Leader peptidase cleaves the amino-terminal leader sequences of many secreted and membrane proteins. We have examined the function of leader peptidase by constructing an Escherichia coli strain where its synthesis is controlled by the arabinose B promoter. This strain requires arabinose for growth. When the synthesis of leader peptidase is repressed, protein precursors accumulate, including the precursors of M13 coat protein (an inner membrane protein), maltose binding protein (a periplasmic protein), and OmpA protein (an outer membrane protein). These precursors are translocated across the plasma membrane, as judged by their sensitivity to added protease K. However, pro-OmpA and pre-maltose binding protein are retained at the outer surface of the inner membrane. Thus, leader peptides anchor translocated pre-proteins to the outer surface of the plasma membrane and must be removed to allow their subsequent release into the periplasm or to translocate into the outer membrane.

Many secreted and membrane proteins are synthesized in a precursor form with a 15-30-amino acid leader or signal sequence at the amino terminus (Milstien et al., 1972). Leader peptides have a basic amino-terminal region followed by a long stretch of uncharged, mainly hydrophobic amino acids. Cleavage by leader peptidase occurs after the protein has crossed the membrane.

Genetic studies of bacteria (Michaelis and Beckwith, 1982) and yeast (Carlson and Botstein, 1982) have shown that the leader sequence is essential for protein export. Mutations within the leader sequence have profound effects on the export of pre-proteins. For example, the introduction of a charged amino acid into the hydrophobic core of the leader sequence of lamB protein (Emr et al., 1978; Emr and Silhavy, 1980), β-lactamase (Koshland et al., 1982), and maltose binding protein (Bassford and Beckwith, 1972; Bedouelle et al., 1980) causes the precursor to accumulate in the cytoplasm. This suggests that the integrity of the leader peptide hydrophobic core is important for the initiation of secretion. However, gene fusion studies (Moreno et al., 1980; Benson and Silhavy, 1983) in which a leader sequence is fused to a cytoplasmic protein have established that the leader peptide alone is not sufficient for translocation.

The removal of the leader sequence is not essential for translocation of prec-proteins across the membrane. Non-cleavable mutants of bacterial lipoprotein (Lin et al., 1980), β-lactamase (Koshland et al., 1982), and M13 procoat (Kuhn and Wickner, 1985) are translocated. Why is it necessary to remove the leader sequence? We have addressed this question by constructing an Escherichia coli strain in which the synthesis of leader peptidase is under the control of the inducible and repressible arabinose B promoter. Precursors to coat protein (inner membrane), maltose binding protein (periplasm), and OmpA (outer membrane) accumulate under conditions where the leader peptidase synthesis is repressed, confirming that leader peptidase processes these proteins in vivo as well as in vitro (Wolfe et al., 1982). The precursors that accumulate are translocated but remain anchored to the outer surface of the inner membrane. Therefore, leader peptidase is not required to catalyze the translocation of pre-proteins across the plasma membrane. Rather, the removal of the leader sequence by leader peptidase is essential for exported proteins to leave the inner membrane and arrive at their correct destination.

**EXPERIMENTAL PROCEDURES**

**Materials**—Protease K and phenylmethylsulfonyl fluoride were from Sigma.

*Bacteria, Phage, and Plasmids*—E. coli strains H560 (F" polA1 _replA_ end1_ tss_ ) and MC1061 (ΔlacX74, araD139, Δara, leu7697, galU, galK, hsr, hsm, strA) were obtained from Dr. Dan Ray (UCLA). M13 was from our collection and M13mp8 was from Pharmacia. The pLNG-1 plasmid containing the _araB_ promoter and the arabinose regulatory elements was a generous gift of Dr. Gary Wilcox (UCLA).

**Growth and Labeling Conditions**—Cells were grown at 37°C to the midlog phase in M9 minimal media (Miller, 1972) containing 0.5% glucose and 5% dimethyl sulfoxide (900 Ci/mmol, Amersham Corp.).

**Synthesis of leader peptidase** was induced by arabinose (0.2%) in the M9 media. To analyze M13 procoat, cultures were grown in M9 minimal medium. Cells were pulse-labeled with 100 μCi/10^6 cells, and then analyzed by SDS-polyacrylamide gel electrophoresis and fluorography.

