JOURNAL OF BACTERIOLOGY, May 1988, p. 2005–2011
0021-9193/88/052005-07$02.00/0

Vol. 170, No. 5

Translational Control of Exported Proteins That Results from OmpC Porin Overexpression

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The regulation of synthesis and export of outer membrane proteins of *Escherichia coli* was examined by overexpressing *ompC* in multicopy either from its own promoter or from an inducible promoter in an expression vector. Overexpression of OmpC protein resulted in a nearly complete inhibition of synthesis of the OmpA and LamB outer membrane proteins but had no effect on synthesis of the periplasmic maltose-binding protein. Immunoprecipitation of labeled proteins showed no evidence of accumulation of uncleaved precursor forms of OmpA or maltose-binding protein following induction of OmpC overexpression. The inhibition of OmpA and LamB was tightly coupled to OmpC overexpression and occurred very rapidly, reaching a high level within 2 min after induction. OmpC overexpression did not cause a significant decrease in expression of a LamB-LacZ hybrid protein produced from a *lamB-lacZ* fusion in which the fusion joint was at the second amino acid of the LamB signal sequence. There was no significant decrease in rate of synthesis of *ompA* mRNA as measured by filter hybridization of pulse-labeled RNA. These results indicate that the inhibition is at the level of translation. We propose that cells are able to monitor expression of exported proteins by sensing occupancy of some limiting component in the export machinery and use this to regulate translation of these proteins.

Recent study of protein export by *Escherichia coli* has been directed primarily to elucidating the components of the export pathway (23) and not to how these components might be involved in regulation of gene expression. Gene regulation coupled to the export pathway may be very important to the cell. Both the outer membrane and the periplasmic compartment of the cell have a physical dimension which is fixed by the growth rate, and both have a finite capacity for protein. Although there are regulatory interactions between some genes for exported proteins, e.g., between *ompF* and *ompC* (19, 24), for the most part there is no apparent global regulation of transcription of genes encoding exported proteins. Many of the genes encoding exported proteins are independently regulated and efficiently transcribed and translated, and in some cases they produce stable mRNA (10). For these reasons, under conditions of maximum expression the products of these genes are among the most abundant proteins in the cell. Examples include the porins, OmpA protein, LamB protein, and the major lipoprotein in the outer membrane and the ribose- and maltose-binding proteins in the periplasm. Since none of these proteins are enzymes whose products can be sensed directly, it is unlikely that the cell can sense the levels of these proteins once they have been exported to their respective compartments. Thus, regulation coupled to export could provide a means for preventing deleterious overproduction of exported proteins and for equitable sharing of limiting components of the export machinery. A system such as the eucaryotic SRP (signal receptor particle) cycle which appears to mediate translation arrest at an early stage in export of proteins into the endoplasmic reticulum could provide such regulation. However, an analogous system for translational control at an early stage of export has not been demonstrated in *E. coli* (26), and the fact that export can be posttranslational (23) argues against such a system.

In this report we describe evidence for translational control of the synthesis of major outer membrane proteins which may be coupled to export and which is very similar to a regulatory system which was recently described for periplasmic proteins (14, 15).

MATERIALS AND METHODS

Plasmids and bacterial strains. Plasmid pGMC1176 consists of pAT153 carrying a 2.2-kilobase (kb) *ompC* fragment from the *E. coli* chromosome. This *ompC* fragment begins just upstream of the *Sal*I site 5′ to *ompC* and extends through the gene to the chromosomal *Hin*dIII site 3′ to the gene. This fragment includes an intact *ompC* promoter and the *micF* gene (19). Plasmid pEMC1 has an insert consisting of a 1.3-kb *Hin*dIII fragment beginning at an *Hin*dIII linker insertion 85 base pairs 5′ to the *ompC* initiation codon and extending to the chromosomal *Hin*dIII site 3′ to the gene. This insert is cloned into the multiple cloning site downstream from the *tac* promoter of the expression vector pKK223-3 (Pharmacia, Inc., Piscataway, N.J.). The *ompC* insert of pEMC1 includes the entire OmpC protein-coding region but lacks the *ompC* promoter and the first 2 bases encoding *ompC* mRNA. Plasmid pGMC65 has an insert which was used as a probe for *ompC* mRNA and consists of a 1.7-kb *Hin*dIII fragment beginning at a *Hin*dIII linker insertion 524 base pairs 5′ to the *ompC* initiation codon and extending to the chromosomal *Hin*dIII site 3′ to the gene. This insert is cloned into the *Hin*dIII site of pAT153. The construction of these plasmids has been described previously (7). Plasmid pA102 has an insert which was used as a probe for *ompA* mRNA and consists of a 1.2-kb fragment which contains nearly all of the *ompA* gene. This insert extends from an *Eco*RV site within the 5′ untranslated leader region to a *Pst*I site approximately 55 bases beyond the 3′ end of the *ompA* transcript. The plasmid was constructed by

