JOURNAL OF BACTERIOLOGY, Nov. 1984, p. 696–701
0021-9193/84/110696-06$02.00/0

Vol. 160, No. 2

Mutation That Suppresses the Protein Export Defect of the *secY* Mutation and Causes Cold-Sensitive Growth of *Escherichia coli*

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Received 4 June 1984/Accepted 13 August 1984

A cold-sensitive mutant was isolated among temperature-resistant revertants of the *secY24* mutant defective in secretion of envelope proteins across the cytoplasmic membrane at 42°C. A single mutation, designated *ssyA3*, is responsible both for the extragenic suppression of *secY* and for the cold-sensitive growth. In contrast to the parental *secY24* mutant, the suppressed cells do not accumulate precursors of envelope proteins at any temperatures. The cells containing the *ssyA3* mutation, whether in combination with *secY24* or not, show an optimal growth at 42°C and a very poor growth at 30°C. At the low temperature, protein synthesis is generally slowed down, probably at the step of chain elongation. The gene *ssyA* was mapped at a new locus between *hisS* and *glyA* on the chromosome. It is possible that the product of this gene interacts both with the protein secretion system and the protein synthesizing system.

Proteins whose destination is the noncytoplasmic compartments must cross the hydrophobic membrane barrier during or after their synthesis by the translation machinery located in the cytoplasm. Genetic approaches in the procaryotic system discovered several genes involved in protein secretion (17, 23). A gene, called *prlA*, *secY*, or Y-reading frame, has been identified at the promoter distal part of the *spc* ribosomal protein operon (2, 11, 21, 22). The gene was initially discovered as a suppressor mutation (*prlA*) which restores the export of the envelope proteins with defective signal sequence (6, 22). Subsequently, we isolated conditionally lethal mutants in which the function of the Y-reading frame had been impaired, and proposed the nomenclature *secY* for this gene (11, 21). Under the restrictive high temperature, these mutants have a defect in secretion of the periplasmic and outer membrane proteins, accumulating unprocessed precursor molecules within the cell. In one of the mutants [*rplO215*(Am)], the expression of the *secY* gene is impaired because of an amber mutation in the preceding gene of the operon (11; K. Ito, D. Cerretti, H. Nashimoto, and M. Nomura, EMBO J., in press). Another mutant [*secY24*(Ts)] contains a missense mutation (*secY24*) with a single base change in *secY* (21), producing an altered *secY* protein (K. Ito, Mol. Gen. Genet., in press).

Extragenic suppressors provide a useful means for analyzing the function of a gene and for identifying other genes that interact with it (12, 27). This approach has already been applied to the genetic analysis of protein secretion (1, 6, 6a). In the present study, we identified a new gene, tentatively designated *ssyA*, as an extragenic suppressor of the *secY24* mutation. The phenotypes of the suppressor mutation suggest that the gene *ssyA* might be involved in general protein synthesis as well as in protein export.

MATERIALS AND METHODS

Bacterial strains. *Escherichia coli* K-12 strains used are listed in Table 1. Generalized transduction was done by using phage P1 *vir* by the method of Miller (18). Isolation of IQ280 is described below. IQ99 was isolated from Tn*5* insertions obtained in strain AT2465 by a procedure described previously (14). IQ328 was one of the *guaA*$^+$ transductants of AT2465, using IQ280 as the donor. IQ337 was one of the *glyA*$^+$ transductants of AT2681, using IQ280 as the donor. IQ345 was one of the tetracycline-resistant transductants of IQ337, using BW280 as the donor. IQ346 was one of the kanamycin-resistant transductants of IQ328, using IQ99 as the donor. IQ349 was one of the tetracycline-resistant transductants of AT2465, using IQ345 as the donor. IQ350 and IQ351 were tetracycline-resistant transductants of MC4100, using IQ345 as the donor.

Media and growth of bacteria. LB medium (5), peptone medium (11), and minimal medium E (25) were used. The minimal medium was supplemented with 2 μg of thiamine per ml, either 0.5% glucose or 0.5% glycerol, and 20 μg each of L-amino acids as indicated in each experiment. Solid media contained 1.5% agar. Tetracycline or kanamycin was added to LB agar at a concentration of 25 μg/ml as required. Cells were grown in a shaking water bath, and the growth was monitored by a Klett colorimeter (no. 54 filter).

Isolation of the suppressor mutant. An overnight culture of IQ85 (*secY24*) in peptone medium was diluted 10-fold with peptone medium, and 0.1-ml portions were plated on minimal agar plates and incubated at 42°C for 1 to 2 days. Colonies that appeared were replica-plated onto LB agar and incubated at 42°C for an additional 3 days. This replica step was necessary because not all of the colonies on the minimal plates could form normal colonies on LB plates at 42°C; many of them still formed the abnormal ghost-like colonies characteristic of the *secY24* mutant. Normal-looking colonies were picked, purified, and verified for their ability to grow at 42°C.

