Primary Role for Endoplasmic Reticulum-bound Ribosomes in Cellular Translation Identified by Ribosome Profiling

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Background: mRNA translation is compartmentalized between cytosolic and ER-bound ribosomes.

Results: Genomic ribosome profiling demonstrates that the mRNA transcriptome is preferentially translated on ER membrane-bound ribosomes.

Conclusion: The endoplasmic reticulum serves a global role in mRNA transcriptome expression.

Significance: Partitioning of mRNAs between the cytosol and endoplasmic reticulum may represent a novel means of post-transcriptional gene regulation.

In eukaryotic cells, the spatial regulation of protein expression is frequently conferred through the coupling of mRNA localization and the local control of translation. mRNA localization to the endoplasmic reticulum (ER) is a prominent example of such regulation and serves a ubiquitous role in segregating the synthesis of secretory and integral membrane proteins to the ER. Recent genomic and biochemical studies have now expanded this view to suggest a more substantial role for the ER cellular protein synthesis. We have utilized cell fractionation and ribosome profiling to obtain a genomic survey of the subcellular organization of mRNA translation and report that ribosomal loading of mRNAs, a proxy for mRNA translation, is biased to the ER. Notably, ER-associated mRNAs encoding both cytosolic and topogenic signal-encoding proteins display similar ribosome loading densities, suggesting that ER-associated ribosomes serve a global role in mRNA translation. We propose that the partitioning of mRNAs and their translation between the cytosol and ER compartments may represent a novel mechanism for the post-transcriptional regulation of gene expression.

The translation of mRNAs into proteins occurs primarily on two populations of ribosomes: those free in the cytosol and those bound to the endoplasmic reticulum (ER). The landmark studies of Palade and colleagues demonstrated that membrane-bound ribosomes function in the translation of proteins destined to enter the secretory pathway. Consequently, subsequent investigations into the contributions of ER-bound ribosome to cellular protein synthesis have focused on integral membrane and secretory protein biogenesis and the mechanisms mediating the recruitment of secretory and membrane protein-encoding mRNAs to the ER.

Genome-scale studies of mRNA partitioning between the cytosol and ER compartments have demonstrated, somewhat unexpectedly, that the mRNA transcriptome is broadly represented on the ER, with mRNAs that encode secretory and membrane proteins being highly ER-enriched and mRNAs encoding cytosolic/nucleoplasmic proteins displaying overlapping subcellular distributions with a prominent cytosolic enrichment. Furthermore, in a limited number of cases, mRNAs that lack an encoded topogenic signal were reported to be highly partitioned to the ER. Combined, these findings indicate that the subcellular organization of mRNA translation may be more complex than generally envisioned and suggest broader roles for the ER in the expression of the mRNA transcriptome. Consistent with this view, ER-associated ribosomes were demonstrated to be competent for de novo initiation, to polymerize amino acids with similar kinetics as their cytosolic counterparts, and to retain their association with the ER upon termination. These findings demonstrate that the mRNA translation cycle can operate on the ER without the obligatory trafficking of ribosomes and/or mRNAs from the cytosol to the ER and raise a number of key questions regarding how mRNA translation is segregated between the cytosol and ER compartments and whether translation is subject to compartment-specific regulation. These questions are of substantial importance; several recent studies have shown that ER-associated ribosomes maintain translational activity under stress conditions that elicit a translational inhibition in the cytosol, and so the subcellular partitioning of mRNAs may have a substantial impact on the expression of the encoded proteins.

The study of in vivo mRNA translation has been substantially advanced by the recent development of a ribosomal footprinting protocol where ribosome-protected regions of mRNAs are isolated and analyzed by deep sequencing to provide a genome-scale, subcodon resolution survey of ribosomal loading of the transcriptome. This method provides a robust proxy for translational activity, is well correlated with protein abundance, and has been applied in yeast and mammalian systems to reveal important information about the basic properties of translation in each. Here, we utilize genome-scale ribosome footprinting of cytosolic and ER-associated

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2 The abbreviations used are: ER, endoplasmic reticulum; GO, gene ontology; nt, nucleotide(s).

This article contains supplemental Figs. S1–S4 and Tables S1–S3.

