Nascent Polypeptide Chains Exit the Ribosome in the Same Relative Position in Both Eucaryotes and Procaryotes

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ABSTRACT We located the polypeptide nascent chain as it leaves cytoplasmic ribosomes from the plant *Lemna gibba* by immune electron microscopy using antibodies against the small subunit of the enzyme ribulose-1,5-bisphosphate carboxylase. Similar studies with *Escherichia coli* ribosomes, using antibodies directed against the enzyme β-galactosidase, show that the polypeptide nascent chain emerges in the same relative position in plants and bacteria. The eucaryotic ribosomal exit site is on the large subunit, ~75 Å from the interface between subunits and nearly 160 Å from the central protuberance, the presumed site for peptidyl transfer. This is the first functional site on both the eucaryotic and procaryotic ribosomes to be determined.

It has been appreciated for some time that, in general, eucaryotic and procaryotic ribosomes share common structural features (for reviews, see references 1, 2). With the advent of immune electron microscopy (3, 4), however, the *Escherichia coli* ribosome has become better understood than its eucaryotic counterparts. In *E. coli*, the functional sites involved in translation are clustered into part of the ribosome, comprising approximately two-thirds of its volume, referred to as the "translational domain." Functional sites contained in the translational domain include the initiation factor binding sites (5, 6), the messenger binding site (7–9), the peptidyl transferase (10–12), the 5S RNA (13), and the L7/L12 proteins (14) that mediate the GTP-dependent steps of translation (for a review of these locations, see reference 15). Together, these sites define the translational domain. Corresponding structural regions are found in eucaryotic ribosomes, as well as in archaebacterial ribosomes (16).

Other aspects of ribosomal organization, particularly those involved with protein secretion and processing, could possibly differ extensively in eucaryotic and procaryotic ribosomes since the rough endoplasmic reticulum has no obvious counterpart in the procaryotic cell. Here we report investigations on the location of the polypeptide nascent chain as it exits from the ribosome in both procaryotes and eucaryotes. We have mapped the exit site of the nascent chain on ribosomes synthesizing the enzymes β-galactosidase and the small subunit of ribulose-1,5-bisphosphate carboxylase (Rubisco) using antibodies directed against these proteins.

The exit sites are at a single region located at comparable sites on the large subunits of both ribosomal types. This site is ~150 Å from the presumed site of the peptidyl-transferase (11). In the duckweed, *Lemna gibba*, the polypeptide nascent chain emerges from the large subunit at a region on the side of the ribosome opposite the translational domain and in the same relative position as found in the *E. coli* ribosome. Hence, in spite of the greater complexity of eucaryotic as compared to procaryotic ribosomes, the overall organization of the exit domain on ribosomes, as reflected by the location mapped for the exit site, seems to be similar in both.

MATERIALS AND METHODS

Preparation of Polysomes from E. coli: 2.5×10^{11} cells from *E. coli* A324-5 (17) were resuspended in 2 ml of buffer A (150 mM NH_4Cl/20 mM Tris·HCl, pH 7.6/10 mM $MgCl_2$) and disrupted in a French pressure cell at 13,800 psi. The cell extract was centrifuged in a SS-34 rotor for 8 min at 8,000 rpm. The supernatant containing the polysomes was layered (0.6 ml per tube) on top of a 15–30% sucrose gradient with 0.5 ml of 60% sucrose cushion on the bottom in buffer B (150 mM NH_4Cl/20 mM Tris·HCl, pH 7.6/5 mM $MgCl_2$). The polysomes were pelleted at 45,000 rpm for 125 min in a SW50.1 rotor (Beckman Instruments, Inc., Spinco Div., Palo Alto, CA). The supernatant was discarded and the pellet rinsed immediately with 5 ml of cold buffer B in order to eliminate the remaining sucrose. The polysomes were resuspended in buffer B (400 μl) and stored at −80°C. The yield of polysomes was A_{260} units per 10^{11} cells.