Tests for accumulation of precursor proteins by pulse labeling. Testing for accumulation of precursor protein was done essentially as described previously (10, 11). Cells grown on glucose-minimal medium supplemented with amino acid mixture (17 amino acids other than leucine, isoleucine, and valine) were pulse-labeled with 30 μCi of [^{3}H]leucine per ml (71 Ci/mmol; Amersham) for 1 min, and subjected to antibody precipitation with antibody against OmpF protein of the outer membrane (provided by Y. Anraku), followed by separation in 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (10) and fluorography (3).

Measurement of the rate of polypeptide chain growth by induction kinetics of β-galactosidase. Cells of IQ328 (*ssyA3*) or AT2465 (*ssyA*$^+$) were grown in glycerol minimal medium

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TABLE 1. *E. coli* strains used

Strain	Genotype	Source or reference
IQ85	*secY24* Tn*10 thiA* Δ*lac araD rpsL rpsE relA*	(21)
IQ86	Tn*10 thiA* Δ*lac araD rpsL rpsE relA*	(21)
IQ99	*zfe*::Tn*5 thi relA spoT*	This study
IQ280	*secY24* Tn*10 ssyA3 thiA* Δ*lac araD rpsL rpsE relA*	This study
IQ328	*ssyA3 thi relA spoT*	This study
IQ337	*ssyA3 argH purF hisG thi rpsL*	This study
IQ345	*ssyA3 zfe-280*::Tn*10 argH purE hisG thi rpsL*	This study
IQ346	*ssyA3 zfe*::Tn*5 thi relA spoT*	This study
IQ349	*ssyA3 zfe-280*::Tn*10 guaA thi relA spoT*	This study
IQ350	*ssyA3 zfe-280*::Tn*10 thiA* Δ*lac araD rpsL relA*	This study
IQ351	*zfe-280*::Tn*10 thiA* Δ*lac araD rpsL relA*	This study
MC4100	*thiA* Δ*lac araD rpsL relA*	(1)
AT2465	*guaA thi relA spoT*	(24)
AT2681	*glyA argH purF hisG thi rpsL*	(24)
BW280	*zfe-280*::Tn*10 nadB thi ung relA spoT*	B. Bachmann[a]
JK84	*hisS glyA argH thi relA lac rpsL*	B. Bachmann (19)

[a] *E. coli* Genetic Stock Center, Yale University School of Medicine.

with 20 amino acids at 42°C (steady state) or at 30°C (temperature shift down). At various times after temperature shift, IPTG (isopropyl-β-D-thiogalactoside) was added to 1 mM. Portions (0.4 ml) were removed at intervals and mixed with 0.6 ml of Z buffer (18) containing 0.1% SDS, 2.5 mg of chloramphenicol per ml, and 2 drops of $CHCl_3$, at 0°C. The activity of β-galactosidase was assayed as described previously (18). The time at which induced β-galactosidase activity first began to appear was calculated by extrapolating the square root of the enzyme activity at a given time (20). The chain growth rates of polypeptide were estimated by dividing the number of amino acid residues in the enzyme (1,023; see reference 13) by the lag time.

RESULTS

Isolation of cold-sensitive mutants as revertants of *secY24* mutant. The mutant *secY24* is temperature-sensitive in cell growth. It forms abnormal ghost-like colonies on agar plates containing rich medium, but it cannot form colonies on minimal agar at 42°C (21). Temperature-resistant revertants were isolated from strain IQ85 (*secY24*) on minimal agar plates at 42°C (see above). Some of the revertants obtained showed very poor growth at 30°C. Such cold-sensitive colonies were obtained at frequencies of 7×10^{-7} to 10×10^{-7} per parental cells or ca. 10^{-2} per Ts^+ revertant. Although systematic genetic classification of the cold-sensitive mutants currently in progress indicates that the *cs* mutations fall into at least eight groups, we have studied one of the isolates, termed IQ280 (Table 1), with a clear growth phenotype in some detail.

