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The 64-kilodalton membrane protein of *Bacillus subtilis* is also present as a multiprotein complex on membrane-free ribosomes

(S complex/protein secretion/protein cross-linking)

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ABSTRACT **The 64-kDa membrane protein of *Bacillus subtilis* is evidently involved in the attachment of secreting ribosomes to membrane. On immunoprecipitation with antibody to this protein, the solubilized particulate fraction, with or without prior chemical cross-linking, yields a complex of four proteins (64, 60, 41, and 36 kDa). This "S complex" was found to be associated with membrane-free ribosomes rather than with membrane, but the 64-kDa protein is also present, without the other proteins of the S complex, in the membrane–ribosome fraction and in the cytosol. Only the form present in the membrane–ribosome fraction is protected from protease. These findings suggest a cycle in which the complex participates in initiation of secretion but not in the later stages. It is not yet clear whether the 64-kDa protein found in the membrane–ribosome complexes is retained from the S complex after initiation and later recycled via the cytosol or whether it is a separate pool.**

In earlier work in our laboratory, aimed at identifying membrane proteins involved in protein secretion, we compared a membrane fraction of *Bacillus subtilis* complexed with ribosomes (complexed membrane, CM) and a fraction free of ribosomes (free membrane, FM) (1). Gel electrophoresis revealed unique protein bands in each of these fractions (1–3). One of the proteins in the CM fraction, of 64 kDa, appeared to be located between membrane and attached ribosomes, since protease and antibodies interacted with it only after treatment that released the ribosomes (4); hence, it seemed likely to be involved in protein secretion. This protein is also present in the cytosol (2).

In this paper, we report that antibody to the 64-kDa protein can precipitate a complex that includes three additional proteins, here called the S complex, from a solubilized membrane–ribosome fraction. However, further fractionation showed that the S complex is present, in large amounts, in membrane-free ribosomes in the unsolubilized fraction, rather than in the membrane, while the 64-kDa protein is present without the rest of the S complex in the membrane–ribosome complexes and also in the cytosol. The S complex therefore appears to play a cyclic role in the initiation of protein secretion, like the signal recognition particle of eukaryotic cells discovered by Walter and Blobel (5), but it also appears to differ in significant respects from that particle.

MATERIALS AND METHODS

Organism and Growth Conditions. *B. subtilis* (ATCC 6051a) was grown in minimal medium A (6) supplemented with $FeSO_4$, 0.4% glucose, and 0.2% Casamino acids. For labeling with $^{35}SO_4^{2-}$ (4 mCi/liter; 1 Ci = 37 GBq), the $(NH_4)_2SO_4$ and $FeSO_4$ were replaced by NH_4Cl and $FeCl_3$, respectively, and cells were grown in the presence of 18 amino acids (without methionine or cysteine) for two generations before harvesting. For labeling with [^{35}S]methionine (1 mCi/liter, 1000 Ci/mmol), 2 μM unlabeled methionine and the other 19 amino acids (each, 0.2 mM) were used in place of Casamino acids. The methionine- or the sulfate-labeled cells sustained exponential growth until harvesting, at 100 Klett (540 nm) units. For labeling of lipids, 2-[^{3}H]glycerol (4 mCi/liter), in the presence of glucose, was added two generations before harvesting.

Membrane Preparations. All sucrose solutions were in TKMD buffer [10 mM Tris·HCl, pH 7.6/50 mM KCl/10 mM $Mg(OAc)_2$/1 mM dithiothreitol]. The CM fraction was prepared in a biphasic sucrose gradient, in a Beckman SW50.1 rotor, as described (1). Fractions CM-I and CM-II were prepared in a triphasic gradient, in a Beckman SW41 rotor (3), as follows. Chloramphenicol (0.1 mg/ml) was added to midlogarithmic phase cells and the culture (1.3 liters) was poured over ice. The cells were washed once in TKM buffer (dithiothreitol-free TKMD buffer) containing chloramphenicol (0.1 mg/ml) and 1 M KCl and twice in TKM buffer containing chloramphenicol and 20% sucrose. The cells were then suspended in 10 ml of buffer A [TKMD buffer with chloramphenicol (0.1 mg/ml), 1 mM phenylmethylsulfonyl fluoride, 0.1 mM *o*-phenanthroline]/20% sucrose, warmed to 37°C, and incubated for 20 min with lysozyme (0.5 mg/ml) and DNase (5 μg/ml). After cooling on ice, the protoplasts were diluted with 2 vol of buffer A and passed through a French press at 12,000 lb/in^2 (1 lb/in^2 = 6.9 kPa).