Preparation of Polysomes from L. gibba: *L. gibba* plants G-3 (18) in growth medium were washed with distilled water at room temperature and poured onto liquid nitrogen or crushed ice at −20°C. ~35 g of *L. gibba* were macerated in a mortar and the paste resuspended in 70 ml of extraction buffer (17% sucrose/0.4 M KCl/30 mM $MgCl_2$/50 mM Tris, pH 9.0). All the operations were carried out at 4°C. The mixture was ground in a Waring Blender (Waring Productions Div., Dynamics Corp. of America, New Hartford, CT) four times

for 15 s, with 1-min intervals. The homogenate was poured through two layers and then eight layers of cold miracloth. The solution was spun for 7 min at 3,000 rpm in a SS-34 rotor. The volume of the supernatant was recorded and 0.1 vol of 20% Triton X-100 was added. The mixture was spun for 20 min at 12,000 rpm in a SS-34 rotor. The supernatant was layered, with a wide pipette, on top of 5 ml of sucrose cushion (60% sucrose/0.2 M KCl/20 mM Tris[pH 7.6]/5 mM $MgCl_2$), and more extraction buffer was added to equilibrate all the tubes. After centrifugation for 3 h at 49,000 rpm in a 50.2 Ti rotor (Beckman Instruments, Inc.), the supernatant was discarded and the pellet washed with resuspension buffer (50 mM KCl/20 mM Tris[pH 7.6]/5 mM $MgCl_2$). The pellet was finally resuspended in 200 μl of resuspension buffer. These polysomes can be further purified by centrifugation in a SW 50.1 rotor on a sucrose gradient (0.5 ml of 60% sucrose cushion at the bottom and 15–30% sucrose gradient in buffer C [0.2 M KCl, 20 mM Tris and 5 mM $MgCl_2$]) by spinning at 45,000 rpm for 2 h.

Purification of the Proteins: The enzyme ribulose-1,5-bisphosphate carboxylase was purified from *L. gibba* G-3 (18). 20 g of *L. gibba* were mixed with 10 ml of buffer D (100 mM KCl, 20 mM Tris-HCl, pH 7.4, and 5 mM $MgCl_2$), 6 g of Dowex 1 (1 × 200–400) and 20 g of sand. The mixture was ground in a mortar and the suspension of the homogenized plant was filtered through two and then eight muslin layers. The filtrate was centrifuged for 20 min at 10,000 rpm in a SS-34 rotor. The clear supernatant was then centrifuged for 30 min at 45,000 rpm in a SW50.1 rotor. The supernatant was concentrated by either polyethylene glycol (PEG) in a dialysis bag or by ultrafiltration in an Amicon apparatus (Amicon Corp., Scientific Sys. Div., Danvers, MA). When the volume was ~5–8 ml, the solution was dialyzed overnight against 100 mM KCl, 20 mM Tris-HCl, pH 7.4, and 5 mM $MgCl_2$. The dialysate (1–2 ml per tube) was layered on top of a 5–30% sucrose gradient in 25 mM Tris-HCl, pH 7.4, and 15 mM $MgCl_2$ and centrifuged in a VTi 50 rotor (Beckman Instruments, Inc.) for 160 min at 49,000 rpm. Under these conditions the ribulose-1,5-bisphosphate carboxylase enzyme complex (M_r ~500,000) moves into the gradient ahead of the bulk of other proteins. The peaks of the enzyme were pooled and dialyzed for 48 h against 2 L of 5% acetic acid (with one change). The acidic solution was then lyophilized. The subunits of the enzyme were separated by preparative SDS electrophoresis of the lyophilized protein. The small (13,000) and large (52,000) subunits were localized on the gel by cutting and staining with Coomassie Blue a 1-cm strip on one side of the slab gel. The unstained gel strips of large and small subunits were soaked in water for 3 h in order to remove the SDS. The gels were then lyophilized for 48 h and ground to a fine powder in a mortar. The enzyme β-galactosidase was purified as previously described (17).