**Immunoprecipitation and SDS-Polyacrylamide Gel Electrophoresis**—After labeling cells with [35S]methionine, samples were mixed with an equal volume of ice-cold 20% trichloroacetic acid and held for 15 min at 0°C. The precipitates were collected by centrifugation, twice suspended in acetone, sedimented, and finally resuspended by boiling in 10 mM Tris-HCl, pH 8.0, 2% SDS. Samples were diluted 20-fold with 10 mM Tris-HCl, 150 mM NaCl, 5 mM EDTA, 2.5% Triton X-100. Proteins were immunoprecipitated (Wolfe et al., 1982) and analyzed by SDS-polyacrylamide gel electrophoresis and fluorography.

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*The abbreviations used are: MBP, maltose binding protein; SDS, sodium dodecyl sulfate; CCEP, carbonyl cyanide m-chlorophenylhydrazone.
RESULTS

Construction of an E. coli Strain with an Inducible and a Repressible Leader Peptidase Gene (lep)—To determine the role of leader peptidase in protein export, we have constructed an E. coli strain in which leader peptidase is only made in the presence of the inducer arabinose. Two genetic modifications were necessary to construct this strain, namely inactivation of the chromosomal copy of lep (Date, 1983) and insertion of a regulated promoter in front of a newly introduced lep gene. This was accomplished by first cloning the lep gene into a vector which expresses leader peptidase under the control of the araB promoter. The gene was then truncated using a convenient restriction enzyme, yielding an enzymatically inactive leader peptidase. The plasmid pRD9, which carries this truncated lep and the gene for ampicillin resistance, was introduced into a polA strain which has a defective DNA polymerase I for replication, ampicillin selects those transformants in which the plasmid has integrated into the chromosome. Integration via homologous recombination produces a duplication of the leader peptidase sequences separated by the vector sequences (Fig. 1). This strain constitutively produces an inactive leader peptidase fragment. Synthesis of active leader peptidase is under the control of an inducible and repressible promoter. Since leader peptidase is the last gene in the operon (March and Inouye, 1985), this promoter controls no other downstream genes.

The leader peptidase expression vector was constructed by subcloning the leader peptidase structural gene (lep) from pTD125 (Date, 1983) into M13mp8 (Fig. 2). The leader peptidase gene with the correct orientation was then cleaved from M13mp8lep with the restriction enzymes SacI and SalI and transferred to the pING-1 vector, a pBR322-derived plasmid which carries the arabinose regulatory elements and the araB promoter (Johnston et al., 1985). This new plasmid is named pRD8. The correct orientation of the leader peptidase gene was confirmed using the restriction enzyme EcoRI. To inactivate the leader peptidase gene, pRD8 was cut using EcoRI and religated to form the plasmid pRD9.

To test whether the expression of the plasmid-coded leader peptidase is strictly regulated by arabinose, cells were pulse-labeled with [35S]methionine for 1 min (Fig. 3A). The addition of arabinose to cells containing the cloned leader peptidase gene (pRD8/MC1061) induced the expression of leader peptidase (lane 6) which was not seen in the absence of inducer (lane 5), in cells with no plasmid (lanes 1 and 2), or in cells with the pING-1 plasmid (lanes 3 and 4). Cells harboring the truncated leader peptidase gene (pRD9/MC1061) synthesized a protein of lower molecular weight (lane 8). Immunoprecipitation with antiserum to leader peptidase (Fig. 3B) showed that the synthesis of leader peptidase was strongly repressed in the absence of inducer.

As described for Fig. 1, pRD9 was integrated into the chromosome of the polA strain H560. Since the plasmid replication requires DNA polymerase I, ampicillin selects the rare chromosomal integrants. We obtained stable ampicillin-resistant colonies at a frequency of $5 \times 10^{-8}/\mu g$ of DNA. The integration of pRD9 at the lep locus rather than at ara was shown by P1 transduction.

To assay directly the synthesis of leader peptidase in the polA constructs, cultures of H560, pRD8/H560, and pRD9/H560 were pulse-labeled with [35S]methionine for 1 min in the presence or absence of arabinose. Extracts were immunoprecipitated with antiserum to leader peptidase and examined by SDS-polyacrylamide gel electrophoresis and fluorography. Leader peptidase was visualized at a low, constitutive level in H560 (Fig. 4, lanes 1 and 2). In pRD8/H560, the normal lep promoter also leads to constitutive synthesis (lane...