After the resultant lysate had been cleared by centrifugation (5 min, 10,000 rpm, Sorvall SS34 rotor), the membrane and ribosomes were concentrated (1 hr, 37,000 rpm, Beckman SW41 rotor) onto a 1-ml 2 M sucrose cushion in TKM buffer. The material at the interface, the sucrose cushion, and the slight pellet were collected together, diluted with 1 ml of buffer A, and centrifuged (16 hr, 37,000 rpm, SW41 rotor) through a triphasic sucrose gradient (1.35–1.6–2.0 M). The FM, CM-I, and CM-II fractions, banding at the successive interfaces, were each collected (about 2 ml), diluted with TKMD buffer, pelleted (150 min, 40,000 rpm, 60 Ti rotor), dispersed in TKMD buffer, and stored at −76°C.

Cross-linking of Proteins. Cells from 1.3 liters of culture were washed with high-salt and low-salt buffers as above (except that the Tris was replaced with triethanolamine) and then suspended in 80 ml of cross-linking buffer [10 mM triethanolamine·HCl, pH 7.6/50 mM KCl/10 mM $Mg(OAc)_2$] with chloramphenicol. After incubation with 40 mM iodoacetamide for 20 min at 37°C to prevent sulfhydryl inter-

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change, dithiobis(succinimidyl propionate) (10 mg/ml stock in dimethyl sulfoxide) was added (0.4 mg/ml) and, after 30 sec, the reaction was stopped by adding 1 M Tris·HCl (pH 8.0) equal in volume to the cross-linking reagent. The CM fraction was then prepared as noted above.

Immunoprecipitation. Rabbit antiserum was prepared to the 64-kDa protein isolated from $NaDodSO_4$ gels as described (2). Immunoprecipitation was carried out as follows: samples (0.1 ml) were made 1% in Triton X-100 and, after 15 min, diluted 1:10 with immunoprecipitation buffer (50 mM Tris·HCl, pH 7.6/150 mM NaCl/5 mM EDTA/0.5% Triton), pretreated with preimmune serum and formalin-fixed *Staphylococcus aureus* Cowan strain, and then precipitated with immune serum and *S. aureus*. The precipitates were washed with immunoprecipitation buffer.

Gel Electrophoresis. Samples for electrophoresis were boiled for 5 min in 1% $NaDodSO_4$ sample buffer, and electrophoresis was carried out in $NaDodSO_4$/polyacrylamide gels as described (7). Gels were autoradiographed as described (3).

Chemicals. Dithiobis(succinimidyl propionate) was purchased from Pierce, radioactive compounds and Triton X-100 were from New England Nuclear, sucrose (ultrapure) was from Schwarz/Mann, lysozyme was from Sigma, DNase was from Worthington, and protease K was from Beckman. All other chemicals were of reagent grade.

RESULTS

The early part of this work was carried out with a CM fraction that had been separated from the FM fraction in a two-step gradient in an SW50.1 centrifuge rotor. Subsequently, we used a three-step gradient, which had been developed to prepare a CM fraction free of contamination by the FM fraction (3). In this procedure, an intermediate-concentration sucrose layer (1.6 M) was introduced between 1.35 and 2.0 M sucrose layers (compared with the 1.35 and 1.8 M layers previously used) and centrifugation was in the longer tubes in the SW41 rotor. This more refined procedure yielded FM, CM-I (at the 1.35–1.6 M interface), and CM-II (at the 1.6–2.0 M interface) fractions. In a typical preparation, these fractions contained 50, 36, and 14% of the total lipid (labeled with [2-^{3}H]glycerol) and 4, 57, and 39% of the RNA (expressed as A_{260} units). As reported below, fraction CM-I, though expected to contain only membrane–ribosome complexes, also contained unattached ribosomes.