Preparation of Antibodies: To prepare antibodies against the small and large subunits of ribulose-1,5-bisphosphate carboxylase, 1 ml of 0.15 M NaCl, and 0.1 M phosphate buffer, pH 7.0, was added to a gel powder of each protein and mixed with 1 ml of complete Freund's adjuvant. These mixtures were subcutaneously injected on the back of rabbits with a large needle. This was followed by an intramuscular boost in the thigh with incomplete Freund's adjuvant. Preparation of antibodies against the enzyme β-galactosidase has been described (19). Purification of the IgG fractions was done by passing the rabbit serum through a Protein A-Sepharose 4B column (Pharmacia Fine Chemicals, Piscataway, NJ) (20).

Preparation of Pairs of Monosomes Linked by IgG: Polysomes (4 A_{260} units) were incubated with 300 μg of IgG at 0°C for 40 min. Then, IgG-reacted polysomes were incubated at 0°C for 30 min with 40 μg of RNase A (Sigma Chemical Co., St. Louis, MO) to cleave the message. Pairs containing two monosomes linked by one IgG were separated from monosomes in a 15–30% sucrose gradient in buffer A (*E. coli*) or B (*L. gibba*) using a VTi 65 rotor (Beckman Instruments, Inc.) (113,000 *g* for 35 min). The dimer peak was passed through a Sepharose 6B column to remove sucrose.

Ribosomes were negatively stained with 1% uranyl acetate as described (21). Electron micrographs were obtained with a Philips 400 microscope at a magnification of 64,500.

RESULTS

Both the plant *L. gibba* G-3 and *E. coli* mutant A324-5 produce large amounts of ribulose-1,5-bisphosphate carboxylase (18) and β-galactosidase (17), respectively. This allowed us to work with polysomes relatively enriched in these proteins. Typical profiles of polysomes from *L. gibba* and *E. coli* are shown in Fig. 1 *A* and *B*, respectively. *E. coli* polysomes have a maximum of A_{256} at approximately 10 ribosomes per message. *L. gibba* polysomes, in contrast to *E. coli* polysomes, contain two classes of ribosomes, cytoplasmic (80S) and chloroplast (70S).

Polysomes were reacted with IgG's against their respective nascent protein chains. The specificity of these IgG's has been

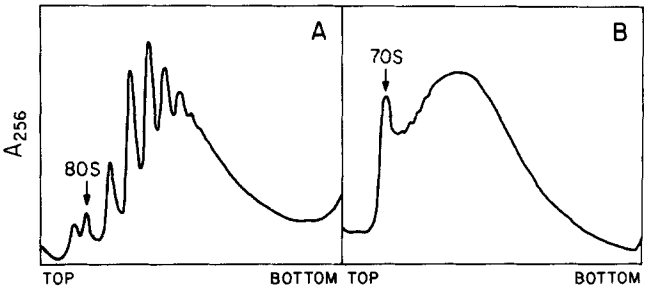

FIGURE 1 Analysis of polysomes. (*A*) *L. gibba*. (*B*) *E. coli*. Polysomes (2.1 A_{260} U) were layered onto a 15–30% sucrose gradient in buffer B (*L. gibba*) or A (*E. coli*) and centrifuged (SW50.1 rotor) for 30 min. at 245,000 *g*.

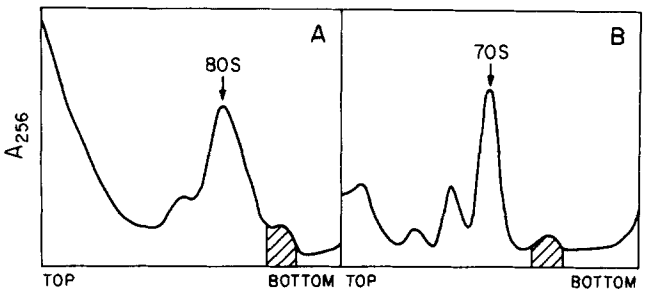

FIGURE 2 Isolation of dimers linked by IgG's (*A*) *L. gibba*. (*B*) *E. coli*. 4 A_{260} U of polysomes were reacted with IgG and digested with RNase A as described in Materials and Methods. The final mixture was layered on top of a 15–30% sucrose gradient in buffer B (*L. gibba*) or A (*E. coli*) and centrifuged (VTi 65 rotor) for 35 min at 113,000 *g*. The shaded dimer peak was collected and negatively stained with 1% uranyl acetate.