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**Fig. 1. Integration of the plasmid pRD9 at the leader peptidase gene locus (lep) of the host chromosome.** The deleted region of lep is represented as darkened areas.

**Fig. 2. Cloning strategy.** The leader peptidase gene (lep) located on pTD125 was cloned into M13mp8 to form the plasmid M13mp8lep and then transferred to pING-1, a pBR322-derived plasmid which contains the regulatory elements (araC) and the promoter of t-ribulokinase (araB). The lep gene on pRD8 was truncated using EcoRI to form the plasmid pRD9.
Arabinose-inducible expression of plasmid-coded leader peptidase. Cells were grown to the mid-log phase with or without 0.2% arabinose as indicated. Aliquots (0.2 ml) were labeled with 20 μCi of [35S]methionine for 1 min and analyzed by gel electrophoresis and fluorography as described under "Experimental Procedures." A, total proteins; B, immunoprecipitates with antisera to leader peptidase (L.P.ase). E. coli strains MC1061 (lanes 1 and 2) and MC1061 containing plasmid pING-1 (lanes 3 and 4), pRD8 (lanes 5 and 6), or pRD9 (lanes 7 and 8) were analyzed.

Leader Peptidase

Strain: H560 pRD8/H560 pRD9/H560

Arabinose - + - + - + - +

FIG. 4. Expression of leader peptidase in pRD9/H560 is tightly regulated by arabinose. Cells were grown in the presence or absence of 0.2% arabinose as indicated. Aliquots (0.5 ml) were labeled with 50 μCi of [35S]methionine and analyzed by gel electrophoresis and fluorography. E. coli strains H560 (lanes 1 and 2) and H560 with integrated pRD8 (lanes 3 and 4) or pRD9 (lanes 5 and 6) were analyzed.

Arabinose - + - + - +

FIG. 5. pRD9/H560 requires arabinose for growth. A culture of pRD9/H560 was grown in M9 medium containing arabinose and fructose, as described under "Experimental Procedures." At time 0, half of the culture was transferred to arabinose-free medium. The absorbance at 600 nm was determined at the times indicated.

E. coli Leader Peptidase

was no detectable synthesis of full-length (and thus active) enzyme (lane 5).

Leader peptidase is essential for cell growth (Fig. 5; also see Date, 1983). A culture of pRD9/H560, grown with arabinose and fructose, was divided into two equal portions. One portion was transferred to arabinose-free medium. The growth rate was virtually identical in the presence or absence of arabinose until 7 h after the change of media. As shown below, this reflects the time needed to dilute the leader peptidase originally present among the daughter cells. Dramatic differences in growth rate in the presence or absence of arabinose were also seen on minimal as well as on rich agar plates.

Precursor Proteins Accumulate When Leader Peptidase Is Limiting—Fig. 6 shows the kinetics of processing of pro-OmpA, pre-MBP, and procoat as the cells become limited for leader peptidase. Cells were pulse-labeled for 1 min at the indicated times after shift to arabinose-free media. Aliquots were then immunoprecipitated with antisera to OmpA and MBP and analyzed by SDS-polyacrylamide gel electrophoresis and fluorography. Under our sample preparation conditions, OmpA binds SDS in two stoichiometries and therefore runs as a doublet, in both heat-modified and heat-unmodified forms (Hindennach and Henning, 1975; Schnaitman, 1974). The precursors only appear in one form. A progressive delay was observed in the processing of pro-OmpA to OmpA (Fig. 6A) and pre-MBP to MBP (Fig. 6B) after the shift to media containing no arabinose. A separate culture was infected with bacteriophage M13. Aliquots were pulse-labeled with [35S]methionine for 1 min at the indicated time after the removal of arabinose. A progressive delay was observed in the processing of procoat to coat (Fig. 6C). These data show that leader peptidase is essential for the processing of three pre-proteins with different cellular destinations, consistent with studies (Wolfe et al., 1982) which showed that pure leader peptidase cleaves these precursors in vitro. The processing and transport of lipoprotein were unaffected by low levels of leader peptidase (data not shown), confirming that it is processed by a separate enzyme, lipoprotein signal peptidase (Tokunaga et al., 1982).
Fig. 6. Precursors of OmpA, MBP, and coat protein accumulate in pRD9/H560 after the shift to arabinose-free medium. Cells were grown in the presence of arabinose and then shifted to arabinose-free medium. Aliquots (0.5 ml) of the cell culture were taken at the indicated times after the arabinose shift, labeled with 50 μCi of \[^{35}S\]methionine for 1 min at 37°C, and analyzed by SDS-polyacrylamide gel electrophoresis and fluorography. A, immunoprecipitation of OmpA and its precursor (pro-OmpA). B, immunoprecipitation of MBP and its precursor (pre-MBP). A separate culture was infected with M13 at 2 h after the shift as described under "Experimental Procedures." At the indicated times, aliquots of this culture were labeled with \[^{35}S\]methionine. C, immunoprecipitation of procoat and coat.