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polyribosomes to identify the contribution of each compartment to global protein synthesis.

EXPERIMENTAL PROCEDURES

Cell Culture and Fractionation—HEK293 cells were grown in DMEM supplemented with 10% FBS. Cell fractionation was performed as described previously (17, 24, 25, 28). Briefly, cells were grown to ~70% confluence and harvested in PBS, 15 mM EDTA. Cells were collected by centrifugation and resuspended in an isotonic cytosol buffer solution containing 0.03% digitonin to release the cytosol contents. Digitonin-permeabilized cells were subsequently washed with cytosol buffer containing 0.015% digitonin, and the ER-associated polyribosomes were released by the addition of an ER lysis buffer containing 2% n-dodecyl-β-D-maltoside. All solutions contained 180 μM cycloheximide, 1 mM dithiothreitol, and 40 units/ml RNase OUT (Invitrogen).

Polysome Gradient Fractionation—Cytosol and ER subcellular fractions were overlaid onto 10-ml 15–50% linear sucrose gradients containing 400 mM KOAc, 25 mM K-HEPES, pH 7.2, 15 mM Mg(OAc)2, 180 μM cycloheximide and centrifuged for 3 h at 151,000 g in a Beckman SW-41 Ti rotor. Gradients were harvested with a Teledyne Iasco gradient fractionator with continuous A260 monitoring. For each experiment, a paired blank gradient containing only lysis buffer was run in parallel, and the A260 profile was used for background subtraction. For radiolabeling experiments, cell cultures were supplemented with 200 μCi/ml [35S]Met/Cys (MP Biomedical) for 2 min, treated with either cycloheximide (180 μM final concentration) or puromycin (250 μM final concentration), and fractionated as described above, and ribosomes were recovered by centrifugation (15 min, 85,000 rpm, TLA100.3 rotor) over a 35% sucrose cushion.

Semiquantitative PCR—RNA from gradient fractions was reverse transcribed using M-MLV reverse transcriptase and then amplified using the following primers: GAPDH, 5'-AGGAACTAACAGT-3', ACTB, 5'-AGGAACTAACAGT-3', 18S, 5'-CTGGAAATG-3'. PCR amplicons were analyzed by agarose gel electrophoresis and visualized using SYBR Green (Invitrogen).

Analysis of Compartmental Translational Profiles—To determine the subcellular distribution of mRNA-associated ribosomes, cells were biosynthetically labeled for 48 h with 6 μCi/ml [3H]uridine (MP Biomedical) and fractionated as above. The buffer composition of each fraction was adjusted to 500 mM KOAc, 25 mM K-HEPES, pH 7.2, 15 mM Mg(OAc)2, 180 μM cycloheximide. Extracts were incubated with oligo(dT)7 resin (Amersham Biosciences) at 40 mg/ml for 1 h at room temperature and centrifuged, and the resin washed extensively in loading buffer and subsequently isolated on Micro Bio-Spin chromatography columns (Bio-Rad). Oligo(dT)7 resin was then eluted with 1 ml of 500 mM KOAc, 15 mM EDTA, 25 mM K-HEPES, pH 7.2. Relative ribosome levels were determined by liquid scintillation spectrometry of the EDTA eluates. To obtain mRNA levels, purified RNA pools were treated with RiboMinus (Invitrogen) according to the manufacturer's protocol, and poly(A) mRNA abundance was assessed by Bioanalyzer (Agilent).