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digestion of pTU201 (6) with *Pst*I to generate a 4.4-kb fragment containing *sulA* and *ompA*. This 4.4-kb fragment was inserted into the *Pst*I site of the multiple cloning site of pUC18 to create plasmid pA101. *Eco*RV digestion of pA101 separated the upstream *sulA* gene from *ompA* and simultaneous cleavage at the *Hin*dIII site adjacent to *Pst*I in the polylinker of the vector released the 1.2-kb *ompA* fragment, which was inserted into pBR322 to create pA102.

The *E. coli* K-12 strain used as background for the expression vector was CS1389, which is an OmpR1 (*ompR151*) Δ*ompC178* derivative of JM101 [Δ(*lac-pro*) *thi supE* F′ *traD36 proAB lacI*^{q}*ZΔM15*] (17). The pEMC1 transformant of CS1389 is CS1499. Other strains include the wild-type strain CS109 (W1485F$^-$) and CS1255, which is a Δ*ompC178* derivative of CS109 (24).

Culture conditions. Cultures for methionine labeling or protein isolation were grown in minimal medium A (18) with supplements as required and 0.2% glycerol as the carbon source or, as noted, in LB medium (18). In some experiments 0.2% maltose was added one generation prior to labeling or harvesting to induce the *mal* operon. Cultures for RNA isolation were grown in modified MOPS medium (5), which contained 13.2 mM phosphate (21) with 1% acid-hydrolyzed casein as the carbon source (MOPS-AHC). All cultures were grown and labeled at 37°C.

For labeling of protein, cultures grown to mid-log phase were induced for various periods of time with 0.5 mM isopropyl-β-D-thiogalactoside (IPTG). Samples of these cultures (2.2 ml) were labeled with 0.2 to 0.4 mCi of [^{35}S]methionine for 30 s, followed by a 15-s or 2-min chase with 0.4% unlabeled methionine. Protein synthesis was stopped by the addition of chloramphenicol (30 μg/ml) and chilling on ice.

Cell fractionation and protein analysis. Cells were harvested and broken by passage through a French press, and the outer membrane fraction was isolated by differential centrifugation (24). In some experiments unlabeled carrier cells were added to facilitate outer membrane isolation. Labeled cultures which were to be used for immunoprecipitation or for analysis of total labeled protein were not harvested; instead, the whole cultures were added to cold trichloroacetic acid (TCA) solution (final concentration, 5%), and the precipitated protein was collected by centrifugation. For total labeled protein, the pellet was washed twice with acetone to remove TCA and dissolved in gel sample buffer. For immunoprecipitation, the precipitated protein was dissolved in sodium dodecyl sulfate (SDS)-Triton buffer and reacted with antisera exactly as described by Ito et al. (16) except that reactions with antisera and IgSorb were each done for 1 h at 22°C. Antisera against OmpA protein and maltose-binding protein (MBP) were kindly provided by P. Ray. Samples for SDS-polyacrylamide gel electrophoresis were prepared by boiling in SDS sample buffer, and proteins were separated as described previously (24). Unlabeled protein samples were loaded so that each well had equal protein, and radiolabeled protein was loaded so that each well had the same number of counts. For quantitative autoradiography data, the film (Kodak XAR-5) was sensitized by exposure to a brief flash of light prior to exposure to the gel. Relative band intensities were obtained by scanning of the film and capture of the image and digitization with a Dade video camera VC68, followed by computerized analysis. To directly quantitate radiolabeled protein, bands were cut from dried gels, hydrated in water, equilibrated with 1 ml of NCS solublizer (New England Nuclear Corp., Boston, Mass.), and counted in a liquid scintillation counter after addition of 10 ml of Ready-Solv NA (Beckman Instrument Co., Fullerton, Calif.).