Cross-linking of Other Proteins to the 64-kDa Protein. To seek proteins that might be associated with the 64-kDa protein, we labeled cells with [^{35}S]methionine and treated them with the cleavable cross-linking reagent dithiobis(succinimidyl propionate), which penetrates the membrane and reacts with aliphatic amines (8). The CM fraction was isolated, solubilized with hot $NaDodSO_4$, and treated with anti-64-kDa antibody. The immunoprecipitate was solubilized with hot 1% $NaDodSO_4$ and the cross-links were cleaved by treatment with 2-mercaptoethanol. Electrophoresis revealed not only the 64-kDa protein (Fig. 1, lane A) but also proteins of 60, 41, and 36 kDa. The 64-kDa band is much heavier than the others because precipitation of the 64-kDa protein did not depend on cross-linking. The unmarked bands appear to be nonspecific precipitates and were also obtained with preimmune serum.

S Complex Recovered Without Cross-linking. The proteins that can be cross-linked with the 64-kDa protein might well be present as a firm physiological complex, which would be dissociated by $NaDodSO_4$ treatment. In hope of retaining such a complex, we used the nonionic detergent Triton X-100 to solubilize the membrane together with EDTA to release the peptidyl-tRNA (and associated membrane proteins if present) from the ribosomes. After treatment with anti-64-kDa antibody, the precipitate was solubilized with boiling 1% $NaDodSO_4$ and analyzed. We examined the more refined fractions CM-I and CM-II rather than the cruder total CM fraction. As shown in Fig. 1, fraction CM-I yielded the same set of proteins, without cross-linking, as obtained from the CM fraction after cross-linking (compare lane B with lane A). Fraction CM-II yielded the 64-kDa protein with little of the S complex, as described below.

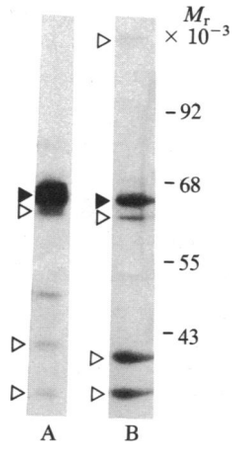

FIG. 1. Immunoprecipitation of the 64-kDa protein together with associated proteins. Cells were labeled with [^{35}S]methionine. Lane A: fraction CM (2 A_{260} units, 6.0 × 10^5 cpm) isolated from cells cross-linked with dithiobis(succinimidyl propionate), solubilized with $NaDodSO_4$, and immunoprecipitated by anti-64-kDa antibody. Lane B: fraction CM-I (2 A_{260} units, 5.0 × 10^5 cpm) from cells not cross-linked was treated with 1% Triton X-100 and then immunoprecipitated. Solid triangle indicates the 64-kDa protein and open triangles indicate the coprecipitated proteins. Autoradiographs were exposed for 14 days (lane A) or 7 days (lane B). Molecular weight standards used were phosphorylase b, bovine serum albumin, immunoglobulin heavy chain, ovalbumin, and carbonic anhydrase.

The four coprecipitated proteins thus form a complex that is stable in the presence of mild detergent and EDTA and will be denoted the "S complex." Quite consistently, the bands at 64, 41, and 36 kDa were similar in intensity while that at 60 kDa was much weaker. A faint band of 130 kDa was also seen in this complex (Fig. 1, lane B) but it was not recovered consistently by immunoprecipitation, and we have found that it cross-reacts with the anti-64-kDa antibody; it may be a dimer of that protein.

Composition of Fractions CM-I and CM-II. Because fractions CM-I and CM-II differed markedly in their content of S complex, it seemed desirable to examine other aspects of their composition. Accordingly, cells were labeled with 2-[^{3}H]glycerol or $^{35}SO_4^{2-}$. The RNA/lipid/protein ratio (A_{260} units/cpm × 10^{-4}/cpm × 10^{-6}) was 1:14:2 for fraction CM-I and 1:5:3 for fraction CM-II (and 1:240:7 for FM). Fraction CM-II evidently carries a higher ribosome/membrane ratio than fraction CM-I.

We also examined the distribution of polysome sizes in a linear 10–30% sucrose gradient, after treatment with Triton X-100 to solubilize the membrane. As shown in Fig. 2, fraction CM-I yielded predominantly monosomes (70S particles) with a few disomes. (In some preparations subjected to repeated freezing and thawing, as in this figure, 50S particles were also prominent, presumably reflecting degradation of the more labile 30S subunit; compare Fig. 3*A*). Fraction CM-II, in contrast to fraction CM-I, yielded few monosomes and mostly longer polysomes (Fig. 2).

