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Point of View

# Compaction of polyribosomal mRNA

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**RNA molecules may form compact secondary and tertiary structures in order to exert their functions. Their folding is assisted by various RNA-binding proteins and was found to be critical for the functionality of many non-coding RNAs (e.g., tRNA and rRNA). For messenger RNA, however, only little evidence was found for compaction to occur while it is being translated. One well-studied mode of compaction is through the interaction between the two ends of mRNA, which give rise to the so-called 'circular mRNA.' Recently, work from several labs revealed that translated mRNAs are compacted far beyond the circular structure. Polyribosomes formed by a continuous cell-free translation system were found by electron microscopy to form a double-row structure in which small subunits of non-adjacent ribosomes are in close proximity. A reconstituted 3D structure of polysomes, which was derived from high-resolution cryoelectron tomography (CET) of *E. coli* extracts, also revealed compact structures in which the large subunits were oriented away from each other. Co-sedimentation and co-precipitation analyses revealed that the 3' UTR of many eukaryotic mRNAs are in strong association with the coding regions, presumably through interaction with translating ribosomes. Taken together, these data imply that in vivo, polyribosomal mRNAs are found in a compact structure in which distant domains are in close contact. How these interactions affect the progress of ribosomes along the coding region, and whether these interactions have regulatory implications are yet to be determined.**

## mRNA Compaction by the Interaction Between the Cap and polyA

Almost every eukaryotic mRNA contains a 7 methyl Guanine (cap structure) that is added to the first nucleotide of the transcript, and a long poly Adenosine sequence that is added to its 3' end (polyA tail). However at distant regions of the mRNA, these features were shown to have a synergistic effect on translation rates.[1] The effects are exerted through the function of their associated proteins: the cap is bound by the cap-binding protein (eIF4E) and the polyA tail is associated with multiple polyA-binding proteins (PABP).[2,3] Each of these proteins was shown to interact with the large scaffold protein eIF4G,[4,5] which was therefore suggested to serve as a bridge between the two ends of the transcript. Indeed, this intra-molecular interaction was shown by atomic force microscopy to induce physical compaction of a reporter mRNA into a "closed loop" structure.[6] The circularization of the mRNA may increase translation rates by facilitating the reloading of terminating ribosomes at the 5' end of the mRNA.[7] Factors involved in translation termination were also shown to be involved in interactions between the mRNA ends.[8] This may also bring the stop codon region into close proximity with the 5' and 3' ends, and thus induce further compaction of the mRNA.

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## mRNA Compaction by Interactions Between Ribosomes

During translation, multiple ribosomes bind the same mRNA and translate its coding region ("polysomes"). Recent works[9,10] have shown that these polysomal complexes may acquire compact structures that do not involve the cap and/or the polyA tail. Continuous Exchange Cell-Free (CECF) translation systems[11] allow translation of an mRNA to be sustained for several hours in vitro. This system mimics intracellular translation with respect to the translation period of an mRNA and therefore the long-term dynamics of polysomal structures can be studied. Kopeina et al.[9] have used this system, and showed by electron microscopy that after two hours of translation, polysomal complexes acquire a compact structure in which the mRNA folds and ribosomes become ordered in two rows. The ribosomes within the two rows were ordered such that the small subunits were on the inside, presumably in contact with each other, and the large subunits faced the solvent. Intriguingly, reporter mRNA that lacked 5' and 3' UTRs acquired the same structure as mRNA with both or only one UTR. This indicates that the formation and stabilization of the double-row structure do not necessitate interaction between untranslated regions or their associated proteins (i.e., eIF4E and PAB). Thus, polysomal mRNAs are compacted through interactions between ribosomes either directly or through associated proteins.

Compaction of polysomal mRNAs was also observed by cryo-electron tomography of bacterial polysomes.[10] These polysomes were generated by translation-competent *E. coli* extract and a series of truncated luciferase mRNAs. Also here, 2D projection

indicated a double-row conformation of the polysomes with the small subunits close to one another. Analysis of several hundred neighboring ribosomes revealed that their small subunits are usually oriented in one of two ways: a parallel or an anti-parallel orientation (i.e., 180° rotation on the y-axis). Although adjacent ribosomes appeared to be well-spaced (by 5 nm on average), some connecting density between the L1 stalk of one ribosome and the small subunit of the other one appeared. Three-dimensional models of polysomes revealed structures more complex than a double row. In some cases, an ordered pseudo-helical conformation in which the large subunits (and hence the protein exit tunnel) are farthest away from each other was observed. Various other conformations were also visualized. These organizations were less ordered than the pseudo-helical conformation, yet a compact structure was usually detected with the large subunits far away from each other. The compaction was nicely presented when the mRNA path was projected through the structure; mRNAs never appear as a straight line, but in various twisted forms. Brandt et al.[10] also examined the structure of polysomes that were isolated from *E. coli* spheroplasts and are therefore more likely to demonstrate the in vivo situation. Although the resolution of this analysis was lower, variable compact polysomes were also observed. Overall, bacterial polysomes appeared in a compact structure in which neighboring ribosomes are in contact with each other. The large subunits are oriented with their protein exit tunnels away from each other; this orientation is likely to maximize the distance between emerging polypeptides.