Time: 0.1 0.5 1.5 5 10 30 (min)

Fig. 7. Post-translational processing of precursors to MBP and OmpA in leader peptidase-deficient cells. E. coli pRD9/H560 was grown in the presence of arabinose and then shifted to arabinose-free medium. After 9 h, an aliquot (900 μl) was pulse-labeled with 90 μCi of \[^{35}S\]methionine for 30 s and chased with an excess of nonradioactive methionine (500 μg/ml). Portions (100 μl) were removed at the indicated chase times and immunoprecipitated with antisera directed against either OmpA (A) or MBP (B).

The radioactive precursors pro-OmpA and pre-MBP observed after a 30-s \[^{35}S\]methionine pulse were converted to their mature forms during a subsequent chase with nonradioactive methionine (Fig. 7). The chase rate of precursor to mature is much faster in the presence of arabinose. This study confirms earlier ones (Josefsson and Randall, 1981; Zimmermann and Wickner, 1983) which showed that post-translational processing can occur.

Protease Accessibility of the Precursors—To map the subcellular location of pre-MBP and pro-OmpA in leader peptidase-depleted cells, we have permeabilized the outer membrane and digested with proteinase K. Cells were grown for 9 h in the absence of arabinose to allow precursors to accumulate, pulse-labeled for 1 min, and then treated at 0°C with Tris, sucrose, and EDTA to permeabilize the outer membrane. Aliquots were incubated at 0°C without further addition (Fig. 8, lane 1) or with proteinase K (1 mg/ml) for various times (lanes 2 and 3) or with proteinase K in the presence of detergent (lane 4). Samples were immunoprecipitated with antiserum to OmpA (Fig. 8A) or to MBP (Fig. 8B). Quantification of the immunoprecipitates showed that over 90% of the pro-OmpA was digested with proteinase K (Fig. 8A, lanes 1–3) under conditions where the bulk of cytoplasmic proteins were resistant to protease (lanes 5 and 6). A small decrease (less than 15%) in the cytoplasmic proteins is attributed to limited cell lysis. pRD9 is fragile to a variety of manipulations when leader peptidase is limiting in the cell. Proteinase K digestion also converted over 90% of the pre-MBP to a species which co-migrated with MBP (Fig. 8B, lanes 1–3). The mature domain of pre-MBP is, like MBP itself, very proteinase-resistant (Dierstein and Wickner, 1985), and only the leader peptide is readily cleaved by proteases. To confirm that the topology of these pre-proteins was being measured, cells were treated before pulse labeling with CCCP, which abolishes membrane potential and prevents translocation. As expected, only the precursor forms of MBP and OmpA were observed, which run at a higher molecular weight than the mature species (data not shown). Under these conditions, pro-OmpA was resistant to proteinase K (Fig. 8A, lanes 7–9) but was digested when the plasma membrane was disrupted by detergent (lane 10). Pre-MBP was also resistant to proteinase K (Fig. 8B, lanes 7–9). We conclude that the topology of these

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2 R. E. Dalhey and W. Wickner, unpublished data.
pre-proteins is different when their processing is blocked by uncoupler versus when it is blocked by a lack of leader peptidase. From these data, it is evident that most of the pre-MBP and pro-OmpA which accumulates in leader peptidase-deficient cell is inserted across the plasma membrane.