Ribosome Footprint Preparation, Sequencing, and Mapping—Cell fractions were adjusted to 100 mM KOAc, 25 mM K-HEPES, pH 7.2, 15 mM Mg(OAc)2, 3 mM CaCl2, 180 μM cycloheximide and treated with 20 μg/ml micrococcal nuclease (Sigma-Aldrich) for 1 h at 37 °C (26). Nuclease activity was inactivated by the addition of 6 mM EGTA, and ribosomes were recovered by centrifugation (60 min, 90,000 RPM in the Beckman TLA 100.3 rotor) over a 500 mM sucrose cushion. The purified ribosome pellet was resuspended in 50 mM sodium chloride, 50 mM Tris, pH 7.5, 5 mM EDTA, 0.5% SDS, 200 μg/ml proteinase K and incubated for 30 min at 37 °C (26). RNA was purified by phenol/chloroform extraction and separated on a 15% acrylamide gel containing 1× Tris-borate-EDTA and 7 M urea. Gels were stained with SYBR Gold (Invitrogen), revealing an ~35-nb band that was absent when ribosomes were dissociated with EDTA prior to nuclease digestion (supplemental Fig. S1). This region of the gel was excised, and RNA was extracted by homogenizing the gel in 100 mM NH4HCO3, freezing to ~80 °C, rapidly reheating to 95 °C, and incubating the gel homogenate, with mixing, for 3 h at room temperature. Acrylamide fragments were removed by centrifugation, and the remaining supernatant fraction was filtered through a SpinX filter (Costar) by centrifuging for 10 min at 12,000 × g. RNA was recovered by EtOH/NaOAc precipitation.

cDNA libraries were prepared for SOLiD sequencing with the Applied Biosystems Small RNA protocol by the Duke University Genome Sequencing & Analysis Core Resource facility. Briefly, the purified 35-nb RNA pool was ligated to oligonucleotide adapters and reverse transcribed. Fragments were then amplified by PCR, size-selected, and applied to beads, which were deposited onto a chip surface for sequencing. Each barcode sample was sequenced using the SOLiD 4 system, obtaining ~115 million 35-base reads between the two samples.

Sequencing of Total mRNA from Cellular Compartments—From the same cellular fractions as were used for ribosome footprinting, a sample of total RNA was purified by TRIzol extraction. Total RNA samples were treated with RiboMinus (Invitrogen) and prepared for bar-coded SOLiD sequencing according to the manufacturer's instructions, where total mRNA was treated with RNase III and prepared for sequencing as described above. In this run, 35 million 50-base reads were obtained between the two samples.

Read Mapping to mRNAs—Total mRNA reads were initially mapped to all RefSeq RNAs (hg19, GRCh37), allowing for 10 maps per read to encompass the multiple annotated isoforms that exist for several genes (27). To select a single mRNA to represent each gene, the longest annotated mRNA with an mRNA read density of at least 75% of the maximum read density was chosen. Reads from mRNA and ribosome footprinting libraries were then remapped to this subset of mRNAs, allowing each read to map to two locations, allowing two mismatches in a 25-nb seed region, and enabling the best and strata options. Mapping details are provided in supplemental Table S1 with abundance and ribosome loading of each mRNA specified in supplemental Table S2. To ensure that the RefSeq database provides a thorough representation of the HEK293 transcriptome.
tome, the density of reads that span exon-exon junctions was compared with those that do not (supplemental Fig. S2); no appreciable differences were observed.

Total mRNA libraries were normalized by the compartmental abundance of mRNA (see Fig. 1C), and ribosome footprinting libraries were normalized by the abundance of poly(A)-associated ribosomes (see Fig. 1D). All transcripts with at least 10 total mRNA reads per kilobase per million reads were considered. The abundance of each species of mRNA or its translation was estimated by the number of reads mapping to that mRNA per million mapped reads normalized by the length of that mRNA (for total mRNA) or coding sequence length (for ribosome footprints) in kilobases (21). Finally, to assess ribosome loading per mRNA, or ribosome density, ribosome loading was divided by the abundance of that mRNA. The cytosol and ER-targeted gene categories were described previously (9). All reads for mRNA and ribosome footprints are available on Gene Expression Omnibus (accession number GSE31539) and are summarized in supplemental Table S2.

Analysis of Ribosome Density Distribution—To evaluate the density of ribosome footprints, the number of reads that span each position relative to the start and stop codon was counted and normalized by total read number. For positional analyses, the ribosome position was taken to be the start of the read +15 nt, which maps to the A site of the ribosome (22). To calculate the processivity of ribosomes in each compartment, the density of ribosomes from 200 to 800 nt to the 3′ of the start codon was counted for all mRNAs with a coding sequence of >1100 nt. A best-fit line was then fitted to the natural log of the smoothed density.