Strain IQ280 forms normal colonies at 42°C, small colonies at 37°C, and little or no colonies at 30°C. To determine whether the ability of IQ280 to grow at 42°C is due to a reversion of *secY24* or to some unlinked suppressor mutation, a transduction experiment with phage P1 was performed. When a wild-type strain (MC4100) was transduced to tetracycline resistance at 37°C by phage P1 grown on IQ280, about 65% of the transductants were found to be temperature sensitive (unable to grow at 42°C). Since the tetracycline resistance marker (Tn*10* insertion) in IQ85 is cotransducible with *secY* at about 60%, the result obtained indicates that the IQ280 revertant still contains the *secY24* mutation. Accordingly, the revertant should contain an unlinked suppressor of the *secY24* mutation. We tentatively designated this unlinked suppressor mutation as *ssyA3* and the gene as *ssyA* (suppressor of *secY*).

When cold-resistant colonies were isolated at 30°C from the *secY24 ssyA3* double mutant (IQ280), some of them exhibited the temperature-sensitive growth characteristic of *secY24*, suggesting that a single *ssyA3* mutation is responsible for both the suppression of *secY* and the cold-sensitive cell growth.

Mapping by F' factors. To obtain an approximate location of the cold-sensitive mutation, strain IQ280 was spot mated (18) with the series of strains carrying various F' factors (15), which together cover almost the entire chromosome, on LB agar containing tetracycline (tetracycline was included to counter select the F' donors). The plates were then incubated at 30°C. Bacterial growth was observed only in the cross in which F'198 factor was used. The conjugants which received F'198 now could grow at 30°C but not at 42°C. Thus, the original phenotype of the single *secY24* mutant was recovered by the introduction of this F' factor. Since the F'198 factor carries the 51- to 55-min region of the *E. coli* chromosome (15), the *ssyA3* mutation should be located in this interval of the chromosome.

Mapping by P1 phage transduction. We determined a more precise location of the *ssyA* mutation by generalized transduction using phage P1. First, its linkage to some of the known markers was examined. P1 lysate prepared from

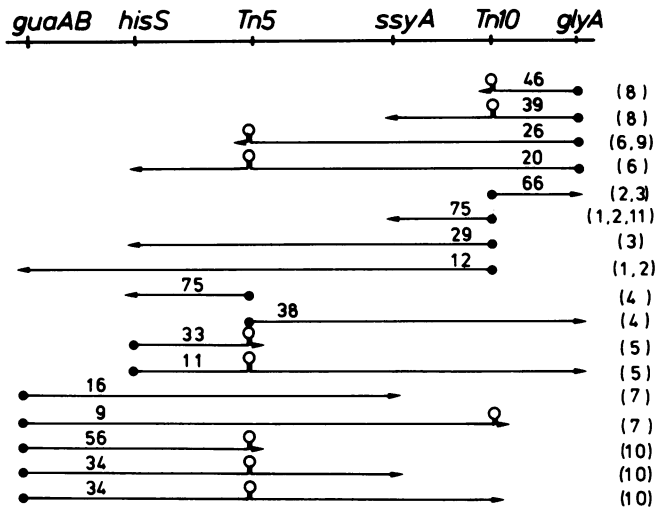

FIG. 1. Mapping of the *ssyA* gene by P1 transduction. The numbers represent average cotransduction frequencies. The closed circles are the selective markers, and arrowheads are the unselected markers. The loops are transposon insertions in the donor strain. The loops are shown for the crosses in which a donor contained a transposon insertion and another marker was used for selection (in these cases the insertion would affect the cotransduction frequencies). The numbers in parentheses indicate the experimental numbers which are described below. The donor and the recipient strains used in each experiment are: 1, IQ349 (*guaA ssyA3* Tn*10*) and MC4100; 2, IQ349 and AT2681 (*glyA*); 3, BW280 (Tn*10*) and JK84 (*hisS glyA*); 4, IQ99 (Tn*5*) and JK84; 5, IQ99 and JK84; 6, IQ99 and JK84; 7, IQ350 (*ssyA3* Tn*10*) and AT2465 (*guaA*); 8, IQ350 and AT2681; 9, IQ346 (Tn*5 ssyA3*) and AT2681; 10, IQ99 and IQ349; 11, IQ345 (*ssyA3* Tn*10*) and MC4100.

IQ280 (*secY24 ssyA3*) was used to transduce strain AT2681 (*glyA*) or AT2465 (*guaAB*) to Gly$^+$ or Gua$^+$, respectively, at 40°C. After purification, transductants were scored for growth at 30°C. Four of 10 Gly$^+$ transductants and 2 of 10 Gua$^+$ transductants were cold-sensitive, indicating that *ssyA* is cotransducible with both *glyA* and *guaAB*. The results also demonstrated that the single *ssyA3* mutation can cause cold-sensitive growth as well as in combination with the *secY24* mutation.

