in the presence of ubiquitin-activating enzyme (E1). Table 1 describes the results of UB–MT cleavage by UαPH as determined by two different assays of E1. After incubation with UαPH, UB–MT (10 pmol/μl) enhanced ^{32}PP$_i$/ATP exchange in the presence of E1 by 1.04 pmol/min, compared with 1.11 pmol/min for standard bovine ubiquitin. Analysis of hydrolyzed UB–MT by E1-activated ubiquitin–[^{3}H]AMP complex formation gave similar results (Table 1). That UαPH hydrolysis of UB–MT gave ubiquitin that was as active as standard ubiquitin in promoting E1-mediated ^{32}PP$_i$/ATP exchange suggests that the C-terminal -Arg-Gly-Gly sequence was intact. To ensure that the cleavage product of the ubiquitin fusion protein was intact, we also determined the N-terminal amino acid sequence of CEP52 cleaved from HUBCEP52. Purified HUBCEP52 was incubated with partially purified UαPH for 60 min. After cleavage, CEP52 was purified by the same procedure as described for HUBCEP52 (10). The purified CEP was subjected to 11 cycles of automated N-terminal sequence analysis, and the sequence was similar to that of the authentic CEP (data not

Table 1. Accuracy of ubiquitin–protein cleavage by UαPH as determined by the activation of free ubiquitin by ubiquitin-activating enzyme E1

Reaction component(s)	^{32}PP$_i$/ATP exchange, pmol/min	Ubiquitin–[^{3}H]AMP formation, pmol/μl
UαPH alone	0	0
UαPH + UB–MT	1.04	9.89 ± 0.05
UαPH + ubiquitin	1.11	10.16 ± 0.07

Reaction mixtures contained UαPH (peak II, 10 μg) and purified UB–MT or bovine ubiquitin (10 pmol/μl) as indicated. Assays were carried as described in *Materials and Methods*. After the incubation at 37°C, the samples were heated at 70°C for 10 min and aliquots were subsequently assayed for the release of active ubiquitin by E1.

shown). No N terminus except isoleucine was detected, suggesting that the fusion protein was quantitatively converted to ubiquitin and CEP52.

DISCUSSION

The λ phage P_L promoter is highly efficient, and numerous studies on P_L-based expression vectors have indicated no significant change in the transcription of RNA (1). Addition of ubiquitin to the N terminus of a protein may affect the translation efficiency or stabilize the structure of the protein so that it is no longer the target of the intracellular proteolytic system. In this context, we propose some mechanisms for increased expression of ubiquitin fusion proteins in *E. coli*.

(*i*) N-end protection. Principles governing the breakdown of proteins in *E. coli* are not well established. However, it is possible that analogous to the eukaryotic system (12), the free N terminus is the degradation signal for short-lived proteins in *E. coli*. The ubiquitin molecule, well known for its resistance to proteases, might protect the fused protein from N-terminal proteolytic attack.

(*ii*) Facilitated folding. Many heterologous proteins expressed in *E. coli* fold improperly and form inclusion bodies wherein the proteins are essentially insoluble (4, 5). The energetically favored folding pathway of ubiquitin may facilitate proper folding of the fused protein. As a result the protein may remain soluble. This hypothesis may also explain the role of ubiquitin on secreted growth factor receptor isolated from cultured human leukemia cells (22) as well as the attachment of ubiquitin to the cell surface receptors (23, 24). It is possible that ubiquitin linked to the growth factor facilitates its secretion; similarly, ubiquitination of receptors may keep these hydrophobic proteins soluble and facilitate their translocation to the cell membrane. Recent studies on the expression in *E. coli* of ubiquitin-fused human steroid receptors (which are generally insoluble when expressed as unfused molecules) showed that up to 30% of the fused receptor protein was soluble and biologically active (Z. Nawaz, D. McDonnell, W. Pike, and T.R.B., unpublished data). Although polyubiquitination of proteins has been proposed as a signal for proteolysis, in view of the fact that ubiquitin is found in all cellular compartments, a nonproteolytic role for ubiquitin is equally plausible. In this respect ubiquitin function may be analogous to that of heat shock proteins. For example, the 70-kDa heat shock protein has been implicated in protecting hydrophobic proteins, facilitating cellular compartmentation and protein secretion (25). Ubiquitin is induced in response to heat shock and may be considered a heat shock protein (26). We suggest that ubiquitin may be one of the "chaperonins" of the eukaryotic system as proposed earlier for the heat shock proteins (25).

(*iii*) Efficient translation. Ubiquitin, Nature's most conserved protein (27), might have evolved the best codon usage for eukaryotes. It is possible that ubiquitin fusion proteins are efficiently translated in *E. coli*. Increased efficiency of translation as a mechanism for increased expression is also attractive because in yeast, where the ubiquitin moiety is cleaved following translation, a severalfold increase in the level of the cloned gene product has been observed under identical conditions of induction. In all cases, ubiquitin fusion led to an increased yield of proteins in yeast (unpublished data). It therefore seems likely that ubiquitin-mediated increased expression of cloned gene products can be adapted for other eukaryotes as well.