Similar experiments were performed to check whether M13 procoat also accumulates in a transmembrane form. Infected cells were grown in the absence of arabinose for 7 h, pulse-labeled for 1 min, and treated with Tris, EDTA, and sucrose at 0 °C to permeabilize the outer membrane. Cells were then incubated at 0 °C with no proteinase K (Fig. 8C, lane 1), with proteinase K for increasing times (lanes 2 and 3), or with proteinase K and detergent (lane 4). Over 90% of these conditions, the plasma membrane remained intact; gene 5 protein and other soluble proteins were not cleaved (lanes 5 and 6), although these levels of protease degraded the cytoplasmic proteins when the cells were disrupted by the addition of Triton X-100 (data not shown). As an additional control for the procoat topology (Date et al., 1980), M13-infected cells were treated with CCCP for 4 min prior to labeling. Under these conditions, procoat (lane 7) was inaccessible to proteinase K (lanes 8 and 9) but was digested when the plasma membrane permeability barrier was abolished by detergent (lane 10). These data show that procoat, like pre-MBP and pro-OmpA, translocates across the membrane in leader peptidase-deficient cells.

Location of Pre-MBP—To determine whether pre-MBP had been released into the periplasm, cells were pulse-labeled for 1 min with [35S]methionine and fractionated by the osmotic shock procedure of Neu and Heppel (1965). Fig. 9 shows that in the presence of ample leader peptidase (+arabinose), mature MBP is the only species detected (lane 4). When these cells were shocked to release the periplasmic proteins, the mature MBP was located exclusively in the shock fluid (lane 6) and was not found in shocked cells (lane 5). In leader peptidase-deficient cells (−arabinose), precursor to MBP was detected as well as mature MBP (lane 1). As observed for the +arabinose study, mature MBP is also released into the shock fluid when the cells are shocked (lane 3). However, the precursor to MBP was not released into the shock fluid (lane 3) but remained in the shocked cell fraction (lane 2). This shows that it is necessary to remove the hydrophobic leader sequence of pre-MBP in order to obtain effective release from the membrane.

Location of Pre-OmpA—Since the pro-OmpA that accumulates when the leader peptidase is limiting in the cell is accessible to added proteinase K (Fig. 8) and is not released into the periplasm (data not shown), we tested whether it was located in the inner or outer membrane. Cells were grown in the presence or absence of arabinose and pulse-labeled with [35S]methionine, and the membranes were isolated as described by Osborn et al. (1972) using isopycnic sucrose gradient centrifugation. Aliquots of the sucrose gradient fractions were immunoprecipitated with antisera to OmpA and leader peptidase. In the presence of arabinose, where there is ample leader peptidase, OmpA is located predominantly in the outer membrane fraction (Fig. 10B, lane 1) with some contaminating OmpA (20%) in the inner membrane fraction (lane 2). This is presumably due to hybrid membranes formed during
The leader peptidases of eukaryotes and prokaryotes have similar processing specificity. This was first demonstrated when the gene for a secreted eukaryotic protein, pre-proinsulin, was introduced into E. coli (Talmadge et al., 1980). Pre-proinsulin was synthesized and the leader sequence was correctly removed to yield proinsulin. In another study, the E. coli leader peptidase was shown to correctly process a mouse IgG x-chain fragment precursor (Watts et al., 1983). In addition, bacteriophage M13 procoat is processed by dog pancreas microsomal membranes (Watts et al., 1983). These studies show that the recognition between pre-proteins and leader peptidase is specific and is conserved during evolution. This suggests that leader peptidase plays an indispensable role in protein export.

To define the role of leader peptidase, we have constructed an E. coli strain in which we can manipulate its expression. The normal leader peptidase promoter was placed in front of an inactive leader peptidase gene while the inducible and repressible arabinose B promoter was introduced to control the expression of active leader peptidase. In the absence of the inducer, the synthesis of leader peptidase was repressed (Fig. 4) and, after a few cell doubling times, the growth rate slowed (Fig. 6). This shows that leader peptidase is essential for cell growth (Date, 1983).

Two lines of evidence suggest that translocation through the bilayer can occur without processing by leader peptidase. The studies presented here show that precursors to coat protein, MBP, and OmpA are still translocated across the plasma membrane when cell growth is limited by low levels of leader peptidase. It might be considered that the effects we see in protein export are due to a secondary rather than a primary effect of limiting leader peptidase in the cell. For example, since protein export in bacteria requires a membrane potential, depolarization of the membrane would prevent transport. However, the export intermediates we have identified are translocated across the membrane. In addition, lipoprotein, which depends on membrane potential and certain sec gene products, is transported normally in leader peptidase-deficient cells. A second line of evidence is that mutants of lipoprotein (Lin et al., 1980), 1,0-lactamase (Koshland et al., 1982), and procoat (Kuhn and Wickner, 1985) which cannot be cleaved by leader peptidase are still translocated. Taken together, these studies provide firm evidence that leader peptidase does not catalyze the translocation of pre-proteins across the membrane.