Finer mapping was carried out by using *guaAB*, *hisS*, and *glyA* markers as well as two transposon insertions, *zfe-208*::Tn*10* and *zfe*::Tn*5*. We first determined the locations of the transposons. When used as a selective marker, the Tn*10* insertion is cotransducible with *glyA* at ca. 70%, with *hisS* at 30%, and with *guaAB* at 10%. Results of a three-factor cross involving, Tn*10*, *glyA*, and *hisS* suggested an order: *guaAB-hisS*-Tn*10-glyA*. The Tn*5* insertion is cotransducible with *glyA* at ca. 40% and *hisS* at 80%. Three-factor crosses suggested an order: *guaAB-hisS*-Tn*5-glyA*. We then separated the *ssyA3* mutation from *secY24* by *glyA*-linked transduction. This step was necessary because the presence of the *secY24* mutation complicates the transduction procedures. The mutation was then combined with either the Tn*10* or the Tn*5* insertion (Table 1).

The results of various crosses are summarized in Fig. 1, and we describe two representative crosses below. In the first cross, IQ349 (Tn*10 ssyA3 guaAB*) was used as the donor, and AT2681 (*glyA*) was used as the recipient. Tetracycline-resistant transductants were selected at 40°C, which were then examined for distribution of the unselected markers. The *ssy3* mutation was cotransducible with Tn*10* at a frequency of 70 to 80%. The distribution of other markers suggested an order: *guaAB-ssyA*-Tn*10-glyA*. In another cross, strain IQ346 (Tn*5 ssyA3*) was used as the donor, and AT2681 (*glyA*) was used as the recipient, selecting for Gly$^+$ transductants at 40°C. The cotransduction frequency of *glyA* and *ssyA3* was ca. 40%, whereas that of *glyA* and Tn*5* was ca. 25%. Thus, the relative order is Tn*5-ssyA-glyA*. Thus the most probable gene order is *guaAB-hisS*-Tn*5-ssyA*-Tn*10-glyA* (Fig. 1). Other crosses with *guaAB* as a selective marker gave results consistent with the above conclusion and are summarized in Fig. 1. The cotransduction frequencies vary somewhat, depending on the particular combinations of donor and recipient and also on the selective markers used, but they are generally consistent with the gene order shown in Fig. 1.

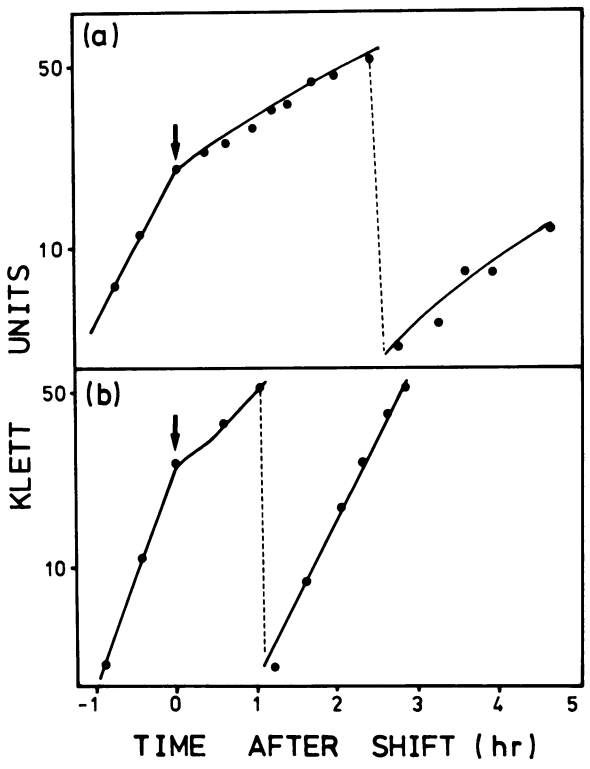

FIG. 2. Growth of the mutant in liquid broth. (a) IQ350 (*ssyA3*) and (b) IQ351 (wild type) were grown in LB broth at 42°C. The temperature was shifted to 30°C at the point indicated by the arrow. The cultures were diluted fivefold with prewarmed medium at Klett unit = 50.

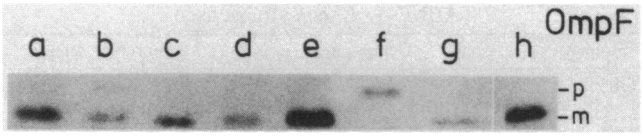

FIG. 3. Processing of the outer membrane protein in the mutant cells. Cells were labeled with [^{3}H]leucine for 1 min, and the labeled proteins were treated with an antiserum against OmpF protein and subjected to gel electrophoresis. Lanes: a and e, wild-type strain (IQ351) at 30°C and 42°C, respectively; b and f, IQ85 (*secY24*) at 30°C and 1.5 h after shift to 42°C, respectively; c and g, IQ280 (*secY24 ssyA3*) 4 h after shift down to 30°C and at 42°C, respectively; d and h, IQ350 (*ssyA3*) 4 h after shift down to 30°C and at 42°C, respectively. p and m indicate precursor and mature forms, respectively.