To test our assumptions that fractions CM-I and CM-II consist of membrane–ribosome complexes and that the higher membrane/ribosome ratio in the CM-I complexes is responsible for their collection at a higher interface than CM-

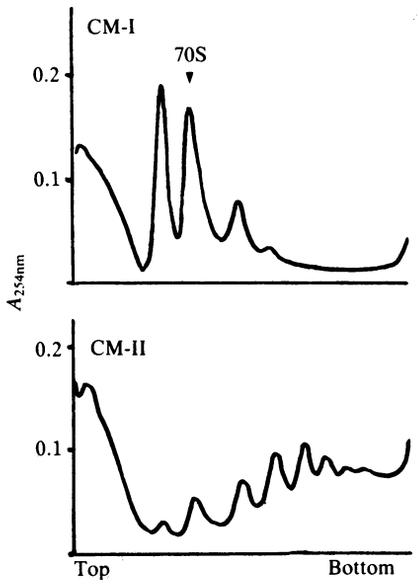

FIG. 2. Distribution of ribosomal particles from solubilized fractions CM-I and CM-II. Samples (2 A_{260} units/0.1 ml) were treated with 1% Triton X-100 for 15 min on ice and then centrifuged (45,000 rpm, 60 min, SW50.1 rotor) through a linear 10–30% sucrose gradient in TKM buffer. Gradients were monitored for absorbance at 254 nm in an Isco fractionator.

II, we examined the distribution of particles without detergent treatment. Unexpectedly, the RNA profile of fraction CM-I (data not shown) was essentially identical with that observed after detergent treatment, suggesting that the monosomes and the membrane particles in fraction CM-I are predominantly separate rather than attached to each other.

To confirm this possibility, we examined the distribution of lipid and of protein in a 10–30% sucrose gradient using cells grown with [2-^{3}H]glycerol or with [^{35}S]methionine. Indeed, as shown in Fig. 3*A*, the glycerol label appeared in the region of the disomes and increasingly throughout the remainder of the gradient but was absent from the monosome region. Moreover, the numerous ^{35}S-labeled membrane proteins (identifiable in Fig. 3*B* because many are larger than the ribosomal proteins) paralleled the distribution of lipid (Fig. 3*A*). (The 38-kDa protein, which is prominent in fraction 12 and above, turned out to be flagellin.) The reason for the accumulation of monosomes at the 1.35–1.6 M interface is discussed below.

Association of the S Complex with Membrane-Free Ribosomes. Fib. 3*B* further shows that, after separation of fraction CM-I in a linear sucrose gradient, the bands of the S complex were prominent in the fluorograms of the monosome region along with the ribosomal proteins (of M_r < 32,000) but were hardly detectable in the later fractions, containing the numerous membrane proteins. The location of the S complex was confirmed by isolation of the monosome fraction, followed by immunoprecipitation with the anti-64-kDa antibody in the presence of Triton X-100 and EDTA: the result was much like Fig. 1*B* (data not shown). We will show elsewhere that the S complex is attached to, and not simply cosedimenting with, the monosomes.

The association of the S complex with monosomes, rather than with membrane, makes possible simpler methods for its isolation. In the initial centrifugation of the lysate for 1 hr, to concentrate membrane and ribosomes on a sucrose cushion prior to fractionation, some of the ribosomes remained in the supernate, and further centrifugation of this material yielded monosomes with the S complex. For small samples, an even simpler source was the 70S fraction obtained directly from a