previously documented (18, 19). After the formation of intrapolysomal IgG dimers, the mixture was treated with RNase in order to cleave the messenger RNA. Dimers of monosomes linked by IgG's were then separated from other components on a sucrose gradient. Fig. 2 shows the resolution obtained with this separation. The shadowed areas contain the IgG dimer peak, as well as some nondigested disomes. The peak at the top of the gradients contains the RNase and unreacted IgG's. The broadened peaks in Fig. 2 *A* result from overlapping of the cytoplasmic ribosomal peaks with those comprising of chloroplast ribosomes which represent a significant proportion of the total ribosomes in the plant.

Electron micrographs of dimers of monosomes connected by IgG's are shown in Fig. 3. The most common views of IgG-linked ribosomes correspond to the nonoverlap projection (10) in both eucaryotic (Fig. 3 *A*) and procaryotic (Fig. 3 *C*) ribosomes. Also shown are monosomes attached to IgG's in their lower line of each figure. In the nonoverlap projection, the exit site of the polypeptide nascent chain maps on the large subunit, ~70 Å from the interface between subunits.

DISCUSSION

It has been appreciated for some time that, on a gross scale, eucaryotic and procaryotic ribosomes share common structural organization (1). More recent comparative studies show that ribosomes from all three lineages, archaebacteria, eubacteria, and eucaryotes, share many common structural features (2, 16) such as the platform and cleft of the small subunit and the central protuberance and L7/L12 stalk of the large subunit.