Growth properties of the cold-sensitive mutant. As described above, the *ssyA3* mutation alone causes cold-sensitive cell growth, forming colonies at 42°C but not at 30°C on agar plates. Growth in liquid medium was followed with strains IQ350 and IQ351, an isogenic pair of *ssyA3* and *ssyA*$^+$ strains, respectively. The *ssyA3* mutant did not show an immediate growth arrest at 30°C, but its growth rate gradually declines after a few hours of incubation at 30°C (Fig. 2). A similar growth curve (not shown) was obtained with the *ssyA3-secY24* double mutant (IQ280).

Effects of the *ssyA* mutation on protein secretion. The *ssyA* mutant was isolated as a suppressor, in terms of cell growth, of *secY24*. We asked whether it suppresses the secretion defect as well by examining whether the mutant accumulates precursor forms of the envelope proteins. Cells were pulse labeled with [^{3}H]leucine at 42°C and treated with an antiserum against the outer membrane OmpF protein. Strain IQ280 (*secY24 ssyA3*) exhibited very little accumulation of the precursor protein (Fig. 3, lane g), in contrast to strain IQ85 (*secY24*), which exhibited marked accumulation (lane f) as reported previously (21). Similar results were obtained with the OmpA protein and the periplasmic maltose-binding protein (data not shown). Thus the *ssyA3* mutation suppresses the protein secretion defect of the *secY24* mutation at 42°C.

We then asked whether the *secY24-ssyA3* double mutant or the *ssyA3* single mutant shows any secretion defect at 30°C, the restrictive temperature for the cold-sensitive mutants. After a 3.5-h incubation at 30°C, neither of these mutants accumulated precursor protein to any detectable extent (Fig. 3, lanes c and d). These results suggest that the cold-sensitive *ssyA3* mutant is able to secrete proteins at the restrictive temperature (30°C), at least up to the point at which the signal sequence is removed proteolytically. Probably, the cold-sensitive growth is caused by some lesion in the cellular function other than protein export.

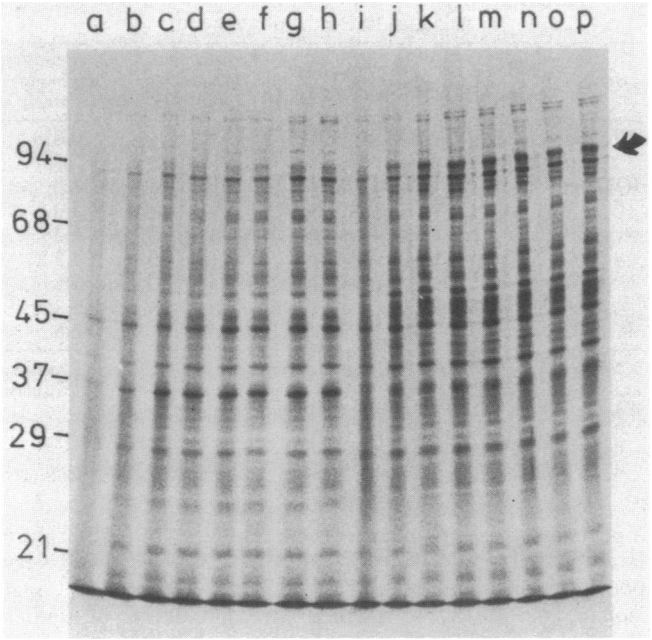

FIG. 4. Incorporation of [^{35}S]methionine into polypeptides of various sizes. IQ280 (*secY24 ssyA3*) (lanes a through h) and IQ86 (wild type) (lanes i through p) were grown in minimal glucose medium with amino acids mixture (except methionine and cysteine) at 42°C, and the temperature was shifted to 30°C. After 4.5 h, cells were pulse labeled with 24 μCi of [^{35}S]methionine per ml (1,400 Ci/mmol; Amersham) for 20 s (lanes a and i) and then chased with unlabeled methionine (200 μg/ml) for 20 s (lanes b and j), 40 s (lanes c and k), 60 s (lanes d and l), 80 s (lanes e and m), 100 s (lanes f and n), 3 min (lanes g and o), or 10 min (lanes h and p). The incorporation was terminated by mixing with an equal volume of cold 10% trichloroacetic acid. After centrifugation, the protein precipitates were washed with acetone and dissolved in SDS sample buffer. The labeled protein bands were visualized by autoradiography. Under the conditions used, polypeptides with molecular weights higher than ca. 20 × 10^3 were separated in the gel. Numbers represent molecular weight of the marker proteins (10). The arrow indicates the protein with a molecular weight of 96,000 used in the next experiment (Fig. 5).