Calculation of Gene Ontology Enrichment—Gene ontology (GO) enrichments were calculated by permutation testing. The mean relative translational efficiency, \( \log_{2}(ER \text{ ribosome load-density}) \), was then fitted to the natural log of the smoothed count for all mRNAs with a coding sequence of 200 to 800 nt to the 3′ of the start codon. The density of reads that span exon-exon junctions was compared with those that do not (supplemental Fig. S2); no appreciable differences were observed.

To evaluate the global translational status of ribosomes in the ER and cytosol, cells were labeled with \(^{35}\text{S}\)Met/Cys for 2 min and then treated with puromycin, which elicits the premature termination of translation and partial polyribosome breakdown (29, 30). Puromycin treatment elicited breakdown of polyribosomes in both compartments, indicating that both populations of polyribosomes were translationally active (Fig. 2). Corroborating this conclusion, \(^{35}\text{S}\)Met/Cys was incorporated into nascent polypeptides in each polyribosome population (Fig. 2, A and B), and polysomal \(^{35}\text{S}\)Met/Cys was abolished upon addition of puromycin (Fig. 2, C and D). To confirm that mRNAs encoding cytosolic proteins were among the population of actively translating polyribosomes that were disassembled by puromycin on the ER, semiquantitative PCR targeting ACTB and GAPDH mRNAs, both of which lack topogenic signals, showed that each mRNA moved to a markedly lighter polysome fraction or lost polysome association altogether in both the cytosol and the ER. GRP94, which encodes a topogenic signal, is similarly reduced in polysomes on the ER, indicating that each class of mRNA is actively translated on the ER. Combined with previous work indicating similar elongation rates for cytosolic and ER-bound ribosomes (17, 22), these experiments suggest that mRNA ribosomal loading is a representative proxy for mRNA translation in each cellular compartment.

Ribosome Footprinting Analysis of Ribosome Loading in Cytosol and ER Compartments of HEK293 Cells—To obtain a genome-scale survey of subcellular mRNA ribosome loading status, we utilized ribosome footprinting coupled with deep sequencing. Here, cytosolic and ER-associated polyribosomes were digested with micrococcal nuclease (31, 32) to yield intact 80 S ribosomes and their associated protected mRNA fragments (26). The ribosome-protected mRNA fragment complexes were isolated by ultracentrifugation and subjected to phenol/chloroform extraction, and the ~35-nt nuclease-protected mRNA fragments were purified by acrylamide gel electrophoresis (supplemental Fig. S1). cDNA libraries were prepared using the SOLiD small RNA expression kit protocol, and the library was deeply sequenced on the SOLiD4 platform (Applied Biosystems). In parallel, total mRNA samples were prepared for deep sequencing so that the abundance of mRNAs in each compartment could be defined, allowing evaluation of ribosome footprints per mRNA, or ribosome density, for individual mRNAs.

Deep sequencing reads representing ribosome footprints as well as total mRNA content in the cytosolic and ER compartments were mapped to RefSeq mRNAs (33). In Fig. 3A, the distribution of mRNAs between the cytosol and ER compartments is plotted and identifies two distinct overlapping popu-
lations: one cytosol-enriched and one ER-enriched. The overall subcellular distributions are similar to those reported previously in cDNA microarray-based studies (10, 11). Notably, the cytosol-enriched mRNA population was substantially represented on the ER, a finding that mirrors earlier observations on the relative population identities of the cytosolic and ER-associated mRNAs (9, 10, 11, 34–36). In the absence of information regarding the ribosome loading status of mRNAs in both compartments, the functional consequences of such mRNA distribution patterns are unknown. In the following, we examine this question through genome-scale analyses of ribosome loading in the cytosol and ER compartments.

To survey the subcellular disposition of ribosome loading onto mRNAs, ribosomal footprint reads from the cytosol- and ER-derived sequencing libraries were counted to yield a measure of transcript-specific ribosome density in each compartment. As shown in Fig. 3B, the subcellular distribution of ribosome density was markedly biased to the ER compartment (Fig. 3A versus 3B), with several mRNAs loaded with ribosomes almost exclusively on the ER. Ribosome loading density, defined as the ribosome footprints per mRNA transcript, was also found to be ER-biased (Fig. 3C), indicating that most mRNAs are more densely loaded with ribosomes when associated with the ER. Of note, those mRNAs that are most efficiently loaded on the ER relative to cytosol are more likely to encode signal sequences and transmembrane domains, suggesting that the relative compartmental efficiency of translation varies for specific populations of mRNAs (Fig. 3C, inset).

To assess the subcellular distributions of mRNAs and their compartment-specific translational status, cumulative density functions of mRNA abundance, number of ribosomes per mRNA, and ribosome loading density in the cytosol and ER compartments were determined. A broad distribution, ~4 orders of magnitude, was observed for each parameter. The cytosol and ER diverge in the relative abundance of each metric. In particular, as a population, mRNAs are more abundant in the cytosol, indicating that this compartment serves as the primary subcellular locale for the majority of transcripts (Figs. 1 and 4A). The number of ribosomes bound to each species of mRNA is largely similar between the two compartments, and the cytosol contains more mRNAs with relatively low quantities of associated ribosomes (Fig. 4B). The ribosome loading per mRNA molecule in each compartment indicates, on average, substantially higher loading of ribosomes on ER-bound mRNAs, which in agreement with the biochemical data presented in Fig. 1, suggests that the ER is a preferred subcellular locale for ribosome loading (Fig. 4C).

**Compartment-specific Translational Selection of mRNAs**—We investigated the nature of the subcellular ribosome loading bias through the lens of two primary cohorts of mRNAs: those encoding cytosolic proteins (i.e., lacking topogenic signals and quantities of combined 80S ribosomes and polyribosomes. C, the subcellular distribution of ribosome-associated mRNA was determined by native oligo(dT) affinity purification of [³H]uridine-labeled polyribosomes and quantification of EDTA-releasable ribosomal subunits. Subcellular mRNA distributions were determined by treating total RNA fractions with RiboMinus, to remove rRNAs, and spectrophotometric quantification of mRNA levels. Values were normalized to a maximum value of 1. Error bars represent ± one S.D.
functioning in the cytosol or nucleus) and those whose translation products are targeted to the ER (i.e. bearing topogenic signals and targeted to cellular membranes or for secretion). By this analysis, cytosolic protein-encoding mRNAs were largely localized to the cytosol, although as noted previously, this population was ~20% represented on the ER (Figs. 3A and 5A). Topogenic signal-encoding mRNAs were substantially ER-enriched, although with a small population displaying a cytosol enrichment (Fig. 5B). Although the global subcellular mRNA distribution is largely consistent with previous studies (9, 11), the distribution of ribosome loading for both the cytosol and the ER cohorts was substantially shifted to the ER compartment. The ribosome loading of cytosolic protein-encoding mRNAs displayed a broad distribution, with the peak gene density occurring at ~45% fractional translation on the ER (Fig. 5C). With but few exceptions, mRNAs encoding ER-targeted proteins were loaded with ribosomes almost exclusively on the ER (Fig. 5D).

To examine compartment-specific properties of translation in further detail, mRNA abundance and ribosome density for these two gene categories in the cytosol and ER were examined (Fig. 6, A and B). In the cytosol, mRNAs encoding ER-targeted proteins are substantially less abundant and are sparsely loaded with ribosomes, indicating that translation is significantly less robust for this cohort in the cytosol than for their counterparts encoding cytosol-targeted proteins. In contrast, mRNAs encoding both cytosol-targeted and ER-targeted proteins are similarly abundant and loaded with ribosomes when associated with the ER, suggesting that ribosomes in this compartment contribute to the synthesis of nearly all cellular proteins. This

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**FIGURE 2. Analysis of compartmental mRNA translational status.** A–D, polyribosome profiles from the cytosol and ER in cycloheximide- (A and B) and puromycin- (C and D) treated cells are illustrated. The downward-facing arrows indicate the migration position of 80S ribosomes. mRNA translational status was determined by [35S]Cys/Met incorporation and is depicted in the line graphs. Total RNA was isolated from gradient fractions, and the relative levels of the indicated mRNAs were determined by semiquantitative PCR.
between ribosome loading in the two compartments for mRNAs encoding cytosolic proteins was observed, suggesting that the translational regulatory machinery may be shared. This correlation is substantially weaker for mRNAs encoding ER-targeted proteins. We also examined the relationship between mRNA localization and the localization of ribosome loading and observed that for mRNAs encoding cytosolic proteins, there is no substantial correlation between the two variables, suggesting that the localizations of mRNAs and their translation are likely under distinct regulation (Fig. 6D). In contrast, there is a significant positive correlation for mRNAs encoding ER-targeted proteins. Each of the divergences discussed here were statistically significant (supplemental Table S2).

To characterize the nature of cytosolic protein-encoding mRNAs that are preferentially loaded with ribosomes in the cytosol or the ER, the enrichment of GOs was examined as a function of ribosome loading density in either compartment. The analysis controls for the relative abundance of mRNAs in each compartment and so is only sensitive to differences in ribosome loading. Identified GOs are noted in Table 1; the relative enrichment for all GOs is provided in supplemental Table S3. The cohort of mRNAs encoding cytosolic products that were more heavily loaded with ribosomes on the ER was enriched for regulatory and dynamic cell functions, particularly the cell cycle. For example, the cell cycle regulators p53 (37) and Myc (38) were both 4.5-fold more heavily loaded with ribosomes on the ER when compared with the cytosol.

Several of the GO-defined cohorts that were efficiently translated in the cytosol were found to be associated with biochemical functions of relevance to plasma membrane function. For example, mRNAs encoding intestinal cell kinase (ICK), a protein kinase that localizes to basal membranes of epithelia (39), are 4-fold more ribosome-loaded in the cytosol. These GOs may represent instances of mRNAs that are translationally activated in particular regions of the cell where their protein products are functional, in this case the areas proximal to the plasma membrane, which is consistent with current views on coupled mRNA localization and translational regulation (40).

ER and Cytosol Diverge in Their Spatial Patterns of Ribosome Loading—In light of the distinct patterns of compartment-enriched ribosome loading noted above and their potential repercussions regarding localized translation, we analyzed the ribosome mapping dataset to derive insight into stage- (initiation, elongation, and termination) specific translational regulation and how this might diverge between the cytosol and ER compartments. This analysis assumes that read density is a representation of occupancy time and therefore the relative kinetics at each position. Total read density was plotted relative to the start and stop codon in the cytosolic (Fig. 7A) and ER-associated mRNAs (Fig. 7B). Both compartments display patterns in which ribosome density increases at the start codon and decreases at the stop codon, consistent with previous ribosome profiling studies (21, 23). Also apparent is a three-base periodicity to the density, indicating that the single-codon processing of the ribosome was accurately captured. The cytosolic compartment displayed a clear density enrichment near the stop codon, suggesting that termination may be slower in the cytosol relative to the ER. The processivity of elongation was
calculated by monitoring the density of ribosomes as a function of coding sequence position (supplemental Fig. S4). This analysis suggested that translation in the cytosol was less processive than that in the ER (Fig. 8), with decay constants of 0.00048 and 0.00019, respectively. These constants correspond to an average ribosome translational lifetime of 2083 amino acids in the cytosol and 5268 amino acids on the ER. We hypothesized that this disparity could lead to enrichment of translation of longer mRNAs on the ER, but no such trend was apparent. Together, these analyses suggest that the composite biochemical reactions of translation in the cytosol and ER possess kinetic differences.

**DISCUSSION**

In the current study, we have used ribosome profiling to investigate the in vivo role of the ER in mRNA translation and report two primary observations: 1) mRNAs encoding cytosolic proteins were broadly represented in the ER ribosome-associated mRNA pool and 2) steady-state ribosome loading on ER-bound mRNAs was substantially higher than in the cytosol. In demonstrating that ER-localized mRNAs display higher ribosome loading than their cytosolic counterparts, these findings expand the landscape of post-transcriptional regulation of gene expression to include the partitioning of mRNAs between the cytosol and ER compartments.

A finding of particular interest in the present study was the observed discordance between mRNA translational status and subcellular localization, indicating that mRNA localization and translational activity can comprise two distinctly regulated processes. The importance of this finding is underscored by the enhanced ribosome loading for ER-associated cytosolic/nucle-
oplasmic protein-encoding mRNAs encoding key regulatory proteins and suggests that the ER may serve as a preferred locale for the synthesis of proteins involved in, for example, the cell cycle and gene expression. When viewed with respect to previous studies demonstrating both an enhanced half-life for ER-associated mRNAs (41) and the finding that ER-associated mRNAs are excluded from the stress granule-directed trafficking pathways (19), the sum of findings to date points toward a distinct, broadly significant role for the ER in the expression of the mRNA transcriptome. We do emphasize, however, that the ribosome footprinting/RNA-Seq analysis is a proxy assay. If, for example, a given mRNA contains abundant pause sites and/or, as noted above, is subject to compartment-specific translational regulation, an enhanced ribosome loading density could reflect a phenomenon other than strict steady-state translational status. These scenarios are under current investigation.

At present, the molecular basis for the enhanced ribosome loading status of ER-associated mRNAs is unknown. One hypothesis for the divergent translational efficiencies of the two compartments is that RNA-binding proteins, many of which modulate a wide range of post-transcriptional processes (42) and confer compartment-specific translational efficiency (43), are compartmentally segregated between cytosolic and ER-bound polysomes. Indeed, it is plausible that one or more of the many RNA-binding proteins shown to change the translational status of mRNAs operate by modulating translation in a compartment-specific manner. A related mechanism was recently described in the case of RPL38, a ribosomal protein that promotes translation of a specific subset of Hox genes (44). Interestingly, despite ribosome footprinting indicating a far greater average ribosome density in the ER, the cytosol and ER polyribosome profiles, as assessed by sucrose gradient fractionation, are similar. This divergence indicates that there may exist a substantial pool of mRNAs that are not ribosome-associated and that this pool is largely segregated to the cytosol. Indeed, it has been demonstrated that ~25% of all yeast mRNAs are not ribosome-associated (45). Should these mRNAs be predominantly localized to the cytosol, perhaps associated with processing bodies or stress granules, this would indicate a similar ribosome loading density in each compartment for those mRNAs that are available for translation. Given, as noted above, that ER-bound mRNAs are resistant to recruitment into stress gran-

![FIGURE 6. Divergent patterns of subcellular ribosome loading for different mRNA cohorts.](image)

### TABLE 1

**Selected compartmental gene ontology enrichments**

| Gene ontology                          | p value  |
|----------------------------------------|----------|
| ER-enriched translation                |          |
| Kinase regulator activity              | 0.0020   |
| Cell cycle arrest                      | 0.0065   |
| System development                     | 0.0095   |
| mRNA metabolic process                 | 0.0180   |
| Cytosol-enriched translation           |          |
| Phosphoinositide binding               | 0.0065   |
| Receptor protein signaling pathway     | 0.0080   |
| Kinase activity                        | 0.0195   |
| Cellular lipid metabolic process       | 0.0245   |
ules (19), it is plausible that compartmental differences in stress granule and processing body association lead to a large population of cytosol-localized mRNAs that are not associated with polyribosomes. This model is consistent with Fig. 2A, where mRNAs in the cytosol are more frequently not associated with polysomes than those on the ER. The ER may therefore represent a cellular compartment for dedicated protein synthesis, with the cytosol compartment engaged in the ancillary functions of mRNA storage, degradation, and related processing.

This study introduces the concept of a subcellular architecture to mRNA localization and translation. Although the functional ramifications and mechanistic details remain to be developed, we suggest that the partitioning of the mRNA transcriptome between the cytosol and ER may represent a novel post-transcriptional regulation mechanism with broad consequences for the expression of the cellular proteome (36).

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FIGURE 7. Spatial patterns of ribosome occupancy on mRNAs. A and B, density of ribosomes near the start and stop codon for cytosolic (A) and ER (B) compartments.

FIGURE 8. Ribosome processivity differs between cytosolic (Cyt) and ER-bound ribosomes. Ribosome processivity was calculated by fitting an exponential decay curve to the ribosome density in each compartment. Error bars represent at 95% confidence interval.
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