Radiolabeling and isolation of RNA. The procedure used for labeling and isolating RNA was essentially that of Stewart and Yanofsky (25). Cultures (10 ml) growing in MOPS-AHC were pulsed with 0.2 to 0.4 mCi of [^{3}H]uridine for 30 s and killed by mixing with an equal volume of a crushed frozen mixture of 5 mM $MgCl_2$–20 mM Tris, pH 7.3, containing 20 mM sodium azide, chloramphenicol (0.4 mg/ml), and 20% sucrose. Cells were pelleted by centrifugation, suspended in 3 ml of the same solution without sucrose, and lysed by repeated freeze-thawing in the presence of lysozyme (0.2 mg/ml; Sigma Chemical Co., St. Louis, Mo.) and DNase (7 μg/ml; RNase-free; Bethesda Research Laboratories, Bethesda, Md.). RNA was isolated by phenol extraction and ethanol precipitation and dissolved in 0.5 M KCl–10 mM Tris, pH 7.3, before and after a second ethanol precipitation. The specific activity of the labeled RNA was determined by precipitation of a sample with cold 5% TCA, collection of the precipitate on a glass filter, and liquid scintillation counting.

RNA-DNA hybridization. CsCl-purified plasmid DNA was digested with appropriate restriction enzymes, and the cloned inserts to be used as probes were isolated by preparative agarose gel electrophoresis. The bands were excised, and the DNA was recovered from the agarose by sequential extraction with Gene Clean (Bio 101 Inc., La Jolla, Calif.) as directed by the manufacturer. DNA was dissolved in 10 mM Tris buffer, pH 8.0, containing 1 mM EDTA, denatured with NaOH, and applied to GeneScreen Plus filter material (New England Nuclear) as directed by the manufacturer with a dot blot manifold. The filters containing immobilized single-stranded DNA were placed in separate vials and treated according to the GeneScreen Plus instructions with 3 ml of hybridization solution (1% SDS, 10% dextran sulfate [Pharmacia], 50% formamide [Fluka Chemical Corp., Ronkonkama, N.Y.], and 1 M NaCl) for 15 min or more at 55°C. Carrier DNA (calf thymus DNA; Sigma) was dissolved in water at 10 mg/ml, sheared by forcing through a 23-gauge needle, clarified by centrifugation, and denatured by boiling for 10 min. The carrier DNA was centrifuged again and kept in a boiling bath until use. The [^{3}H]RNA was denatured by boiling for 3 min. The carrier DNA and [^{3}H]RNA (80 to 100 μl of each) were rapidly added to each vial and allowed to hybridize overnight at 55°C. The filters were removed and washed as follows: twice in 2× SSC (0.3 M NaCl and 0.03 M sodium citrate), once for 1 h in 2× SSC containing RNase A (20 μg/ml) which had been boiled for 10 min to inactivate DNase, once for 15 min at 50°C in 2× SSC containing 1% SDS, twice in 2× SSC, and once in 0.1× SSC, followed by air drying. Filters were incubated for 5 min with 0.05 ml of water and 0.5 ml of NCS solublizer and then counted in a liquid scintillation counter after addition of 10 ml of Ready-Solv NA.

RESULTS

Overexpression of OmpC protein from a multicopy plasmid inhibits expression of other outer membrane proteins. The initial observation which prompted this study was that cells transformed with a multicopy plasmid carrying the *ompC* gene expressed a greatly elevated amount of OmpC protein in the outer membrane (Fig. 1, lane 5). This overexpression resulted in an almost complete inhibition of expression of OmpA protein. Additional experiments (not shown) indicated that other proteins which were similarly inhibited

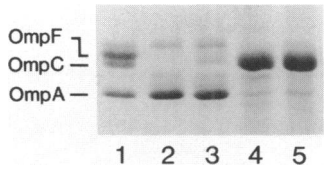

FIG. 1. Effect of prolonged overexpression of *ompC*. The central portion of a Coomassie blue-stained gel of the outer membrane fractions is shown. Lines at the side indicate OmpF, OmpC, and OmpA proteins. Lane 1, CS109 (wild type); lane 2, CS1389 (OmpC$^-$ OmpF$^-$); lane 3, CS1499 (CS1389 transformed with the *ompC* expression vector plasmid pEMC1), no induction; lane 4, CS1499 induced for 10 h with IPTG; lane 5, CS1255 (OmpC$^-$) transformed with the multicopy *ompC*$^+$ plasmid pGMC1176. Cultures were grown in minimal glycerol medium without maltose.

included LamB protein and the Lc porin encoded by lambdoid phage. OmpF porin was completely inhibited to the point where the cells became resistant to the OmpF-specific phage K20. The inhibition of OmpF protein was probably due in part to the presence of the *micF* RNA gene on the plasmid, which has been shown to inhibit OmpF expression when present on a multicopy plasmid (19).