Protein synthesis defects in the *ssyA* mutant. In the course of the above experiments, we noticed poor incorporation of [^{3}H]leucine into the *ssyA* mutant cells at 30°C. Patterns of SDS-gel electrophoresis indicated that proteins with higher molecular weights were labeled poorly. Thus, we considered that protein synthesis might be defective at the step of polypeptide chain elongation, and examined this possibility by a pulse-chase experiment. After 4.5 h of incubation at 30°C, strain IQ280 was pulse labeled for 20 s with [^{35}S]methionine and chased with unlabeled methionine for various periods. Incorporation of [^{35}S]methionine into polypeptides of various sizes was studied by SDS-gel electrophoresis and autoradiography (Fig. 4). In wild-type cells, the polypeptides of all sizes were labeled fully after 20- to 40-s chases (Fig. 4, lanes j and k), and further chases did not alter the patterns of the radioactive polypeptides (lanes k through p). In contrast, strain IQ280 did not exhibit clear patterns of labeled proteins at the early times during chase. This was especially evident for larger polypeptides (Fig. 4, lanes a through f). Full labeling of the high-molecular-weight polypeptides was observed only after a few minutes of chase (Fig. 4, lanes g through h). Thus, it seems that the nascent polypeptide chains grow more slowly in the mutant cells than in the wild-type cells. Similar results were obtained with an *ssyA3* single mutant.

To measure the rates of polypeptide growth more quantitatively, we carried out a double-labeling experiment. Strains IQ350 (*ssyA3*) and IQ351 (*ssyA*$^+$) were grown first at 42°C and then shifted to 30°C. Cells were prelabeled with [^{14}C]leucine and pulse labeled (50 s for IQ350 and 30 s for IQ351) with [^{3}H]leucine 4 h after the shift, followed by chase with unlabeled leucine. Samples were removed at various times after the initiation of chase, and subjected to SDS-gel electrophoresis. After staining, a major band in the 96 × 10^3 molecular-weight region was cut out from each gel, and its ^{3}H and ^{14}C radioactivities were determined (Fig. 5). The time at which a constant ^{3}H/^{14}C ratio was achieved indicated the time at which the pulse-labeled polypeptide chain was completed.

In the wild-type strain the chain was completed ca. 2.2 min after initiation of chase. In the *ssyA3* mutant completion