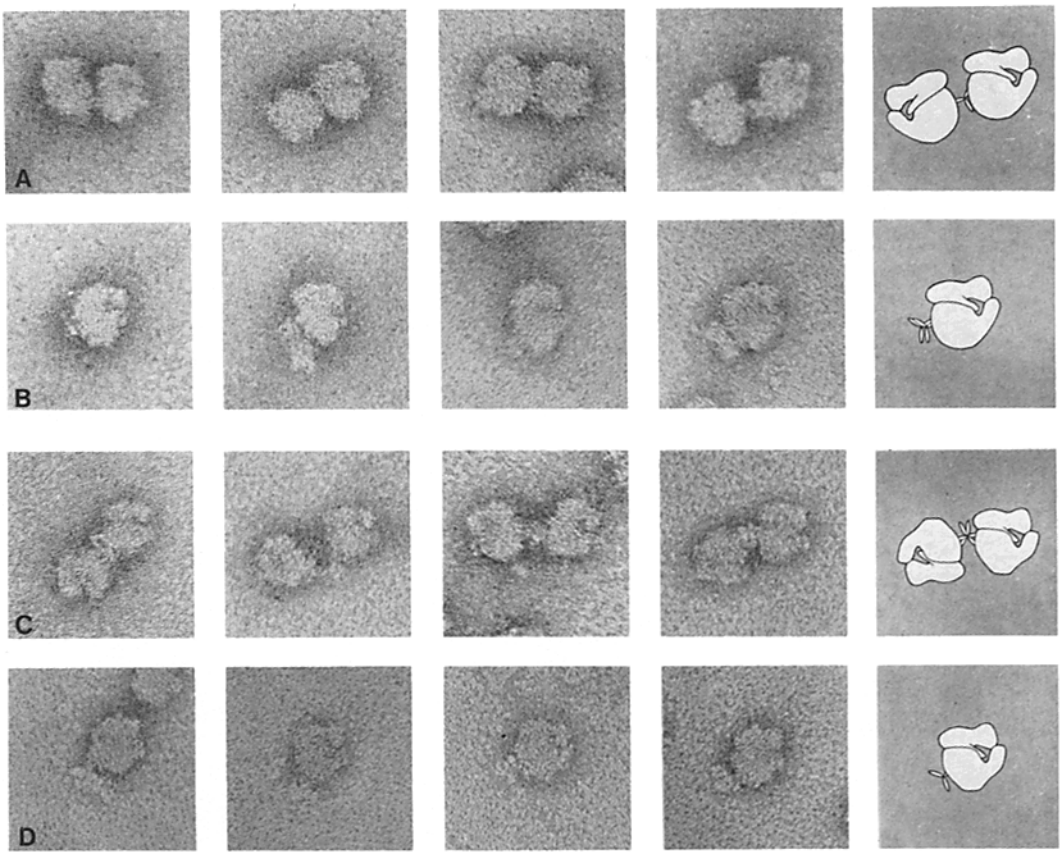

FIGURE 3 Electron micrographs of ribosomes reacted with IgG's against their nascent protein chains. In *A* and *B* eucaryotic, ribosomes are reacted with IgG's directed against Rubisco and in *C* and *D* procaryotic ribosomes are reacted with IgG's directed against β-galactosidase. Pairs of ribosomes (the nonoverlap projection) linked by an IgG are shown in *A* and *C* and single ribosomes with an attached IgG are shown in *B* and *D*.

Other ribosomal features, such as the archaebacterial bill and the eucaryotic lobes, are present in only some lineages and absent in others (16). Less is known about the functional correspondence although it has been generally assumed that parts of the ribosomes directly involved in translation (e.g., tRNA binding site, elongation factor binding sites, mRNA binding site) are similar. No information exists about the ribosomal locations of those functions involved in protein secretion and transport, where ribosomal function might possibly differ in procaryotes and eucaryotes. The results in this paper provide the first information on the sites of these functions.

The nascent polypeptide chain exists from a similar ribosomal site in both procaryotic and eucaryotic ribosomes. In the 60S subunit of *L. gibba* the nascent polypeptide chain emerges 160 Å from the central protuberance. In *E. coli* 50S subunits, the nascent chain exists 140 Å from the central protuberance, the site of the peptidyl transferase center (11). The conclusion that the nascent chain exit site is quite distant from the peptidyl transferase center is consistent with protease protection experiments on the nascent polypeptide chain. These experiments showed that the first 30–40 residues at the carboxy-terminus of the nascent chain are protected from degradation, e.g., in *Bacillus subtilis* (22), rabbit reticulocytes (28), and rat liver (24).

From the distance between the exit site of the nascent chain and the peptidyl transferase determined in our mapping experiments (and from the uncertainties in ribosomal dimensions [±15%]), we calculated that 39 ± 6 and 44 ± 7 residues could be protected in procaryotes and eucaryotes, respectively, if we assume that the nascent chain is fully extended. Hence the nonextended protein conformations, for example the alpha helix which measures 1.5 Å/residue, would require many more residues to span this distance and are not consistent with the protection measurements. This suggests that the nascent chain may traverse the ribosome in the fully extended conformation.

The nascent chain exist site is located in an area where a membrane binding site has been reported in lizard oocytes (25). The exit site and the membrane binding site probably correspond to the two types of interactions in the attachment of eucaryotic ribosomes to membranes of the RER. One of these is through the nascent chain and the second possibly involves integral membrane proteins (26–28) and a signal recognition particle (29). Together, these two sites comprise the region involved in the secretion of the proteins that we have named exit domain (19). When ribosomes are bound with the exit site contacting the membrane, the parts of the ribosome involved in translation, i.e., the translation domain, faces the cytoplasm. This is consistent with the requirement that the translational surface of the ribosome has access to ligands in the cytoplasm such as mRNAs, tRNAs, and factors.

In conclusion, immune electron microscopy has shown that the exit site of the nascent chain is located at similar regions in both procaryotes and eucaryotes and, in combination with other results, delineates the exit domain of the eucaryotic ribosome. It is hoped that these observations will be useful in ultimately understanding the molecular mechanisms of protein synthesis and secretion.

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