The universality of this observation may become more apparent when more gene products are expressed as ubiquitin fusion proteins in yeast and *E. coli* and their yields are analyzed. Unpublished data from the expression of human lymphocyte CD4 receptor, the natural HUBCEPs, and human poly(ADP-ribose) polymerase in *E. coli* suggest that the fusion of ubiquitin to these proteins increased their yield up to 5-fold. Moreover, addition of ubiquitin may not only help stabilize the unstable proteins but also increase the yield of relatively stable proteins. Thus, ubiquitin fusion technology has the potential for general application in augmenting the yield of cloned gene products in prokaryotes as well as eukaryotes.

We have proposed multiple roles of ubiquitin in eukaryotes (28). A clue to the additional roles of ubiquitin became more apparent after the discovery of natural HUBCEPs. It is intriguing that the ubiquitin portion is highly conserved, while the CEP structure is 60% conserved between yeast and human (ref. 10 and references therein). CEP, a metalloprotein, may be under fewer evolutionary constraints, and perhaps relatively unstable in the absence of metal. We propose that the presence of ubiquitin on CEP may stabilize the protein during translation until the metal is coordinated and the ubiquitin is cleaved.

1. Shatzman, A. R. & Rosenberg, M. (1987) *Ann. N.Y. Acad. Sci.* **478**, 233–248.
2. Tabor, S. & Richardson, C. C. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 1074–1078.
3. Graziano, M. P., Casey, P. J. & Gilman, A. G. (1987) *J. Biol. Chem.* **262**, 11375–11381.
4. Gross, M., Sweet, R. W., Sathe, G., Yokoyama, S., Fasano, O., Goldfarb, M., Wigler, M. & Rosenberg, M. (1985) *Mol. Cell. Biol.* **5**, 1015–1024.
5. Kane, J. F. & Hartley, D. L. (1988) *Trends Biotechnol.* **6**, 95–101.
6. Casadaban, M. J., Martinez-Arias, A., Shapira, S. K. & Chon, J. (1983) *Methods Enzymol.* **100**, 293–308.
7. Nagai, R., Perutz, M. F. & Poyart, C. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 7252–7255.
8. Hershko, A. & Ciechanover, A. (1986) *Prog. Nucleic Acid Res. Mol. Biol.* **33**, 19–56.
9. Ozkaynak, E., Finley, D., Soloman, M. J. & Varshavsky, A. (1987) *EMBO J.* **6**, 1429–1439.
10. Monia, B. P., Ecker, D. J., Jonnalagadda, S., Marsh, J., Gotlib, L., Butt, T. R. & Crooke, S. T. (1989) *J. Biol. Chem.* **264**, 4093–4103.
11. Redman, K. L. & Rechsteiner, M. (1988) *J. Biol. Chem.* **263**, 4926–4931.
12. Bachamir, A., Finley, D. & Varshavsky, A. (1986) *Science* **234**, 179–186.
13. Butt, T. R., Khan, M. I., Marsh, J., Ecker, D. J. & Crooke, S. T. (1988) *J. Biol. Chem.* **263**, 16364–16371.
14. Jones, D. T. & Reed, R. R. (1987) *J. Biol. Chem.* **262**, 14241–14249.
15. Ecker, D. J., Butt, T. R., Marsh, J., Sternberg, E. J., Margolis, N., Monia, B. P., Jonnalagadda, S., Khan, M. I., Weber, P. L., Mueller, L. & Crooke, S. T. (1987) *J. Biol. Chem.* **262**, 14213–14221.
16. Winge, D. R., Nielson, R. B., Gray, W. R. & Hamer, D. H. (1985) *J. Biol. Chem.* **260**, 14464–14470.
17. Berka, T., Shatzman, A., Zimmerman, J., Strickler, J. & Rosenberg, M. (1988) *J. Bacteriol.* **170**, 21–26.
18. Rose, I. A. (1988) in *Ubiquitin*, ed. Rechsteiner, M. (Plenum, New York), pp. 135–153.
19. Haas, A. L. & Rose, I. A. (1982) *J. Biol. Chem.* **257**, 10329–10337.
20. Rose, I. A. & Warms, J. V. B. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 1477–1481.
21. Jonnalagadda, S., Butt, T. R., Marsh, J., Sternberg, E. J., Mirabelli, C. K., Ecker, D. J. & Crooke, S. T. (1988) *J. Biol. Chem.* **262**, 17750–17756.
22. Okabe, T., Fujisawa, M., Mihara, A., Saton, S., Fujiyoshi, N. & Takaku, F. (1986) *J. Cell Biol.* **103**, 4429.
23. Siegelman, M., Bond, M. W., Gallatin, M., St. John, T., Smith, H. T., Fried, V. A. & Wiessman, I. L. (1986) *Science* **231**, 823–829.
24. Yarden, Y., Escobedo, J. A., Kuang, W. J., Yang-Feng, T. L., Daniel, T. O., Tremble, P. M., Chen, E. Y., Ander, M. E., Harkins, R. N., Francke, U., Fried, V. A., Ullrich, A. & Williams, L. T. (1986) *Nature (London)* **323**, 226–232.
25. Pelham, H. (1988) *Nature (London)* **322**, 776–777.
26. Bond, U. & Schlesinger, M. J. (1985) *Mol. Cell. Biol.* **5**, 949–956.
27. Sharp, P. M. & Wen-Hsiang, L. (1987) *J. Mol. Evol.* **25**, 58–64.
28. Butt, T. R., Ecker, D. J., Sternberg, E., Marsh, J., Jonnalagadda, S. & Crooke, S. T. (1988) in *The Ubiquitin System*, eds. Schlesinger, M. & Hershko, A. (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 12–18.