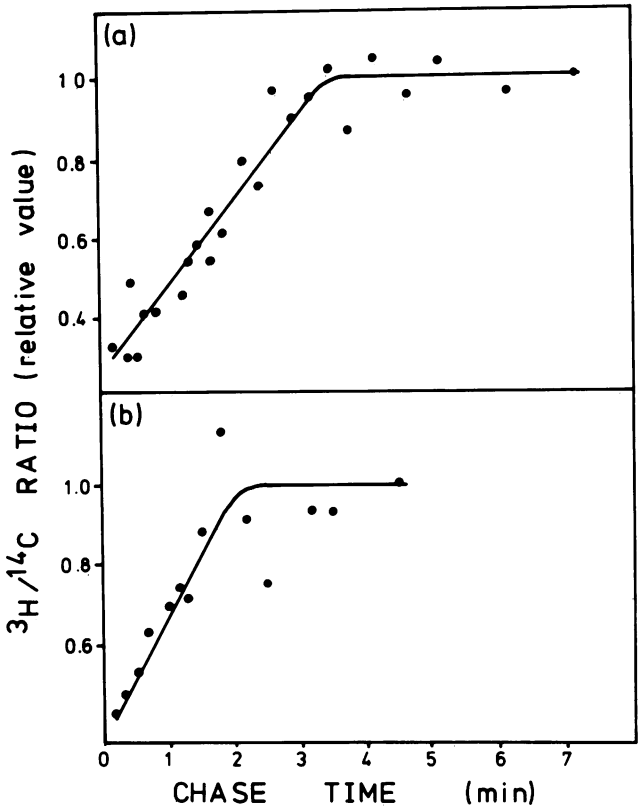

FIG. 5. Measurement of polypeptide elongation rate by double-labeling. (a) IQ350 (*ssyA3*) and (b) IQ351 (wild type) were grown in glucose minimal medium with 17 amino acids (except leucine, isolecine, and valine) at 42°C. At Klett unit = 10, the temperature was shifted to 30°C and 0.2 Ci of [^{14}C]leucine per ml (342 mCi/mmol; Amersham) was added. At Klett unit = 50 (4 h after the shift in the case of IQ350), cells were pulse-labeled with [^{3}H]leucine for 50 s (for IQ350) or 30 s (for IQ351) and then chased with 400 μg of leucine and 100 μg of isoleucine per ml. At various times, 0.5-ml portions were withdrawn and mixed with trichloroacetic acid. Proteins were separated in 10% SDS-polyacrylamide gels (using *N,N'*-diallyltartardiamide instead of *N,N'*-methylenebisacrylamide) and stained with Coomassie brilliant blue. A major band at the 96,000-dalton region was cut out and solubilized in 0.25 ml of 2% HIO_4 neutralized with NH_4OH. ^{3}H and ^{14}C radioactivities were counted in Bray's liquid scintillator with a Beckman model LS7000 scintillation counter.

required about 3.6 min. From the molecular weight (96×10^3) of the protein band examined, average rates of polypeptide chain growth may be calculated to be 6.6 amino acids per s and 4.0 amino acids per s for the wild-type and the mutant cells, respectively.

We also estimated the chain growth rate of polypeptide by an independent method. The method is based on the notion that the time between the addition of IPTG (an inducer of the lactose operon) and the first appearance of β-galactosidase activity reflects the time needed for the completion of the first peptide chains of the enzyme (4). Strain IQ328 (*ssyA3*) and its parent, AT2465, were grown at 42°C and shifted down to 30°C. At various times after the shift, IPTG was added, samples were withdrawn at short intervals, and β-galactosidase activities were determined (Fig. 6). The polypeptide chain growth rate was estimated (see above) and is summarized in Table 2. At 42°C, both strains showed almost the same rates of peptide chain growth (20 to 21 amino acids per s). After shift to 30°C, however, the mutant cells showed progressively slower rates of peptide elongation. At 4 to 5 h after temperature shift, the chain elongation rate in the mutant (2.6 to 3.2 amino acids per s) was almost one-half that in the wild-type strain (5.7 amino acids per s). The reduction in the elongation rate appears to be correlated with the reduction in growth rates.

Thus the chain elongation rates determined by the two independent methods agree well. These results indicate that the *ssyA3* mutant synthesizes proteins at a slower rate than the wild-type strain at 30°C.

DISCUSSION

The gene, tentatively designated as *ssyA* has been identified by isolating temperature-resistant revertants from the *secY24* temperature-sensitive mutant. The mutation *ssyA3* has been mapped at 54.5 min between *glyA* and *hisS* on the chromosome. The mutation restores the temperature-sensitive growth defect, as well as the temperature-sensitive protein secretion defect of the *secY* mutant. Another phenotype of the *ssyA3* mutation is cold-sensitive cell growth. The mutant, in combination with either *secY*$^+$ or *secY24*, shows

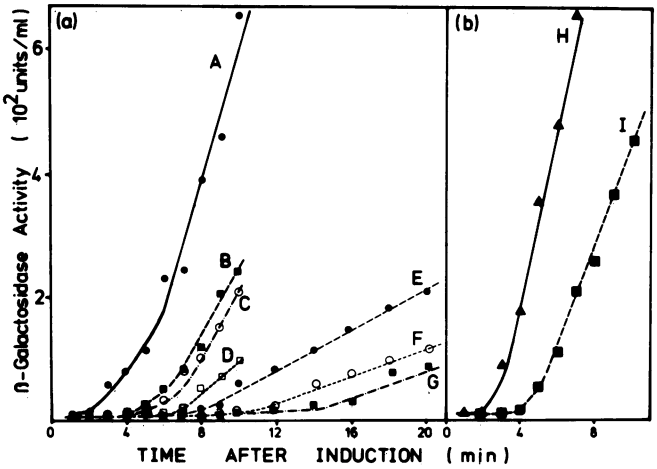

FIG. 6. Induction kinetics of β-galactosidase. Cells of (a) IQ328 (*ssyA3*) or (b) AT2465 (*ssyA*$^+$) were grown at 42°C and parts of the cultures were shifted to 30°C. At 42°C or after incubation at 30°C for the period indicated below, induction of β-galactosidase was initiated by addition of IPTG. A, 42°C; B, 30°C for 15 min; C, 30°C for 1 h; D, 30°C for 2 h; E, 30°C for 3 h; F, 30°C for 4 h; G, 30°C for 5 h; H, 42°C; I, 30°C (steady state).