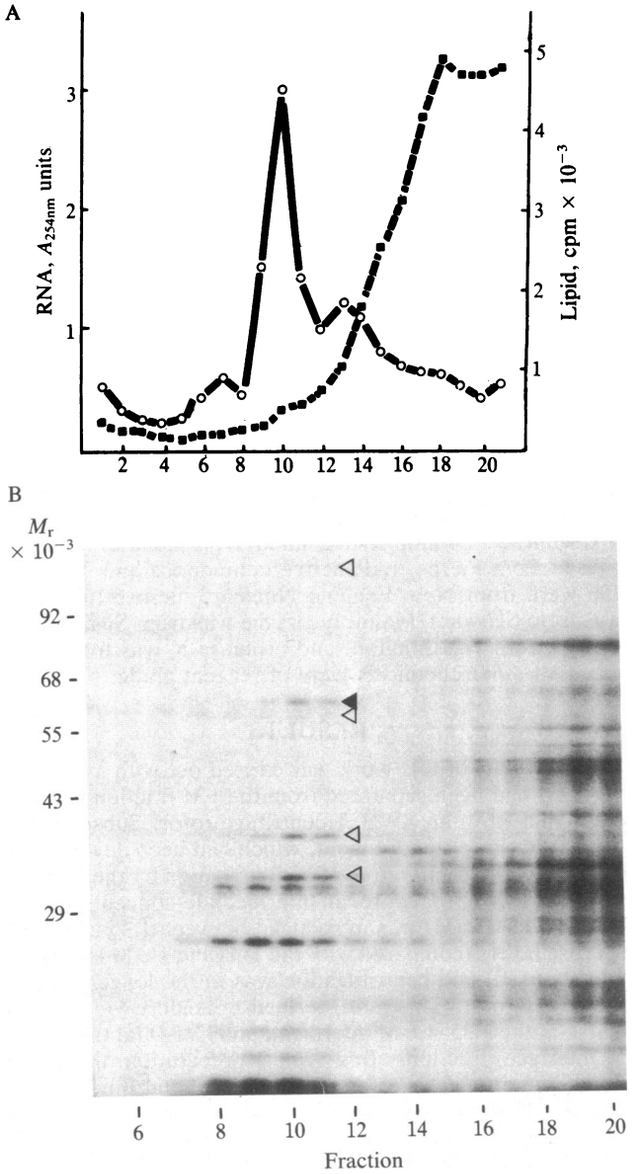

FIG. 3. Distribution of ribosomes, proteins, and lipid in fraction CM-I. (*A*) Fraction CM-I (5 A_{260} units, 2.2 × 10^5 cpm, 0.1 ml) from cells labeled with [2-^{3}H]glycerol was sedimented (without solubilization) through a linear 10–30% sucrose gradient (45,000 rpm, 60 min, SW50.1 rotor) and, after fractionation (8 drops), the absorbance at 254 nm (○) of and the radioactivity (■■) in each fraction were determined. (*B*) Fraction CM-I (1 A_{260} unit, 1.0 × 10^6 cpm, 0.1 ml) from cells labeled with [^{35}S]methionine was fractionated as in *A* and the protein pattern of each fraction was analyzed by electrophoresis. The autoradiogram was exposed for 7 days. Solid triangle, 64-kDa protein; open triangles, S-complex proteins (from top to bottom) of 130, 60, 41, and 36 kDa.

total lysate after sedimentation through a 10–30% sucrose gradient.

In some experiments, the yield of monosomes was very high (up to 70% of the total ribosomes recovered), probably due to disruption of polysomes during lysis. We could not compare polysomes and free ribosomes (without mRNA) because lysozyme alone, without the French press, did not yield good lysis.

The 64-kDa Protein Without the S Complex. Fraction CM-II, treated with Triton X-100 and EDTA and then precipitated with anti-64-kDa antibody like fraction CM-I above, exhibited a heavy band of the 64-kDa protein, with much less of the proteins of the S complex (which were largely or en-

tirely contributed by the small 70S portion of this fraction). When a polysomal region was isolated, to eliminate this contamination, its total proteins included a heavy 64-kDa band (relative to the other membrane protein bands, of $M_r >$ 32,000) but essentially none of the other proteins of the S complex (Fig. 4, lane C). Fraction CM-II contained less of the 64-kDa protein per A_{260} unit than fraction CM-I, which is consistent with the prominence of the ribosomal bands in Fig. 4 (lane C).

We have previously reported (2) that the 64-kDa protein is present in the cytosol fraction as well as in the CM fraction. However, we now find the S complex associated with membrane-free ribosomes, and these might not all have been removed by the 1-hr centrifugation used to prepare that cytosol. We therefore prepared a ribosome-free cytosol by a 4-hr centrifugation. Immunoprecipitation with the anti-64-kDa antibody confirmed the presence of the 64-kDa protein. Moreover, as in fraction CM-II, it was not accompanied by the S complex (data not shown).

Protection of the 64-kDa Protein. Earlier observations, on the CM fraction from a biphasic gradient, suggested that the 64-kDa protein is located in the membrane at a site covered by attached ribosomes, since it was protected from protease by ribosomes unless released by EDTA (4). Since the 64-kDa protein is now seen to be associated also with ribosomes alone, from which it can be released by EDTA, protection by membrane–ribosome apposition became uncertain. However, when the protection was examined further (with protease K, which can be neutralized after the reaction) the 64-kDa protein was found to be protected in CM-II (Fig. 4, lanes C and D), in which it is associated (without the S complex) with membrane and polysomes. In contrast, it is not protected in fraction CM-I (Fig. 4, lanes A and B), in which it is mostly present (as the S complex) in the membrane-free monosome fraction. This difference strongly supports the earlier inference that the 64-kDa protein in ribosome–membrane complexes is protected by a location between ribosomes and membrane.

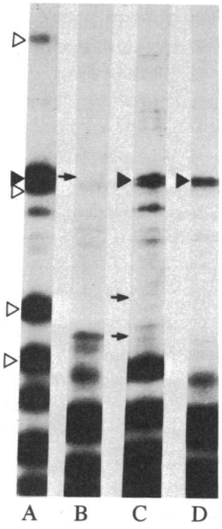

FIG. 4. Susceptibility of the 64-kDa protein to protease K. Fractions CM-I (0.8 A_{260} unit, 7.0×10^5 cpm) and CM-II (2.0 A_{260} units, 1.4×10^5 cpm) from cells labeled with [^{35}S]methionine in 0.1 ml of TKMD buffer were incubated with or without protease K at 0.02 mg/ml for 20 min on ice. After inhibition of the protease by adding 2 mM phenylmethylsulfonyl fluoride each sample was sedimented through a 10–30% sucrose gradient in TKM buffer and analyzed as in Fig. 3*B*. Representative lanes from each gel are shown: A and B, monosome–S-complex fraction of fraction CM-I; C and D, a fraction from fraction CM-II containing long polysomes, with no S complex. Lanes A and C, no protease K; lanes B and D, plus protease K. Autoradiograms were exposed for 14 days. Symbols as in Fig. 3*B*; arrows represent the positions where the 64-kDa (lane B) and the 41- and 36-kDa proteins (lane C) would have run.

DISCUSSION

Because the 64-kDa protein of the complexed membrane fraction (CM) of *B. subtilis* appeared to be involved in the attachment of the ribosomes to the membrane (4), it seemed likely to function in a complex of proteins, and we have used antibody to this protein to identify such a set (the S complex), of 64, 60, 41, and 36 kDa. This set of proteins was identified in two ways, either after cross-linking in cells by a cleavable reagent that can penetrate the membrane or after solubilization of the membrane of untreated cells by a nonionic detergent and dissociation of the ribosomes by EDTA.

To study the distribution of the S complex we employed a modification (3) of the earlier fractionation, using a triphasic sucrose gradient that yielded fractions CM-I and CM-II at successive steps (1.35–1.6 M and 1.6–2.0 M sucrose) in the gradient, instead of the earlier CM fraction at 1.35–1.8 M. When analyzed in a linear 10–30% sucrose gradient after solubilization of the membrane, the ribosomes of fraction CM-I were found to be almost entirely present in a 70S monosome peak, while fraction CM-II contained polysomes but few monosomes (Fig. 2). However, similar analysis without solubilization of the membrane showed that the ribosomes in these fractions were not all complexed with membrane, as previously assumed: most of the ribosomes of fraction CM-I were monosomes essentially free of lipid (Fig. 3*A*). Unexpectedly, the proteins of the S complex were found in that monosome peak rather than in membrane (Fig. 3*B*), as confirmed by immunoprecipitation.

The presence of the S complex and the monosomes in the same region of a gradient does not prove that they are attached. However, attachment has been demonstrated by cross-linking the S complex to ribosomal proteins (unpublished work). In addition, electron microscopy, in collaboration with Deirdre Furlong, has shown that anti-64-kDa IgG can cause aggregation of the monosome fractions described here but not of a control preparation of ribosomes without the S complex.

Because effective lysis required use of the French press, our monosome peak undoubtedly included fragmented polysomes as well as free ribosomes, and so we cannot accurately relate the S complex to the various classes of ribosomal particles in the cell. However, since the monosomes constituted well over half the total ribosomes recovered from our lysates, while other studies have shown that the polysomes comprise up to 90% of the ribosomes in exponentially growing bacteria, the heavy bands of the S complex seen in our monosome peaks (Fig. 3) suggest that a large fraction of the polysomal ribosomes in the cell carry the complex.

When we found earlier that the 64-kDa protein in the CM fraction is not accessible to protease until after treatment with EDTA (4) we assumed that this treatment was dissociating and releasing membrane-bound ribosomes and leaving the 64-kDa protein in the membrane. This assumption was shaken by the finding that much of the 64-kDa protein, in fraction CM-I, is associated with monosomes without membrane and is released by EDTA. However, further tests showed that the 64-kDa protein is indeed protected from protease when in the membrane–ribosome complexes of fraction CM-II, though not in the monosomes of fraction CM-I (Fig. 4). Clearly, some of the 64-kDa molecules in the cell are located at a site of close apposition of ribosomes to membrane, while others are not.

These molecules of the 64-kDa protein, located between the ribosomes and membrane, are not accompanied by the other proteins of the S complex. Moreover, in the cytosol,

where we verified the presence of the 64-kDa protein by examining preparations carefully freed of ribosomes, it was also not accompanied by the S complex. These findings suggest that the S complex plays a cyclic role in protein secretion, mediating initiation of that process by promoting attachment to the membrane. Moreover, it may well be that the 64-kDa protein is retained on the membrane in that cycle after the rest of the complex is released and then is recycled via the cytosol; but we have not established this second cycle. Curiously, even though the 64-kDa protein is associated (in the S complex) with membrane-free ribosomes, in the membrane–ribosome complexes it is evidently embedded (without the S complex) in the membrane, for dissociation and release of the ribosomes by exposure to low Mg^{2+} or puromycin (without Triton) leaves that protein with the membrane (1, 2).

In mediating the initiation of protein secretion, the S complex resembles the signal recognition particle (SRP) of eukaryotic cells, which was discovered and definitively related to the initiation of translocation by Walter and Blobel (5). On the other hand, there appear to be major differences. The SRP contains six polypeptides and a 7S RNA, while we have been unable to demonstrate a significant amount of RNA in the immunoprecipitated S complex (prepared with or without EDTA). Moreover, the SRP on the eukaryotic ribosome causes translational arrest at the emerging signal sequence of secreted proteins, and added membrane relieves this arrest (5, 9); but with an *Escherichia coli* system forming secretory proteins (10), we could not demonstrate translational arrest by *B. subtilis* S complex. However, bacteria are rich in RNase, and even though in some preparations we used inhibitors of RNases (bentonite and heparin), it is possible that the S complex as isolated has lost an RNA and/or a protein(s) and hence cannot produce the effects of the native complex.

Sucrose step gradients, run to equilibrium, have been used extensively for fractionation of membranes on the basis of density (11). The biphasic fractionation that we used previously (1), in an SW50.1 rotor, evidently also separated components on the basis of density (though it was not run to equilibrium), since it yielded a free membrane fraction (without ribosomes) at the sample–1.35 M sucrose interface and ribosomes free of lipid in the pellet (1), while the CM fraction at the 1.35–1.8 M interface probably consisted largely of membrane–ribosome complexes, separated by density from the other two fractions. Hence in the present experiments, using the SW41 rotor to accommodate larger samples and a triphasic gradient to produce a more refined fractionation (3), we were surprised to find a large number of monosomes, unattached to vesicles, in the CM-I fraction. Initially we considered the possibility that the density of the monosomes in fraction CM-I had been decreased by the attached S complex (perhaps including unrecognized lipid). However, on equilibrium density centrifugation (SW50.1 rotor, 35,000 rpm, 60 hr), after fixation by glutaraldehyde, these monosomes did not differ in position from ribosomes without S complex, from the pellet of a biphasic gradient (unpublished observation). Evidently the triphasic fractionation, which employed longer tubes and a lower centrifugal force than the biphasic fractionation, distributed particles more by S value than by density, and so the monosomes (in fraction CM-I) did not sediment as far as the polysome–membrane complexes (in fraction CM-II).

We have tested briefly for cross-reactivity with *E. coli*. Antibody to the 64-kDa protein of *B. subtilis* precipitated a complex of proteins (a 60-kDa band being the heaviest) from an S-100 extract of *E. coli* (12) in the presence of Triton and EDTA. However, the 70S region from a lysate of *E. coli*, unlike that from *B. subtilis*, did not yield a similar complex (unpublished observations).

We have briefly presented earlier the finding of a complex of proteins coprecipitated by antibody to the 64-kDa protein from *B. subtilis* (4, 13, 14). Adler and Arvidson (15) have recently reported similar findings in another Gram-positive bacterium, *S. aureus*: antibody to a 60-kDa protein (presumably from membrane) of that organism coprecipitates a set of proteins. Moreover, it also precipitates the 64-kDa protein and the associated complex of *B. subtilis*.

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