The high level of OmpC expression had no apparent deleterious effect on the cells, since the growth rate and the appearance of the cells by light microscopy were normal. However, the level of expression of OmpC protein under these conditions was near the maximum that the cells could tolerate. When transcription of *ompC* was increased by adding NaCl to a final concentration of 2% to LB cultures of strains carrying the multicopy *ompC* plasmid, there was an immediate and almost complete inhibition of growth, as indicated by culture turbidity (data not shown). When incubation was continued after addition of the NaCl, cells showed evidence of filament formation and lysis. In control experiments, addition of NaCl to a strain transformed with the plasmid vector without the *ompC* insert or to a wild-type Omp$^+$ strain had no effect on growth or cell morphology.

To determine whether the inhibition of expression of OmpA protein seen in cells carrying the multicopy *ompC* plasmid was due to inhibition of transcription from the *ompA* promoter, a multicopy *ompC* plasmid (pGMC1137, which is pAT153 carrying the 2.6-kb chromosomal *ompC* *Hin*dIII fragment) and a control plasmid (pAT153 with no insert) were transformed into a strain carrying a chromosomal *lac* deletion which was lysogenic for an *ompA-lacZ* operon fusion phage (3). The strain carrying pGMC1137 produced 7.0 U of β-galactosidase per mg of protein, assayed as described by Miller (18), while the strain carrying pAT153 with no insert produced 8.0 U/mg of protein and the background strain produced 11.3 U/mg of protein. These results indicate that the inhibition of OmpA expression was not at the level of transcription.

Inducible overexpression of *ompC*. To provide a system in which overexpression of OmpC protein could be experimentally manipulated, a multicopy plasmid in which expression of *ompC* was driven from an inducible hybrid *trp-lac* (*tac*) promoter was constructed. This plasmid, designated pEMC1, consists of a wild-type *ompC* gene lacking its own promoter inserted into the polylinker site of the *tac* expression vector pKK223-3 (7). Since the *ompC* insert encodes most of the 5′ untranslated leader, the mRNA from this plasmid is essentially identical to wild-type *ompC* mRNA.

Expression of OmpC protein from plasmid pEMC1 was examined in the background of strain CS1389. This strain carries an *ompR1* mutation to prevent transcription of the chromosomal porin genes (12) and is deleted for the chromosomal copy of *ompC*. It is derived from JM101, which carries a *lacI*^{q} mutation to reduce uninduced expression from the *tac* promoter.

Very little OmpC protein was expressed from pEMC1 in the absence of inducer (Fig. 1, lane 3). Prolonged growth in the presence of IPTG (Fig. 1, lane 4) resulted in accumulation of OmpC protein in the outer membrane in an amount comparable to that seen with the strain carrying multicopy *ompC* expressed continuously from its own promoter (Fig. 1, lane 5).

The effect of expression of *ompC* from pEMC1 on growth is shown in Fig. 2. The cultures in this experiment were grown in minimal medium containing glycerol and maltose, in which the doubling time for wild-type cells is about 2.5 h. The IPTG-induced culture carrying pEMC1 grew at a rate comparable to that of the control cultures for more than one generation but then abruptly ceased growing. This indicated that *ompC* overexpression from the *tac* promoter was deleterious, just as had been observed with osmotic induction of overexpression from the *ompC* promoter. The only difference between the two systems was a lag of more than a generation following IPTG induction of the *tac* promoter before inhibition of growth was observed, indicating that it is the sustained high level of OmpC protein production rather than the initiation of overexpression which is deleterious.

The relative proportions of newly synthesized proteins being added to the outer membrane were examined by briefly pulse-labeling cells with [^{35}S]methionine after various times of induction with IPTG. Analysis of the outer membrane proteins by gel electrophoresis and autoradiography (Fig. 3B) showed a rapid increase in OmpC protein. OmpC synthesis reached a high level within 2 min and was maximal after 10 min. There was a simultaneous decrease in the appearance of newly synthesized OmpA and LamB. These results were quantitated by excising the bands of interest and measuring the radioactivity in a scintillation counter. There was a similar decrease in both LamB and OmpA (Fig. 4) concomitant with the increase in OmpC.

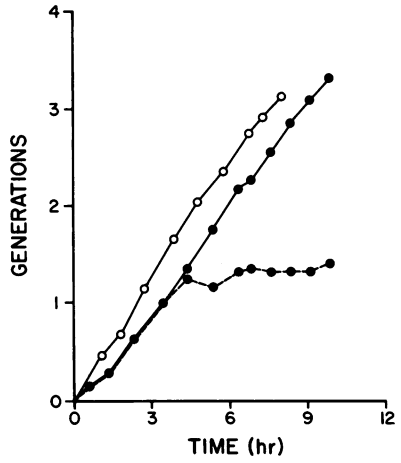

FIG. 2. Effect of induction of the *ompC* expression plasmid pEMC1 on growth. Generations indicate a doubling of turbidity. Cells were grown on glycerol minimal medium, and maltose was added one generation before time zero. Symbols: ○, CS1389 (no plasmid); ●, CS1499 (pEMC1); ---, culture induced with IPTG at time zero. The culture conditions in this experiment were identical to those in the experiments shown in subsequent figures.

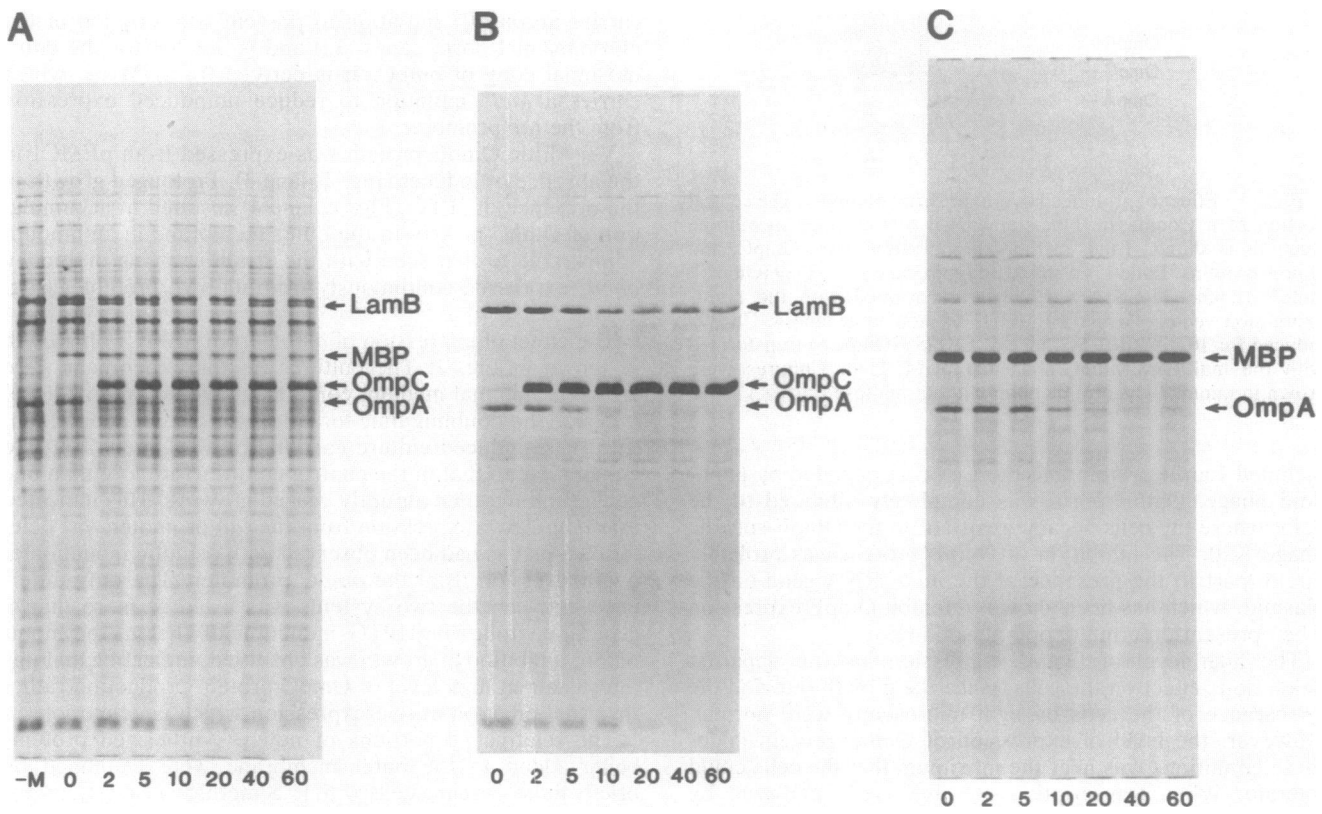

FIG. 3. Relative synthesis of major outer membrane proteins is rapidly altered after induction of OmpC overexpression. Cultures were pulse-labeled with [^{35}S]methionine, followed by a 15-s chase after various times of IPTG induction, divided into three portions, and analyzed by SDS gel electrophoresis and autoradiography. (A) Total labeled protein; (B) outer membrane fraction; (C) double immunoprecipitate by anti-OmpA and anti-MBP from total labeled protein. The numbers at the bottom of the lanes indicate IPTG induction time (in minutes); lane −M, culture not induced with maltose or IPTG.

To determine whether the decrease in newly synthesized OmpA and LamB in the outer membrane was due to inhibition of synthesis rather than a block in export of the proteins, the relative rates of synthesis of total labeled proteins were examined (Fig. 3A). Following induction of OmpC expression, there was a decrease in the total amount of LamB and OmpA proteins synthesized. The LamB protein migrated close to another band and is difficult to see in Fig. 3A, but could be clearly seen in the original radioautograph. Densitometric measurement of the decrease in LamB in a similar experiment is shown in Fig. 5. These results indicated that the decrease in the amount of LamB and OmpA in the outer membrane was not due to an export block or to loss of these proteins into the culture medium. There was a decrease in synthesis of a small polypeptide seen at the bottom of the gels of the total labeled protein and the outer membrane fraction. We assume this to be the murein lipoprotein. The relative synthesis of most of the other proteins of the cell was unaffected, indicating that on a short time scale the primary effect of OmpC overexpression is on exported proteins.

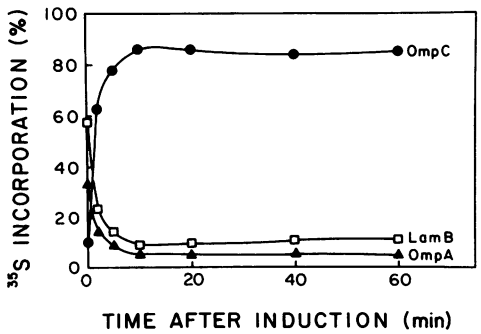

FIG. 4. Quantitation of relative synthesis of the major outer membrane proteins following OmpC overexpression. Protein bands were excised from the dried gel shown in Fig. 3 and counted as described in the text. The data are plotted as the ratio of the counts in a given band to the sum of the counts in all three bands for each time point.

The total labeled protein results also show that the synthesis of MBP, a protein exported to the periplasm, was not affected by OmpC overexpression. This observation was confirmed by coprecipitating MBP and OmpA from whole-cell lysates with specific antibodies and examining the precipitated proteins (Fig. 3C). The data clearly show a decrease in synthesis of OmpA relative to MBP. Thus, it appears that the effect of OmpC overexpression is restricted to a subset of exported proteins, namely outer membrane proteins.

It should be noted that in Fig. 3C there is no evidence for accumulation of a slower-migrating precursor form or faster-migrating degradation products of OmpA or MBP following induction of OmpC overexpression. In a control experiment (not shown) cells were treated with phenethyl alcohol (11), and in this case both the precursor and mature form of OmpA protein were detected in immunoprecipitates. These

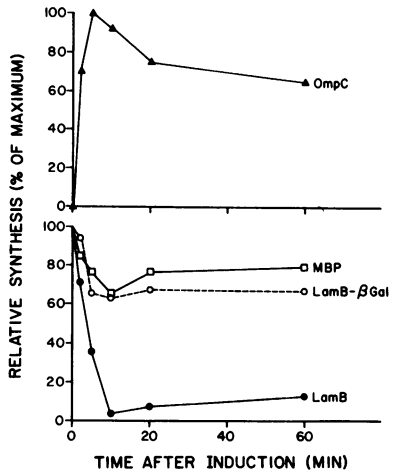

FIG. 5. Relative synthesis of OmpC, LamB, MBP, and LamB-LacZ fusion protein (LamB-βGal). Strain CS1389 was made lysogenic for the *int*⁻ λ *lamB-lacZ* fusion phage 61-4 (20) and then transformed with pEMC1. The culture was pulse-labeled, followed by a 2-min chase at various times after IPTG induction, and whole-cell lysates were analyzed by gel electrophoresis and radioautography exactly as described in the legend to Fig. 3A. Relative intensities of the bands were determined by scanning the radioautograph. A portion of the culture which had not been induced with either IPTG or maltose was analyzed in parallel. The corresponding band intensities (basal-level synthesis plus nonspecific background) were subtracted to yield net intensities of the bands of the experimental cultures. The results are plotted as percentages of maximum net intensities.

results are in contrast to the precursor accumulation which has been seen when export is blocked by *secA* mutation (22), by mutations in signal sequences (1, 9), or by expression of deleterious fusion proteins (2). If the effect of overexpression of OmpC is to block the export of other outer membrane proteins, then the lack of precursor accumulation implies that there is a concomitant decrease in synthesis of the precursor forms of these proteins.

Effect of induced OmpC overexpression on transcription of *lamB* and *ompA*. To determine the level at which decreased synthesis is regulated, the effect of induction of OmpC overexpression on transcription of both *lamB* and *ompA* was examined. For *lamB*, this was done by measuring expression of the *lamB-lacZ* gene fusion 61-4 (20). This fusion encodes a LamB-LacZ hybrid protein consisting of the first two residues of the LamB signal sequence fused to the amino-terminal region of β-galactosidase. Since the gene fusion includes all of the sequences necessary for regulation of *lamB* transcription and initiation of translation but an insignificant amount of the protein-coding region, expression of protein from this gene fusion should provide a measure of *lamB* transcription analogous to that provided by an operon fusion.

Expression of the fusion protein was measured by following the incorporation of [^{35}S]methionine during induction of OmpC overexpression. The incorporation was measured by scanning densitometry of radioautographs of gels of whole-cell extracts, which was facilitated by the fact that the fusion protein, OmpC, LamB, and MBP are all well-resolved bands. The results of this analysis are shown in Fig. 5. Synthesis of the fusion protein showed only a small decrease, similar to that seen for MBP, and was clearly different from that of LamB. This result suggests that the decreased synthesis of LamB is not due to a decrease in transcription or to a decrease in the initiation of translation.

The effect of OmpC overproduction on *ompA* transcription was addressed more directly by determining the rate of synthesis of *ompA* mRNA. This was done by briefly pulse-labeling the cells with [^{3}H]uridine, extracting total RNA, and measuring the counts hybridized to single-stranded *ompC* or *ompA* DNA probes immobilized on filters (25). The results obtained from two representative [^{3}H]RNA preparations are shown in Table 1. We consistently observed a small decrease in *ompA* mRNA at later time points, but this decrease was never greater than 50% and was not substantial enough to account for the decrease in OmpA protein synthesis. We conclude that the decrease in OmpA protein is not primarily a result of a decrease in *ompA* transcription.

DISCUSSION

Most studies on protein export by *E. coli* have involved the use of mutations which affect either the secretion machinery or the structure of the protein which is being exported. We have taken a somewhat different approach, involving overexpression of a wild-type outer membrane protein to jam the export machinery. The consequences of this overexpression were examined in cells in which the secretion machinery was also wild type. Thus the experimental system resembles a normal physiological situation which occurs when multiple genes encoding major outer membrane proteins are expressed at a high level.

An example of such a physiological situation, the induction of high-level LamB expression in cells which are approaching bulk saturation with respect to other outer mem-

TABLE 1. Hybridization of [^{3}H]mRNA to filter-bound DNA[a]

Strain	IPTG induction (min)	Hybridization[b] (cpm)				Relative hybridization[c]			
		ompA		*ompC*		*ompA*		*ompC*	
		1	2	1	2	1	2	1	2
CS1499 Δ*ompC*(pEMC1)	0	44.8	52.4	294	583	1.00	1.00	1.00	1.00
	2	88.5	70.1	11,800	17,900	1.57	1.22	32.2	28.2
	5	44.3	40.9	10,200	12,500	0.94	0.73	33.1	20.0
	10	37.9	44.8	8,290	13,800	0.81	0.80	27.4	22.2
CS1389 Δ*ompC*	0	31.2	27.7	1.9	28.1	0.64	0.48	0.01	0.04
BRE69 Δ*ompA*	0	3.7	−0.2	211	359	0.06	0	0.89	0.37

[a] In two separate experiments (columns labeled 1 and 2), pulse-labeled [^{3}H]RNA was isolated and portions (1×10^6 to 5×10^6 cpm) were hybridized to filters on which 0.8 pmol of *ompA* DNA or 3.0 pmol of *ompC* DNA had been immobilized.

[b] Washed filters were counted for 10 min, and background (36.8 cpm) was subtracted. Average of duplicate hybridizations for each of two experiments.

[c] Specific hybridization (cpm hybridized/cpm added) relative to specific hybridization of CS1499 at 0 min of induction.

brane proteins, has been described by Diedrich and Fralick (8). They found that the primary effect of high-level induction by maltose was a decrease in transcription of *ompC*. Since several exported proteins are induced by maltose, it is not clear that this was entirely a consequence of LamB expression.

Our results demonstrate a different form of coupling between the expression of different major outer membrane proteins, so that when one protein is highly overexpressed, there is a rapid and nearly complete inhibition of synthesis of the other proteins.

This inhibition has several unique characteristics compared with most other export defects which have been studied. First, it occurs very rapidly. Even with brief pulse and chase times we observed no significant lag between the onset of OmpC overexpression and the inhibition of OmpA and LamB. Second, there was no detectable accumulation of precursors or degradation products of either OmpA or MBP, as has been observed in most studies in which export has been inhibited. Finally, the inhibition appears to be specific for outer membrane proteins, since there was no inhibition of MBP or of most other proteins seen in the total cell protein.

There are similarities between the effect of overexpression of OmpC and the phenotype of a *trans*-dominant *ompC* mutation [*ompC*(td)] affecting the C-terminal region of OmpC protein (7). These include specificity limiting the effect to major outer membrane proteins, lack of accumulation of precursors, and the observation in both systems that inhibition is at the level of translation rather than transcription. The effects are also similar to observations made on the regulation of expression of periplasmic proteins. Hengge-Aronis and Boos (14, 15) have shown that expression of a C-terminally truncated form of the periplasmic phosphodiesterase encoded by *glpQ* resulted in inhibition of synthesis of several periplasmic binding proteins. In the case of MBP, this inhibition was shown to be at the level of translation. This inhibition exhibited a specificity which is the reverse of what we observed, in that it affected periplasmic proteins but did not affect LamB, OmpA, or the porin proteins. More recently, these workers extended the generality of these results by demonstrating that overexpression of MBP results in translational inhibition of other periplasmic proteins (R. Hengge-Aronis and W. Boos, submitted for publication).

Taken together, these observations indicate that *E. coli* has systems for translational regulation of exported proteins which are coupled to the export process. A constraint on any model proposed to describe these regulatory systems is the growing body of information (reviewed in reference 23) which indicates that, in *E. coli*, insertion of precursors into the cytoplasmic membrane is not cotranslational and can occur late in translation or even after completion of the protein.

The export process in *E. coli*, as distinct from translation, can be divided into early and late stages. The early stage can be operationally defined as the stage at which a block leads to accumulation of a cytoplasmic precursor. Examples include blocks such as jamming of export by LacZ fusion proteins (2), by mutations affecting the hydrophobic region of signal sequences (1, 9), and by *secA* mutations (22). Blocks at the early stage are nonspecific, affecting periplasmic and outer membrane proteins alike.

In the systems cited above, the late stage of export can be operationally defined as the stage at which the pathways of export of outer membrane proteins and periplasmic proteins diverge. Other characteristics include the lack of accumulation of cytoplasmic precursors following a block in the export pathway and feedback control mediated at the level of translation.

Like other feedback control systems, these translational regulatory systems for exported proteins must be divided into two temporally distinct events. The first event occurs at a late stage of export and involves sensing the functioning of the export pathway. Since the final products are translocated away from the cytoplasmic membrane, this must occur when intermediates in the pathway sense the occupancy of the export machinery. One mechanism for this might be competition for a potentially limiting component of the export machinery. The rapid response time of the regulatory system suggests that such a component must be present in a small amount relative to the amount of protein being exported. The second event in the regulatory system involves transmission of a regulatory signal to the polysome to effect elongation of nascent chains destined to share the export pathway.

The temporal separation of these two events is clearly shown by two different phenotypic dominance experiments. First, it was found that a mutation introducing a defective signal sequence into the OmpC(td) protein was dominant over the translational inhibition caused by OmpC(td) (7). This indicated that the OmpC(td) protein caused a block which was later in the export pathway than the block caused by its defective signal sequence. The OmpC(td) block is an example of the first event in the feedback control system in which the nonfunctional state of the export pathway is sensed.

Second, it was shown that the translational inhibition of MBP caused by expression of the C-terminally truncated GlpQ protein was dominant over the accumulation of MBP precursor resulting from activation of a temperature-sensitive *secA* mutation (15). Thus, although the GlpQ block is subsequent to the block imposed by the *secA* defect, the translational inhibition acted at a step earlier than the *secA* block. The action of translation inhibition at an early step in the export pathway is the second event in the feedback control system.

The temporal separation of these events implies the existence of a factor(s) involved in transmitting the signal from the export machinery to the polysome. The specificity of the translational control system for either periplasmic or outer membrane proteins indicates that different factors sense the two pathways. These in turn must differentially recognize polysomes synthesizing the two classes of proteins. The observation that mutation of a specific arginine residue near the amino-terminal end of the LamB signal sequence results in an export-dependent decrease in translation (4, 13) suggests that the signal sequence might be part of the target on the polysome. However, the signal sequence appears to lack the information necessary to confer outer membrane or periplasmic specificity. Another component of the polysome, either the mRNA or the rest of the nascent polypeptide, must contain the additional information required to recognize the specific regulatory factors.

ACKNOWLEDGMENTS

We thank P. Ray for providing specific antisera, S. Benson and E. Bremer for strains, and W. Boos for providing data prior to publication.

This research was supported by grant DMB-8605335 from the National Science Foundation.

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