TABLE 2. Measurement of peptide elongation rate by β-galactosidase induction kinetics

Strain	Temp (°C)	Time after shift[a] (h)	Growth rate (μ[b])	Calculated lag time[c] (min)	Chain elongation rate[d] (amino acids per s)
AT2465	42	SS	1.17	0.82	20.8
	30	SS	0.92	3.00	5.7
IQ328	42	SS	0.97	0.81	21.0
	30	0.25	0.87	3.40	5.0
		1	0.70	3.28	5.2
		2	0.46	4.02	4.2
		3	0.42	4.77	3.6
		4	0.29	5.25	3.2
		5	0.15	6.45	2.6

[a] Cells were grown in amino acids-supplemented glycerol minimal medium at 42°C, and shifted to 30°C. After the temperature shift, β-galactosidase induction was initiated at the time indicated. SS, Steady state.
[b] μ, Doublings per hour.
[c] Determined as described by Schleif et al. (20).
[d] Calculated assuming that β-galactosidase contains 1,023 amino acids (13).

a peculiar growth property. Its optimal growth temperature is as high as 42°C. It grows poorly even at 37°C and very poorly at 30°C. The mutant studied here is not an exceptional isolate, and we have obtained several mutants of similar phenotypes and similar map position by the procedure described in this paper (K. Shiba, unpublished results).

At 30°C, protein synthesis is gradually slowed down. The pulse-chase experiments and the measurement of the induction kinetics of β-galactosidase suggested that the polypeptide chain elongation rate is slowed down at 30°C. This defect in protein synthesis appears to be exerted over the general cellular proteins, not specifically on the exported proteins. Protein export itself appears unimpaired, as evidenced by the normal processing of envelope proteins observed in the mutant. Since it is generally known that the rate of peptide chain growth is constant at a given temperature over a variety of growth conditions (9), the reduced elongation may not be a nonspecific consequence of the reduced cell growth. Conversely, the slow growth may be caused by the slow peptide elongation.

The mutation site is close to *hisS*, which is involved in protein synthesis. However, *ssyA* and *hisS* are distinct genes, since Tn*5* can insert between them (Fig. 1). Also, recombinant plasmids complementing *ssyA3* do not complement the *hisS* mutation (K. Shiba, unpublished data).

The mechanism by which the *ssyA* mutation suppresses *secY24* is not known. Generally, such suppression can be explained by either: (i) altered subunit interactions in macromolecular assembly, (ii) activation of an alternative by-pass reaction, (iii) stabilization of the mutant gene product, (iv) indirect physiological effect, or (v) suppression at the level of transfer of genetic information. It is unlikely that the *ssyA3* mutation activates an alternative protein export pathway, because it cannot suppress the *rplO215*(Am) mutation, an amber mutation in *rplO* which reduces expression of *secY* (the *rplO* gene itself is dispensable for growth; Ito et al., in press).

A slow translation could facilitate protein secretion because more time may be allowed for the signal peptide to function (8). However, since the chain elongation rate appears essentially normal at 42°C, the temperature at which the suppression is observable, nonspecific suppression by a slow translation seems to be an unlikely explanation as well.

We consider the following possibility as a current working model. The gene product of *ssyA* may interact with the *secY* gene product. At the same time it may be involved in general

model. The gene product of *ssyA* may interact with the *secY* gene product. At the same time it may be involved in general protein synthesis. The altered *ssyA3* product could somehow help the function of the altered *secY24* protein at 42°C by its altered properties of interaction. On the other hand, the altered *ssyA3* gene product cannot function as the putative factor of general protein synthesis at 30°C. The *ssyA* gene might specify an as-yet-unidentified component in the protein synthesizing system. Ferro-Novick et al. (6a) isolated the *secC* mutation as a suppressor of a temperature-sensitive *secA* mutation. The *secC* mutant is cold sensitive and unable to synthesize exported proteins at the low temperature. The translation arrest appears to be mediated by the signal sequence of the protein being synthesized. The cold sensitivity in the *ssyA3* mutant should be of a category different from that revealed in the *secC* mutants, because the protein synthesis defect was observed generally, not specifically for the exported proteins.

The signal recognition particles in eucaryotic cells interact with ribosomes, as well as with the signal sequence and the receptor protein in the membrane (7, 16, 26). Thus, it is conceivable that a component of the general protein synthesis machinery (ribosomes or related factors) in the bacterial cell also interacts directly with the secretion machinery. The possibility that the *ssyA* gene might specify such a component of protein synthesis will be of special interest in our further analysis of the gene function.

ACKNOWLEDGMENTS

We acknowledge B. Bachman and W. Wickner for supplying bacterial strains, and J. Asano and M. Mihara for excellent technical assistance.

This work was supported by grants from the Ministry of Education, Science and Culture, Japan.

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