Removal of leader peptides is necessary for the release of many pre-proteins from the membrane. Both pre-MBP and pro-OmpA, although translocated, are retained in the inner membrane and are only released after cleavage has occurred (Figs. 9 and 10). It is likely that the pre-proteins remain anchored to the membrane via their hydrophobic leader sequences. In vivo studies with pre-MBP support this notion. As shown in one of the accompanying papers (Dierstein and Wickner, 1985), pre-MBP binds to detergent whereas mature MBP does not. This suggests that the leader peptide directly binds to an amphipathic surface. In addition, a non-cleavable mutant precursor of 1,0-lactamase was translocated across the membrane but not released into the periplasmic space (Koshland et al., 1982). However, this is not true for all proteins, since a non-cleavable mutant of prolipoprotein was found predominantly in the outer membrane (Lin et al., 1980). Most inner membrane proteins are not cleaved by leader peptidase during their assembly (Wolfe and Wickner, 1984), possibly because they never leave this membrane. M13 procoat is an exception in that it is an inner membrane protein which does undergo cleavage; perhaps this is necessary to allow the coat protein to leave the membrane during virion extrusion.

**E. coli Leader Peptidase**

![Diagram showing localization of pro-OmpA in pRD9/H560](image)

**Fig. 10.** Localization of pro-OmpA in pRD9/H560. Cells (10 ml) were grown in the presence or absence of arabinose for 9 h, pulse-labeled with 175 μCi of [35S]methionine for 1 min, mixed with crushed ice, and made into spheroplasts by lysozyme treatment as described by Osborn et al. (1972). The spheroplasts were disrupted by sonication, and the membrane fraction was isolated by centrifugation (145,000 x g, 0 °C, 60 min). Membranes were further fractionated by isopycnic sucrose gradient centrifugation, and the fractions were analyzed by SDS-polyacrylamide gel electrophoresis and fluorography. Immunoprecipitation with antiserum directed against leader peptidase (LPase) is shown A and with OmpA in B. OM and IM indicate the outer membrane and inner membrane fractions, respectively, which were pooled from the sucrose gradient. Samples from cells grown with or without arabinose are indicated.
Our observation that procoat, pre-MBP, and pro-OmpA are translocated across the membrane in the absence of leader peptidase suggests that cleavage is not necessary to render insertion irreversible. For procoat, this was a surprising result, since it had been suggested that insertion across the membrane is reversible. In M13am7-infected cells, procoat largely accumulates at the cytoplasmic surface of the plasma membrane (Date et al., 1980), yet the genetic overproduction of leader peptidase accelerates its conversion to coat. It was therefore proposed that procoat integration across the membrane is reversible, that the equilibrium of this reaction favors the non-translocated procoat, and that leader peptidase must compete with the reverse reaction for a limited amount of transmembrane procoat (for details, see Zimmermann et al., 1982)). However, when leader peptidase is limiting, most of the procoat that accumulates is transmembrane rather than cytoplasmic. Further studies are necessary to illuminate the kinetics of coat protein biosynthesis during M13am7 infection.

The strategy we employed to construct a strain which expresses leader peptidase in an inducible and repressible manner may have wide application to controlling other genes in either prokaryotes or eukaryotic cells. This inducible control may be a more effective tool to study an essential gene than conventional temperature-sensitive mutations. In many cases, temperature-sensitive mutants are difficult to isolate. The approach reported here requires: 1) cloning the gene into a vector with a promoter that controls its expression; 2) modification of the gene to render the protein product inactive; and 3) introducing this vector into the chromosome via homologous recombination at the gene locus. A strain constructed in this manner produces an inactive protein constitutive and an active protein under the control of an inducible and repressible promotor.

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REFERENCES

Bassford, P., and Beckwith, J. (1979) _Nature_ 277, 538-541
Date, T. (1983) _J. Bacteriol._ 154, 76-83
Miller, J. H. (1972) _Experiments in Molecular Genetics_, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY