An *E. coli* Ribonucleoprotein Containing 4.5*S* RNA Resembles Mammalian Signal Recognition Particle

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The signal recognition particle (SRP) plays a central role in directing the export of nascent proteins from the cytoplasm of mammalian cells. An SRP-dependent translocation machinery in bacteria has not been demonstrated in previous genetic and biochemical studies. Sequence comparisons, however, have identified (i) a gene in *Escherichia coli* (*ffh*) whose product is homologous to the 54-kilodalton subunit (SRP54) of SRP, and (ii) an RNA encoded by the *ffs* gene (4.5*S* RNA) that shares a conserved domain with the 7*SL* RNA of SRP. An antiserum to Ffh precipitated 4.5*S* RNA from *E. coli* extracts, implying that the two molecules reside in a complex. The 4.5*S* RNA can also bind to SRP54 and can replace 7*SL* RNA in an enzymatic assay. The product of a dominant mutation in the *ffs* gene (4.5*S* RNA^{dl1}) is also coprecipitated by the antiserum to Ffh protein and is lethal when expressed from an inducible promoter. After induction of 4.5*S* RNA^{dl1}, the earliest observed phenotype was a permanent induction of the heat shock response, suggesting that there was an accumulation of aberrant proteins in the cytoplasm. Late after induction, translocation of β-lactamase was impaired; this may be an indirect effect of heat shock, however, because translocation of ribose binding protein or of the porin, OmpA, was unaffected. An unusual separation of the inner and outer membranes, suggestive of a defect in cell envelope, was also observed. Protein synthesis did not cease until very late, an indication that 4.5*S* RNA probably does not have a direct role in this process.

IN MAMMALIAN CELLS THE SYNTHESIS OF ESSENTIALLY ALL secretory proteins occurs on ribosomes bound to the membrane of the endoplasmic reticulum (ER). The signal recognition particle (SRP), an 11*S* cytoplasmic ribonucleoprotein (RNP) consisting of six distinct proteins and one RNA subunit, and the SRP receptor (also termed docking protein), a heterodimeric ER membrane protein, function as adaptors between the cytoplasmic translation apparatus and the membrane bound protein translocation machinery (*1*). Together, SRP and SRP receptor catalyze the events that lead to the formation of a proper ribosome-membrane junction. Initially, the 54-kD subunit of SRP (SRP54) binds to the signal sequence on the nascent protein as it emerges from the ribosome (*2*). Next, the complex of SRP, nascent chain and ribosome interacts with the SRP receptor at the ER membrane. In a guanosine triphosphate (GTP)–dependent reaction, the ribosome and nascent protein are transferred to other, less well defined components in the membrane, and transport occurs concomitantly with polypeptide chain elongation (*3*).

Unlike mammalian cells, both yeast and bacteria have a posttranslational mode of translocating many proteins (*4, 5*). Protein precursors bearing signal sequences (indistinguishable from, and functionally interchangeable with, those of mammalian secretory proteins) are released from the ribosomes into a soluble cytoplasmic pool. Preproteins are bound to chaperonins, which are thought to maintain them in a soluble and loosely folded state (*6, 7*) where the signal sequence remains accessible for interaction with a membrane-bound translocation apparatus (translocon). There is some evidence that the chaperonins are in part functionally redundant; some are expressed constitutively (such as SecB), whereas others can be induced over a basal level by externally imposed conditions such as heat shock (*6*). Although genetic and biochemical studies have shown that chaperonins and membrane proteins may be involved in the translocation pathway (*8–10*), neither approach has identified. SRP or SRP receptor-like components have not been identified in these organisms. Surprisingly, homologues of SRP54 and the SRP receptor have been identified in *Escherichia coli* [the genes *ffh* and *ftsY*, respectively (*11, 12*)] and yeast (*13*) because of their sequence similarity. In addition to these protein homologues, a domain of 7SL RNA (Fig. 1, top, domain IV) can be found in small RNA's from yeast, archaebacteria, and bacteria (*14*). In *E. coli*, the conserved domain IV is present in the abundant 4.5S RNA (Fig. 1, center). The three *E. coli* SRP homologues described so far are essential for viability (*15–17*). Given these similarities, we undertook studies to determine whether the products of the *ffs* and *ffh* genes interact with each other and whether they may constitute a protein targeting apparatus in *E. coli*.

Association of 4.5*S* RNA with Ffh in *E. coli*. An antibody to a fusion protein consisting of a fragment of Ffh fused to glutathione transferase specifically recognizes an *E. coli* 48-kD protein, the size expected for the *ffh* gene product (*18*). Indirect immunoprecipitations were performed under native conditions from extracts of ^{32}P-labeled cells. RNA extracted from the immunoprecipitates was analyzed directly by polyacrylamide gel electrophoresis (PAGE) (Fig. 2A). The RNA recovered from immunoprecipitates of unlabeled cells was also analyzed by RNA (Northern) blots hybridized with a 4.5*S* RNA specific oligonucleotide (Fig. 2B). The immune serum (Fig. 2, A and B, lanes 2), but not the preimmune serum (Fig.

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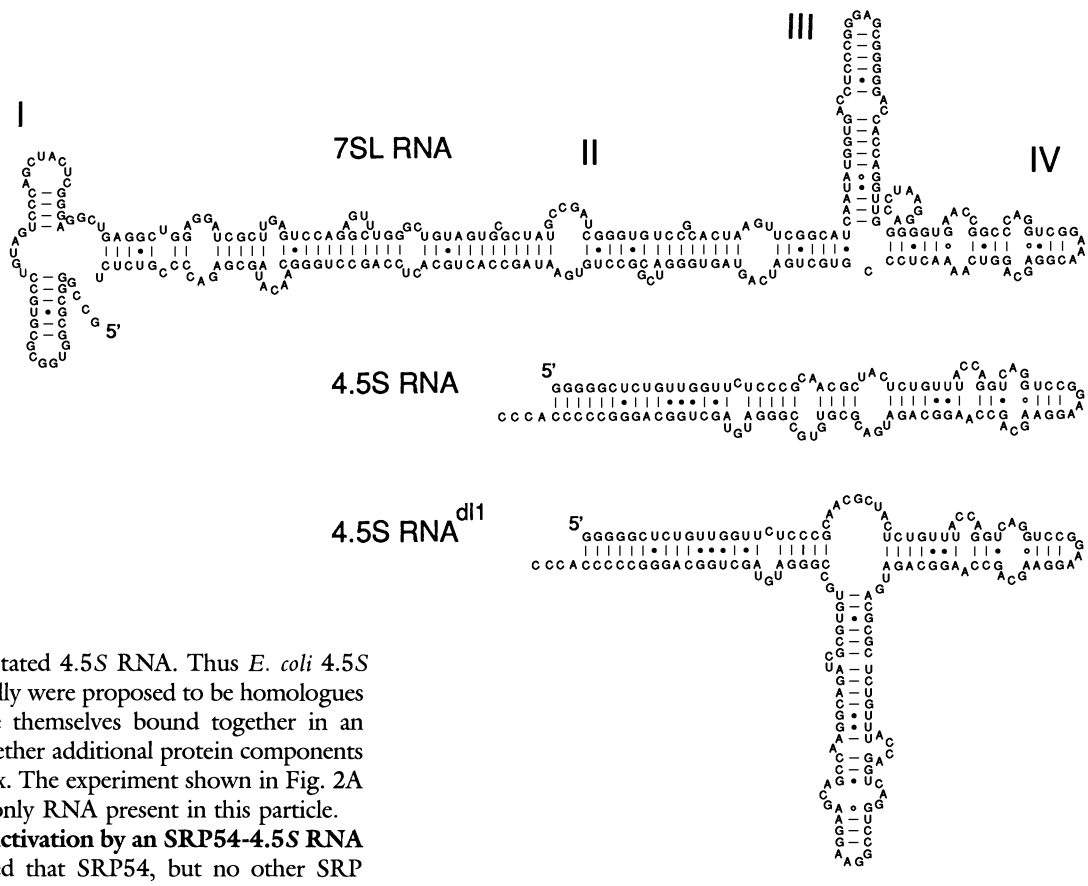

Fig. 1. Proposed secondary structures of human 7*S*L RNA (Top), *E. coli* 4.5*S* RNA (Center) and 4.5*S* RNA^{dl1} (Bottom). U·G and A·G base pairs are indicated by closed circles and open circles, respectively. Domains I to IV of the 7*S*L RNA are indicated (*14*). An alternative secondary structure for 4.5*S* RNA^{dl1} consists of a single long stem containing one internal copy of the domain IV motif.

2B, lane 3), specifically precipitated 4.5*S* RNA. Thus *E. coli* 4.5*S* RNA and Ffh, which individually were proposed to be homologues of essential SRP subunits, are themselves bound together in an RNP. We do not yet know whether additional protein components are associated with this complex. The experiment shown in Fig. 2A implies that 4.5*S* RNA is the only RNA present in this particle.

Guanosine triphosphatase activation by an SRP54-4.5*S* RNA complex. We recently observed that SRP54, but no other SRP protein, binds tightly to 4.5*S* RNA under stringent high salt conditions (*19*). This result was unexpected because efficient binding of SRP54 to mammalian 7*S*L RNA has been observed only when SRP19 (by itself an RNA binding protein) was also bound (*12, 20*). It may be that parts of mammalian 7*S*L RNA [such as domain III (*14*)] that are absent in 4.5*S* RNA may partially obscure the SRP54 binding site, whereas the binding of SRP19 to the RNA relieves the interference. Since the only resemblance between mammalian 7*S*L RNA and *E. coli* 4.5*S* RNA lies in the domain IV, motif, we think it likely that SRP54 and Ffh bind to their cognate RNAs through this conserved stem.

To determine whether the binding of prokaryotic 4.5*S* RNA to mammalian SRP54 functionally mimics the putative interaction between 7*S*L RNA and SRP54, we used a guanosine triphosphatase (GTPase) assay. SRP54 and both subunits of the SRP receptor are GTP binding proteins (*21*), yet neither SRP nor SRP receptor have significant GTPase activity by themselves. Rapid GTP hydrolysis was observed, however, when both SRP and SRP receptor were combined. This constitutes a sensitive biochemical assay for a functional interaction of SRP with SRP receptor. Using partially reconstituted SRP's, we determined that a particle consisting only of 7*S*L RNA, SRP54, and SRP19 is sufficient to elicit GTPase activity in the presence of SRP receptor (*22*). Moreover, we observed that SRP19 was dispensable, indicating that SRP54 bound to 7*S*L RNA directly under the low salt conditions of this assay. Because *E. coli* 4.5*S* RNA bound very efficiently to SRP54, we asked whether the chimeric SRP54-4.5*S* RNA particle could replace the partially reconstituted SRP (Fig. 3). Only the reaction containing SRP54-4.5*S* RNA and SRP receptor (Fig. 3, lane 8), but not either component alone (Fig. 3, lanes 2 to 4), nor any pairwise combination of the three components (Fig. 3, lanes 5 to 7), was active in hydrolyzing GTP. An unrelated control RNA, calf liver transfer RNA (Fig. 3, lane 12), could not replace 4.5*S* RNA in this assay.

A dominant lethal 4.5*S* RNA mutant. To study the physiological role of 4.5*S* RNA in vivo, we constructed a mutant allele of the *ffs* gene in which the phylogenetically conserved domain IV has been duplicated (*23*) (Fig. 1, bottom). We reasoned that dominant mutations could be created in this way because domain IV might contain an important site whose duplication might sterically impede function. Analysis of a dominant mutant allele would have advantages over gene disruption experiments because mutant RNA produced from a strong promoter would accumulate rapidly as a stable toxic product. Thus, phenotypic changes should occur more rapidly, compared to those following shut off of 4.5*S* RNA synthesis (*15, 24*). In the latter case, the stable RNA first must be diluted beyond a minimal threshold by cell division and hence may complicate the analysis of primary defects.

When the wild-type *ffs* gene was coupled to the strong tac promoter and induced by isopropyl-β-D-thiogalactopyranoside (IPTG), there was a rapid accumulation of 4.5*S* RNA but no change in the growth rate (Fig. 4A, open squares) (*25–27*). However, expression of the mutant 4.5*S* RNA from the same plasmid led to an inhibition of cell growth (Fig. 4A, filled squares). Cell lysis was observed by light microscopy analysis and by a reduction in light scattering. The plating efficiency on IPTG of cells carrying the mutant plasmid was reduced by a factor of more than 10^6. This indicated that induction of the mutant 4.5*S* RNA was a lethal event, rather than a transient growth arrest. We refer to this mutant allele of the 4.5*S* RNA as *ffs*^{dl1}, and the RNA gene product as 4.5*S* RNA^{dl1} (*d*ominant *l*ethal).

We next examined the association of 4.5*S* RNA^{dl1} with Ffh in vivo. RNA analysis indicated that 4.5*S* RNA^{dl1} accumulated as a stable RNA (Table 1) in cells bearing the *ffs*^{dl1} gene. Antibodies to Ffh specifically immunoprecipitated both 4.5*S* RNA^{dl1} and the wild-type 4.5*S* RNA (Fig. 2A, lane 5, and Fig. 2B, lanes 5 and 6) from these extracts. This result indicated that 4.5*S* RNA^{dl1} resembles wild-type 4.5*S* RNA sufficiently to assemble with Ffh. The

lethal phenotype of 4.5*S* RNA^{dl1} induction, however, suggests that this complex must be defective in at least one essential function.

Induction of the stress response by 4.5*S* RNA^{dl1}. Cells carrying the *ffs*^{dl1} allele contained about half a copy of 4.5*S* RNA^{dl1} per copy of wild-type 4.5*S* RNA as a result of readthrough transcription from the uninduced promoter (Fig. 2A, lane 5). These cells show no discernable aberrant phenotype (*26*). The first observable change was an induction of the characteristic set of heat shock proteins (Fig. 5A), which occurred by 15 minutes after the addition of IPTG. At that time, induced cells had accumulated approximately equimolar amounts of 4.5*S* RNA^{dl1} and 4.5*S* RNA (Table 1). Since overproduction of the wild-type 4.5*S* RNA had no observable effect on cell physiology (*25–27*), heat shock induction was not due simply to the overexpression of a stable RNA species. Heat shock induction reached a maximum 30 minutes after the addition of IPTG. Immunoprecipitation of a protein specifically induced by heat shock (groEL) (Fig. 5B) from the ^{35}S-labeled extracts demonstrated that synthesis of heat shock proteins was quantitatively indistinguishable from that observed in cells shifted to high temperature (Fig. 5B, lane 1). Furthermore, it was not a transient response as would be observed after shifting cells to 42°C. Instead, the stress response

Table 1. Time course of 4.5S RNA^{dl1} induction in S971(pSN3). The molar ratio of mutant 4.5*S* RNA^{dl1} to wild-type 4.5S RNA (mt/wt) was estimated by visual comparison of ethidium bromide staining of RNA in native gels as described (*25*) and by comparison with standards. RBP, ribose binding protein; MBP, maltose binding protein; N.D. not determined.

Time (min)	RNA (mt/wt)	Phenotype
0	0.5	Wild type
15	1	Heat shock induced
30	2	Heat shock fully induced No effect on overall protein synthesis No effect on growth
60	5	Defect in pre–β-lactamase, but not in pre-RBP or pre-OmpA translocation
120	9	Inhibition of synthesis of lamB and MBP
180	N.D.	Outer and inner membranes separate Heat shock remains fully induced Still no effect on overall protein synthesis
240	N.D.	Filamentous cells form Protein synthesis and cell growth cease

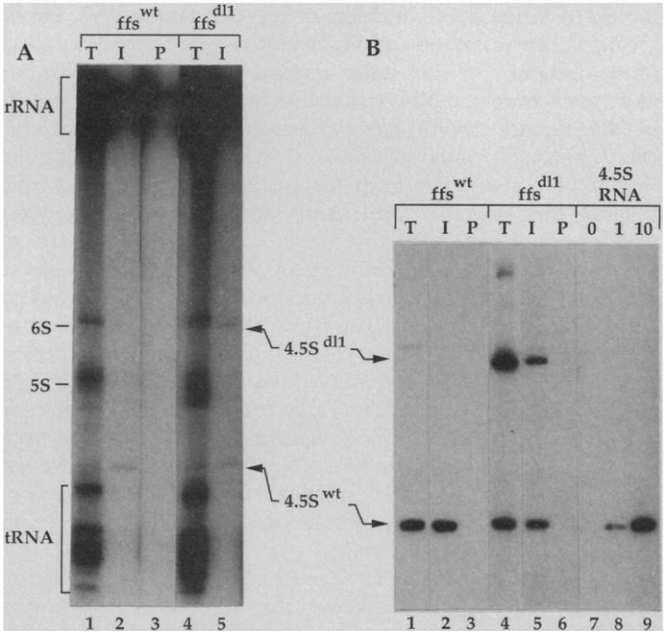

Fig. 2. Immunoprecipitation of 4.5*S* RNA and 4.5*S* RNA^{dl1} from native *E. coli* lysates. Lysates were prepared from S971 cells containing either pSN0 [tac vector (*23*)] or pSN3 (*ffs*^{dl1}) as indicated. Cells were grown in the presence of [^{32}P]phosphate (**A**) or in LB medium (**B**) (*40*). RNA was prepared from the total lysate [T, 7.5 μl of lysate (A) or 30 μl (B)] or from immunoprecipitates [30 μl of lysate (A) or 100 μl (B)] produced with preimmunine antiserum (PI) or antiserum to Ffh (I). The RNA samples were analyzed by electrophoresis on a 10 percent nondenaturing polyacrylamide gel. In (A) the RNA was visualized directly by autoradiography. In (B) RNA was transferred to a nylon membrane and hybridized with a 5′ end-labeled (^{32}P) oligonucleotide probe complementary to 4.5*S* RNA. Lanes 7 to 9 in (B) contain 0, 1, and 10 ng of purified 4.5*S* RNA, respectively.

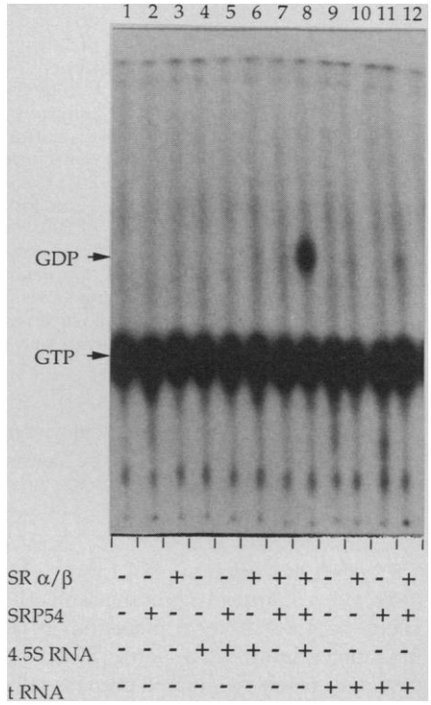

Fig. 3. A chimeric particle consisting of *E. coli* 4.5*S* RNA and mammalian SRP54 elicits GTPase activity in the presence of SRP receptor. Reconstitution reactions were performed in which SRP54 was mixed with 4.5*S* RNA or tRNA (400 nM each) (*41*). In some reactions, either SRP54 or RNA was omitted. GTPase assay contained 20 nM SRP receptor (*41*) and 20 nM reconstituted SRP54-4.5*S* RNA, and were performed at 25°C in 50 mM triethanolamine–acetic acid (pH 7.5), 50 mM postassium acetate, 2.5 mM $Mg(OAc)_2$, 1 mM DTT, 0.1% Nikkol, and 1 μM [α-^{32}P]GTP. An 0.5-μl portion of each reaction was analyzed by chromatography on polyethyleneimine thin layer plates, which were developed in 0.75 M KH_2PO_4-H_3PO_4, pH 3.3 and autoradiographed.

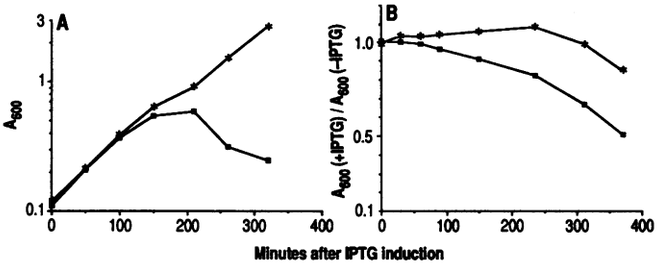

Fig. 4. Growth of cells after expression of wild-type and dominant lethal alleles of 4.5*S* RNA. (**A**) Cultures of S971 carrying either pSN1 (tac-*ffs*, asterisks) or pSN3 (*ffs*^{dl1}, closed squares) were diluted into minimal medium (M63 plus all the amino acids except methionine and cysteine, 0.2 percent glycerol, 0.2 percent maltose, and ampicillin at 50 μg/ml) and grown at 37°C to an optical density between 0.1 and 0.2 A_{600}, at which point 1 mM IPTG was added to induce expression of the two *ffs* alleles (time, 0 minute). Cell growth was monitored by measuring A_{600}; the cultures were diluted to keep the A_{600} between 0.2 and 1.0. (**B**) Isogenic strains (23) MC4100 htpR$^+$ (pSN4) (*ffs*^{dl1} carrying lacI^Q, asterisks) and MC4100 htpR15 (pSN4) (filled squares) were grown at 30°C as described in (A). At time "0 min", 1 mM IPTG was added to half of the cells and optical density was monitored in both induced (A_{600}, with IPTG) and uninduced (A_{600}, without IPTG) cultures. The ratio of optical densities is shown. The effects of *ffs*^{dl1} expression take longer to observe in (B) than in (A) because the high level of lacI^Q produced from pSN4 inhibits full induction of the tac promoter.

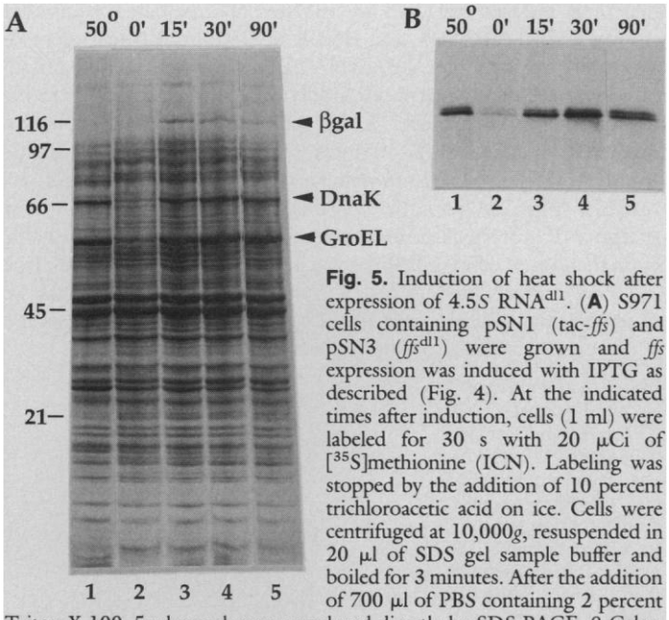

Fig. 5. Induction of heat shock after expression of 4.5*S* RNA^{dl1}. (**A**) S971 cells containing pSN1 (tac-*ffs*) and pSN3 (*ffs*^{dl1}) were grown and *ffs* expression was induced with IPTG as described (Fig. 4). At the indicated times after induction, cells (1 ml) were labeled for 30 s with 20 μCi of [^{35}S]methionine (ICN). Labeling was stopped by the addition of 10 percent trichloroacetic acid on ice. Cells were centrifuged at 10,000*g*, resuspended in 20 μl of SDS gel sample buffer and boiled for 3 minutes. After the addition of 700 μl of PBS containing 2 percent Triton X-100, 5-μl samples were analyzed directly by SDS-PAGE. β-Galactosidase (βgal), DnaK, and GroEL are indicated on the right. (**B**) This extract (100 μl) was used for immunoprecipitations with antibodies to GroEL (*42*). Gel electrophoresis of the supernatant from the immunoprecipitation showed that GroEL was quantitatively recovered.

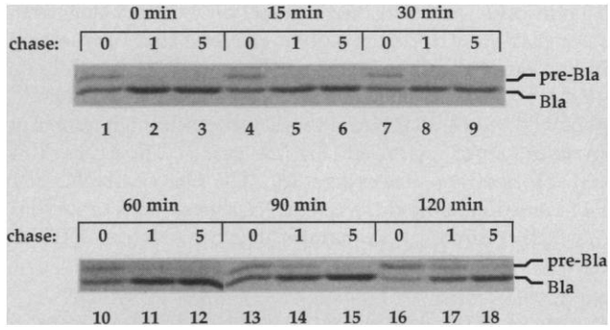

Fig. 6. Delay of translocation of β-lactamase until late after induction of 4.5*S* RNA^{dl1} expression. Expression of S971-SN3 (*ffs*^{dl1}) was induced with IPTG as described (Fig. 5). At the indicated times after induction, cells were pulse-labeled with [^{35}S]methionine as described (Fig. 4). After 30 s, nonradioactive 1 mM methionine was added. After the indicated chase periods (0, 1, or 5 minute) cells were processed for immunoprecipitation with antibody to β-lactamase as described (Fig. 5B).

remained permanently induced, suggesting that the cells had been subjected to a harsher and more continuous insult of the sort observed after a shift of cells to 50°C (*28*). Heat shock induction has also been observed in cells depleted of 4.5*S* RNA; however, in this case induction was delayed until 2.5 to 3.5 hours after shutoff of 4.5*S* RNA transcription (*29*).

To assess influence of heat shock on cell growth, we evaluated the effects of 4.5*S* RNA^{dl1} induction in cells that are defective in mounting a heat shock response [htpR$^-$ cells (*30*)]. The amount and pattern of newly synthesized proteins in htpR$^-$ cells at intervals up to 6 hours after induction of 4.5*S* RNA^{dl1} was indistinguishable from that of uninduced cells (*26*). The ability to induce the heat shock regulon, however, prolonged the ability of cells to grow after induction of the toxic 4.5*S* RNA^{dl1} (Fig. 4B).

The heat shock response has been thought to be induced by the accumulation of misfolded proteins (*31*). Given the structural resemblance of Ffh and 4.5*S* RNA to SRP components, we reasoned that misfolded proteins could result from a defect in a targeting function of the Ffh-4.5*S* RNP. Periplasmic or membrane proteins may fail to become targeted to their proper intracellular locus and accumulate as misfolded precursors in the cytoplasm. A precedent for such an effect is that cells defective in the membrane translocation apparatus (*32*) induce heat shock in response to cytoplasmically retained preproteins. We therefore tested directly whether the translocation of periplasmic or membrane proteins would be affected by induction of 4.5*S* RNA^{dl1}.

We did not observe translocation defects for a periplasmic protein (ribose binding protein, RBP) or an outer membrane protein (OmpA) when we tested for precursor accumulation after short pulse labeling with [^{35}S]methionine and subsequent immunoprecipitation with specific antibodies to those proteins (*26*). In contrast, we observed accumulation of pre–β-lactamase, the precursor of the periplasmic protein β-lactamase, in a similar labeling experiment (Fig. 6, compare lanes 1 to 3 with 16 to 18). This translocation defect occurred 60 to 90 minutes after 4.5*S* RNA^{dl1} induction (Fig. 6, compare lanes 11 and 14 to lane 8), whereas the heat shock response was detectable 15 minutes after 4.5*S* RNA^{dl1} induction (Fig. 5). Because of this lag time between heat shock induction and pre–β-lactamase accumulation, it is, however, questionable whether the defect results directly from 4.5*S* RNA^{dl1} induction.

The observation of a block in β-lactamase secretion was especially surprising in that this protein translocated efficiently posttranslationally (*4*). GroEL has been shown to serve as a chaperonin for β-lactamase by binding to pre–β-lactamase before its translocation (*33*). It has also been suggested that an inability to mount a heat shock response can lead to a defect in β-lactamase translocation (*34*). It is thus conceivable that insufficient available GroEL would cause the observed translocation defect of pre–β-lactamase (*35*). Hence the defect in translocation of β-lactamase may be a secondary effect of the disruption of 4.5*S* RNA function. Further support for this idea comes from the observation that, not long after the pre–β-lactamase translocation defect was first detected, other pleiotropic effects were also observed (Table 1). These effects included the shutoff of transcription from the maltose regulon, presumably resulting from depressed cyclic AMP (adenosine monophosphate) levels (*36*).

At late times in the experiment, an aberrant separation of the outer and inner membrane was also evident by electron microscopy (Fig. 7, compare A with B). This could be due to an impairment of membrane protein biogenesis, culminating in the observed defects in cell envelope. Still later, a block in cell division caused the appearance of filamentous cells. Taken together, these results show that the induction of heat shock is kinetically well separated from other events that occur after 4.5*S* RNA^{dl1} induction. We have not yet determined the primary stimulus of heat shock induction, although presumably it involves the improper folding of certain proteins during their biogenesis.

Function of Ffh-4.5*S* RNP. It has been suggested previously, on the basis of two lines of evidence, that 4.5*S* RNA performs an essential role in protein synthesis. First, the kinetic analysis of cell death in 4.5*S* RNA depletion experiments showed that protein synthesis ceased before DNA synthesis and cell growth (*15, 24*). In contrast, our data, as well as that in (*29*) indicate that heat shock as well as other pleiotropic defects precede cessation of protein synthesis. Hence, the Ffh-4.5*S* RNP is not likely to be required for maintenance of translational capability. Second, extragenic suppressors of an *ffs* mutant map to specific components of the translational apparatus (*37*). In particular, suppression of the lethality caused by amounts of 4.5*S* RNA below those required for cell growth was achieved by mutations either in the gene encoding elongation factor

G or in any of several genes encoding tRNA synthetases. A common feature of these suppressors is that they slow down a specific step in the elongation cycle of protein synthesis, namely the step after translocation of the nascent peptidyl-tRNA from the A to the P site in the ribosome but before exit of tRNA from the E site (*37*). Presumably the suppressor mutations increase the time window during which the particle that contains 4.5*S* RNA can interact with the ribosome. While these data are consistent with models involving protein elongation, they are equally consistent with another model discussed below.

We and others (*38*) have observed an inhibition of β-lactamase translocation at late time points after 4.5*S* RNA function had been perturbed. For the reasons described above this is most likely an indirect effect of heat shock and not evidence of an SRP activity in *E. coli*. If the Ffh-4.5*S* RNP particle is required for translocation of only a small subset of proteins, we would not have observed a widespread translocation defect and a more extensive search is required to find the correct substrates.

Our biochemical data indicate that the Ffh-4.5*S* RNP structurally resembles mammalian SRP and that, in at least one assay, the 4.5*S* RNA of *E. coli* is able to replace mammalian 7*S*L RNA. Thus it seems reasonable to propose that, by analogy with the mammalian SRP, the Ffh-4.5*S* RNP recognizes signal sequences on nascent polypetide chains (through direct binding to Ffh) and targets them

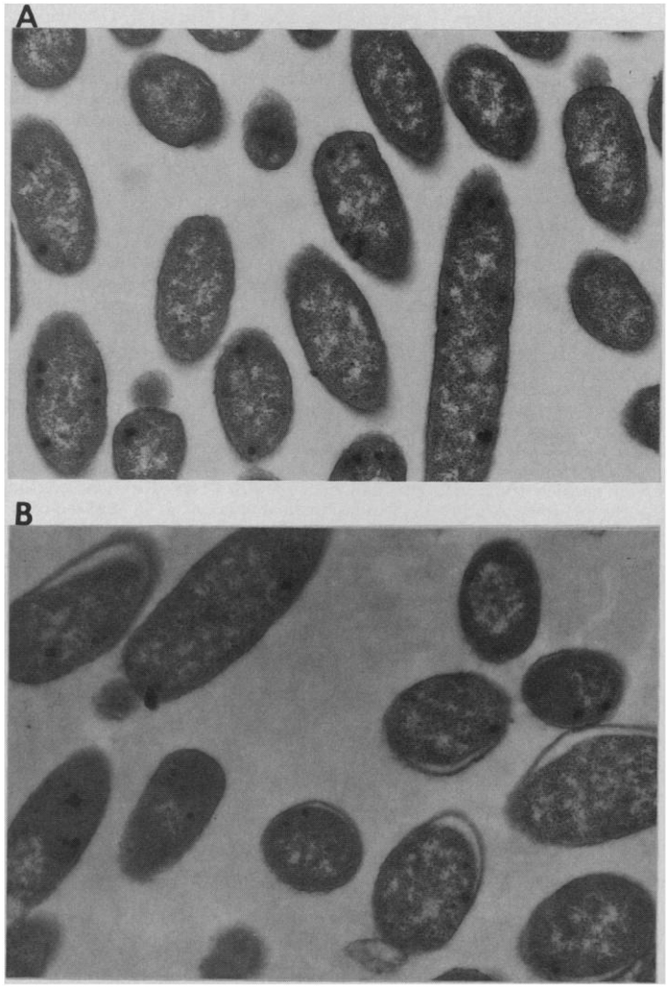

Fig. 7. Electron microscopy of *E. coli* expressing wild-type 4.5*S* RNA and 4.5*S* RNA^{dl1}. S971 carrying pSN1 (tac-*ffs*, A) or pSN3 (*ffs*^{dl1}, B) were grown in the presence of IPTG for 3 hours (Fig. 4) before being prepared for electron microscopy (*43*).

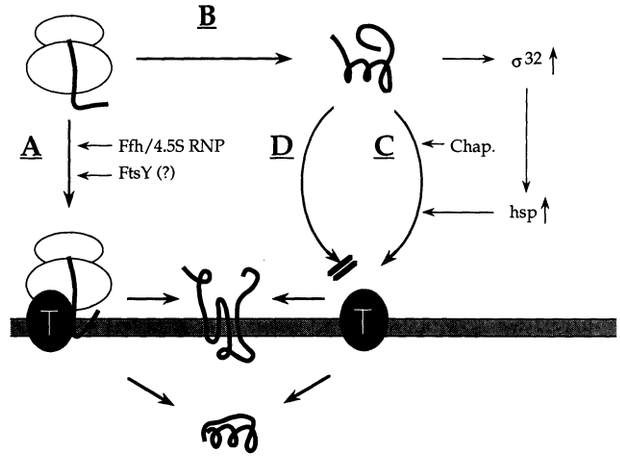

Fig. 8. Model of Ffh-4.5*S* RNP action. T, Translocon, composed of the membrane associated components of the translocation apparatus (SecA, SecY, SecE); σ^{32}, product of the htpR gene; hsp, heat shock proteins induced by σ^{32}; Chap, chaperonins (*6*).

cotranslationally to a defined intracellular locus for further processing. The concept that Ffh-4.5*S* RNP interacts with nascent chains is consistent with the above genetic analysis and is supported by the observation that the fraction of 4.5*S* RNA that is bound to polysomes can be released by puromycin, an agent that releases nascent proteins from ribosomes (*37*).

If the Ffh-4.5*S* RNP defines a prokaryotic SRP involved in membrane targeting and secretion, two major issues must be resolved, namely (i) how an SRP-dependent targeting pathway relates to the well-established posttranslational translocation pathway, and (ii) why neither *ffh*, *ffs*, or *ftsY* were identified during the extensive genetic analyses of the secretion pathways in *E. coli*. Keeping these questions in mind, we present a molecular model that could account for the observed phenomena.

According to the model (Fig. 8), the Ffh-4.5*S* RNP provides an obligatorily cotranslational activity in pathway A, which recognizes signal sequences on certain nascent polypeptide chains. The role of the RNA moiety may be to interact with the ribosome and to position the presumed signal sequence binding site of Ffh in order to ensure efficient recognition. The signal sequence would only be released from Ffh after interaction with an appropriate receptor, which would ensure that the interaction of the signal sequence and ribosome with the translocon is properly set up. Delivery of the ribosome–nascent chain complex to the translocon might be mediated by the putative SRP receptor homologue, FtsY.

If the Ffh-4.5*S* RNP function is perturbed, the preprotein is nevertheless synthesized and released from the ribosome (Fig. 8, path B). Some preproteins might undergo posttranslational translocation, perhaps if chaperonins can bind to them and maintain translocation competence (path C). Alternatively, some preproteins may be incapable of posttranslational translocation altogether (path D). Accumulation of such mistargeted and hence misfolded secretory or membrane (pre)proteins in the cytoplasm would induce heat shock. Induction of heat shock helps cells to survive longer (Fig. 5C), probably by partially relieving the distress resulting from improper protein targeting by stimulating the posttranslational pathway (*39*) or protein degradation. Hence path C is used as a rescue pathway for substrates that are inefficiently targeted cotranslationally. Alternatively, but not mutually exclusively, path A and path B-C are both operational, each for a distinct set of substrates under normal physiological conditions (see below).

In this view the likelihood of identifying *ffh* and *ffs* in a genetic screen would be dependent on the pathways used by the test

substrate for export. Analysis is further complicated because chaperonins can partially replace one another and include components that are not under heat shock control (such as *secB*) (*39*). If the analyses are limited to the study of passenger proteins that can effectively use a posttranslational pathway, then no requirement for an SRP-like function would be detected. Furthermore, if only a few proteins have an absolute requirement for the cotranslational pathway, then blocking Ffh or 4.5*S* RNA function would not necessarily lead to a vast accumulation of presecretory proteins. Hence *ffh* and *ffs* might be overlooked in genetic screens that assume substantial preprotein accumulation, such as those that detect up-regulation of *secA* expression (*9*). In contrast, genetic analysis has been most successful in yeast and in *E. coli* in defining components of the translocon (SecA, SecY, SecE) (*10*), where path A and path B-C are proposed to converge.

Implicit in this model is the assumption that nascent chains that utilize path A can be distinguished from those that utilize path B-C. The distinguishing feature could be encoded in the structure of the signal sequences that bind with different affinities to components of either pathway (7). Alternatively, discrimination could be kinetically controlled. Perhaps reduction in the rate of nascent chain elongation after exposure of the signal sequence (for example, by the use of rare codons) would favor interactions with the Ffh-4.5*S* RNP. If so, changes in cell physiology that affect the rate of protein synthesis could influence the degree to which each targeting pathway is utilized.

If our model is correct, then SRP-dependent protein targeting is an ancient mechanism for localizing proteins during their synthesis. By coupling protein synthesis with membrane translocation, such a targeting mechanism would alleviate constraints that particular folding characteristics might impose on their translocation. Certain proteins, most prevalent in unicellular organisms, have evolved so that they can be maintained translocation competent through interactions with other molecules (chaperonins) that keep them from misfolding. Once the ability of a particular protein to utilize a posttranslational pathway has been optimized through evolution, SRP-dependent targeting would become less important for that protein. SRP-dependent targeting must have remained obligatory for a class of as yet unidentified proteins, however, since the yeast and *E. coli* SRP and SRP receptor homologues are essential for cell viability.

It is conceivable that cellular processes other than secretion or membrane integration require cotranslational targeting and also use the Ffh-4.5*S* RNP. Such processes could include, for example, the transfer of proteins from the ribosome to chaperonins, in cases where the interaction of the protein with chaperonins is required for subsequent events, like folding, oligomerization or posttranslational membrane translocation. In this case release from the ribosome could lead to rapid misfolding that would effectively prevent chaperonins from binding and carrying out their metabolic function. If such a function could be demonstrated for the Ffh-4.5*S* RNP in *E. coli*, then mammalian SRP might also have a broader function that previously appreciated.

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6. We use the term "chaperonin" to refer to a set of proteins that may have diverse recognition properties. A given chaperonin could recognize either the signal sequence directly (or portions of the mature protein in an unfolded conformation, or both (7) [L. L. Randall, T. B. Topping, S. J. S. Hardy, *Science* **248**, 860 (1990)]. While SecB may be dedicated to the secretion process, other chaperonins (like GroEL, DnaK) are involved in other cellular processes as well and may substitute for SecB in a less specific manner; see H. D. Bernstein, T. A. Rapoport, P. Walter, *Cell* **58**, 1017 (1989).
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21. SRβ contains a GTP binding site as judged by sequence analysis. GTP binding to all three proteins (SRα, SRβ, and SRP54) was experimentally demonstrated by ultraviolet light (UV) cross-linking of ^{32}P-labeled GTP to the respective proteins [T. Connolly and R. Gilmore, *Cell* **57**, 599 (1989); J. Miller and P. Walter, unpublished data].
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23. The plasmids used in our work were pSN0 [pKK223-3 of (*25*)], which contains the tac promoter adjacent to a polylinker and terminator in a pBR322-based vector; pSN1 [pKK235b-15 of (*25*)], which is derived from pKK223-3 by the insertion of the wild-type *ffs* gene into the Eco RI site in the polylinker; pSN3, which is derived from pKK235b-15 by the insertion of a double-stranded oligonucleotide into the *ffs* gene sequence to create the *ffs*^{dl1} allele; and pSN4, which is derived from pSN3 by insertion of the lacI^{Q} gene into the single Pvu II site. The 30-nt synthetic oligonucleotide oSN3 [4.5*S* RNA nucleotide sequence 31 to 60 (*27*)] and oSN7 (4.5*S* RNA anti-sense sequence 75 to 46) were annealed, filled in with the Klenow fragment of *E. coli* DNA polymerase, and ligated in molar excess to pSN1, which had been cut Mlu I and filled in with Klenow fragment. This ligation was transformed into S971 [HfrH lacI^{Q}, of (*37*)] and ampicillin-resistant colonies were screened for their inability to grow on media containing 1 mM IPTG. Sequencing of DNA confirmed that the Mlu I site had been filled in, and the 4.5*S* RNA sequence from 31 to 75 was inserted into that site in the sense orientation. This increased the size of 4.5*S* RNA from 114 to 163 bases. pSN4 was constructed from pSN3 by cloning an Eco R1 fragment containing the lacI^{Q} gene from pFSIQ [F. Stevenson and I. Kuhn in *Vectors: A Survey of Molecular Cloning Vectors and Their Uses.*, R. L. Rodriguez and D. T. Denhardt, Eds. (Butterworth, Boston, 1988)] into the Pvu II site. Both pSN0 and pSN1 were provided by J. Brosius, and S971 was provided by S. Brown.
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30. HtpR (also known as rpoH) encodes σ^{32}, the sigma factor responsible for induction of the heat shock regulon. The isogenic strains MC4100 htpR^{+} and MC4100 htpR15 [T. Tobe, K. Ito, T. Yura, *Mol. Gen. Genet.* **195**, 10 (1984)] used in our work were constructed and provided by K. Tilly (Rocky Mountain Laboratory, NIH-NIAID, Hamilton, Montana).
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35. The mechanism might resemble that of heat shock induction per se. Current models suggest that the induction occurs via stabilization of σ^{32} (*30*). In unstressed cells σ^{32} is thought to be rapidly degraded via a pathway that is mediated by heat shock proteins. The binding of misfolded proteins to heat shock proteins competes with σ^{32} degradation, thus leading to increased levels of σ^{32} and consequently heat shock protein synthesis (*31*). Similarly, misfolded proteins, as yet unidentified, produced as a consequence of perturbed 4.5*S* RNA function induction might bind GroEL and thus make it unavailable for interaction with pre-β-lactamase.
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39. Similarly, it was shown that induction of heat shock proteins can at least partially replace a requirement for SecB, a protein that is essential in path C when cells are grown in rich medium (E. Altman and S. Emr, personal communication).
40. S971(pSN0) (tac vector) or S971(pSN3) (ffs^{dfl}) were grown to midlog phase either in low phosphate medium [H. M. Goodman, J. N. Abelson, A. Landy, S. Zadrazil, J. D. Smith, *Eur. J. Biochem.* **13**, 461 (1970)] for 1 hour in the presence of 0.32 mCi of [^{32}P]phosphate or in LB medium. Cells (1 ml) were poured over ice, centrifuged, washed in cold water, and frozen in liquid nitrogen. The cells were thawed in 1 ml of native buffer [50 mM triethanolamine–acetic acid (pH 7.5), 100 mM potassium acetate, 16 mM magnesium acetate, 1 mM EDTA, 0.1 percent Nikkol detergent, 1 mM dithiothreitol (DTT)] containing: 0.5 mM phenylmethylsulfonyl fluoride, Trasylol at 1000 U/ml, and leupeptin, pepstatin, chymostatin, and antipain at 1 μg/ml each. After sonication with a microprobe (Branson Sonifier) for two 10-s pulses, the lysate was clarified by centrifugation for 10 minutes at 10,000*g*. The supernatant (100 μl) was added to 40 μl of a 25 percent slurry of protein A Sepharose beads (Sigma) containing bound antibody. Antibody (4 μl of serum) was first bound to the beads for 1 hour in phosphate buffered saline (PBS) containing 2 percent Triton X-100, washed in PBS containing 1 M NaCl, and then washed twice in native buffer. After incubation at 4°C for 1 hour with constant rotation, the beads were washed twice with native buffer, transferred to new tubes, and eluted with 1 percent SDS, 50 mM tris-HCl (pH 7.5), 25 mM EDTA, and 10 μg of wheat germ tRNA. RNA was prepared by phenol-chloroform extraction and ethanol precipitation. For the experiment of Fig. 3B the RNA was blotted to Duralon-UV membrane (Stratagene), ultraviolet cross-linked, and hybridized with oSN7 that had been labeled at the 5′ end with ^{32}P (*23*) at 42°C in 7 percent SDS, 30 percent formamide, 0.2 M Na_2HPO_4-H_3PO_4, pH 7.2, 0.3 M NaCl, 1 mM EDTA essentially as described [G. M. Church and W. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 1991 (1984)].
41. SRP54 was prepared from disassembled SRP as described (*20*). The 4.5*S* RNA purified by phenol extraction of S971(SN1) cells induced overnight with IPTG and then subjected to nondenaturing polyacrylamide gel electrophoresis. SRP receptor was purified on SRP affinity columns [R. Gilmore, P. Walter, G. Blobel, *J. Cell Biol.* **95**, 470 (1982)]. Reconstitution reactions were performed in 5 μl under conditions that allow for reassembly of SRP (*20*).
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43. Cells (1 ml) were centrifuged at 10,000*g* and kept overnight in PBS containing 2 percent glutaraldehyde [R. E. Coggeshall *Anat. Rec.* **194**, 201 (1979)], post-fixed in reduced osmium [M. J. Karnovsky *J. Cell Biol.* **28** 137A (1965)], dehydrated in a graded ethanol series, and infiltrated and embedded in a mixture of Poly-Bed and Acraldite (1:1). Thin sections were grid-stained in saturated alcoholic uranyl acetate for 5 minutes and 0.6 percent lead citrate for 10 minutes before examination.
44. We thank N. Craig for advice at the early stages of this work, D. Crumrine for performing the electron microscopy, and R. Gilmore for communicating unpublished results. Supported by a Markey Charitable Trust Predoctoral Grant (M.P.); a fellowship from the Damon Runyon–Walter Winchell Cancer Fund (H.D.B.); an EMBO long-term fellowship (D.Z.); a fellowship of the Juvenile Diabetes Foundation (K.S.); and grants from the Alfred P. Sloan Foundation and NIH (P.W.).

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