## mRNA Compaction by Interactions Between the 3' UTR and the Coding Region

The 3' UTR of eukaryotic mRNAs is known to coordinate many regulatory responses that lead to changes in gene expression. These responses include coordination of mRNA levels, intracellular mRNA localization and translation regulation. Sequences within the 3' UTR serve as binding sites to proteins or short RNA molecules, which in turn affect expression levels. The exact mechanisms by which these molecules affect translation rates are not clear. We recently presented data indicating extensive association between the 3' UTR and the coding region.[12] We used an experimental procedure in which we isolated polysomal mRNA from yeast cells and cleaved the phosphodiester bond that links the 3' UTR to the coding region. This specific cleavage was obtained by using RNase H and oligodeoxynucleotide that is specific to the stop codon region of each mRNA. The cleavage reaction was resolved by centrifugation in a sucrose gradient that separates complexes according to their mass. We expected that the 3' UTR would not sediment deeply into the gradient because it is not known to be associated with heavy polysomal complexes. Surprisingly, 3' UTR fragments from all tested mRNAs sedimented deeply into the gradient, and to the same position where the coding domain had sedimented. For example, the 3' UTR of PIL1 mRNA sedimented as if it had a mass of 7 ribosomes, the same number of ribosomes that are associated with the entire transcript. Clearly the 3' UTR is not associated with such a number of ribosomes because in such a case the entire transcript would have been associated with 14 ribosomes. The more likely explanation is that the 3' UTR remains strongly associated with its ORF even when the covalent poshpodiester bond between them is cut. This association is maintained throughout the experimental procedure and consequently the 3' UTR co-sediments with the polysomal coding region in the sucrose gradient. Interestingly, the extent of co-sedimentation varied between mRNAs though we could not find a correlation to any obvious features (e.g., number of ribosomes, length of the 3' UTR or length of the 5' UTR). We supported these results by an alternative assay in which TAP-tagged polysomes were immunoprecipitated after the cleavage reaction. 3' UTR fragments were found to co-immunoprecipitate with polysomes, further demonstrating the association between them. The association is unlikely to be through known interactions between the polyA-binding protein and eIF4E since various interventions with the cap-polyA association, including removal of the polyA tail or of an entire 5' UTR, did not affect the co-sedimentation of the 3' UTR with the coding region. We conclude that the 3' UTR interacts with the coding region, most likely with the ribosomes associated with it. This interaction leads to compaction of the transcript and thereby brings distant domains closer.

## Open Questions

The detection of a compact polysomal structure in which different regions interact with each other raises several questions. First, *what mediates the compaction?* Ribosomal components are probably central for the interactions. The data presented by Brandt et al.[10] reveal a connection between the L1 stalk of one ribosome and the small subunit of other ribosomes. Closer inspection of the interaction site on the small subunit of some complexes suggested that the interaction is either with a short region of the 16S rRNA (U407-A434) or with ribosomal protein S4. Indeed, ribosomal proteins are prime candidates for being involved in such long-range interactions. These proteins are usually very basic and contain multiple RNA-binding domains with high affinity to diverse RNA sequences. Moreover, most are positioned at the periphery of the ribosome and exposed to the neighboring environment. Protiens from one ribosome may therefore interact with RNA molecules from other ribosomes or from the 3' UTR.

*How is the movement of ribosomes orchestrated during translation?* The translation process involves multiple translocations of multiple ribosomes through the coding region. To maintain an ordered structure in which small subunits are oriented similarly, the translocation of ribosomes should be coordinated. Moreover, the associations between different ribosomes, and between ribosomes and the 3' UTR, may impose a physical limitation to ribosomal translocation and to the decoding process. As all experiments were carried out with stalled ribosomes, conclusions regarding the dynamics of the process are limited. We speculate that the associations break for short periods of time, allowing ribosomes to translocate, and once translocation has terminated the associations resume. Such a flexible connection will allow the exchange of association partners. The 3' UTR, for example, may switch partner-ribosomes during the translation process and thereby cause extensive effects.

*How broad is this phenomenon?* Double-row polysomes were observed in polysomes from either prokaryotes or eukaryotes, and all reporter mRNAs appeared to acquire a compact polysomal structure. Moreover, the 3' UTRs of all 11 endogenous mRNAs appeared to be associated with the coding region.[12] Thus, compaction is a general and broad property of polysomal mRNA. However, it is conceivable that the extent of compaction and the 3D structure of polysomes will vary between mRNAs. The association of 3' UTRs with their coding regions varied significantly between the different mRNAs. In addition, if compaction is related to protein folding, then the protein folding process will have significant impact on the 3D structure. For example, α-helices may fold already inside the ribosome exit tunnel,[13] therefore for these domains keeping the large subunits away from each other is less critical. Finally, polysomes that synthesize membrane-associated or secreted proteins are associated with cellular membranes. The membrane association may impose constrains on the compaction process and may lead to other 3D structures of polysomes.

*What is the significance of polysomes compaction?* One explanation is that compaction maintains the polysomes in a desired conformation. The works of Kopeina and Brandt reveal that in the commonly desired conformation, the large subunits are kept away from each other. As an outcome of this orientation, polypeptides that emerge from the ribosomes are located far away from each other. This was suggested to minimize the chances of aggregation neighboring proteins in the process of synthesis.[10] Another advantage of the interaction between distant domains is the possibility of cross-talk between different regulatory domains. Regulatory sequences could easily affect the translation process if they are in constant interaction. The 3' UTR is highly enriched with regulatory sites that serve as binding sites for RNA-binding proteins or miRNAs. These molecules transduce signals from the environment to the mRNA and affect its expression levels. miRNAs, for example, were shown to quickly reduce their target protein production. The mechanism by which this reduction occurs is still not clear, but it surely necessitates communication between the 3' UTR and the translating ribosomes. Thus, extensive associations between the 3' UTR with the coding region will have important implications on the speed and efficiency of the translation reduction.



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