Conformation Transitions of Eukaryotic Polyribosomes during Multi-round Translation

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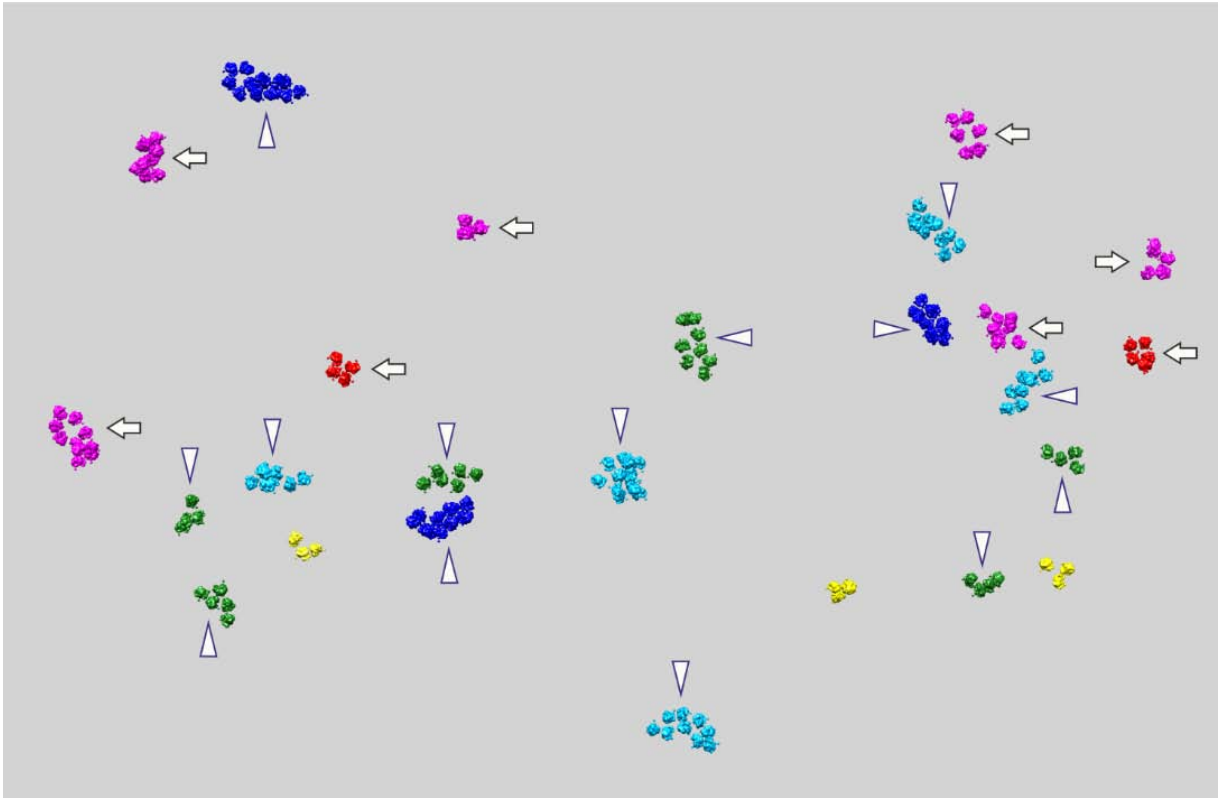
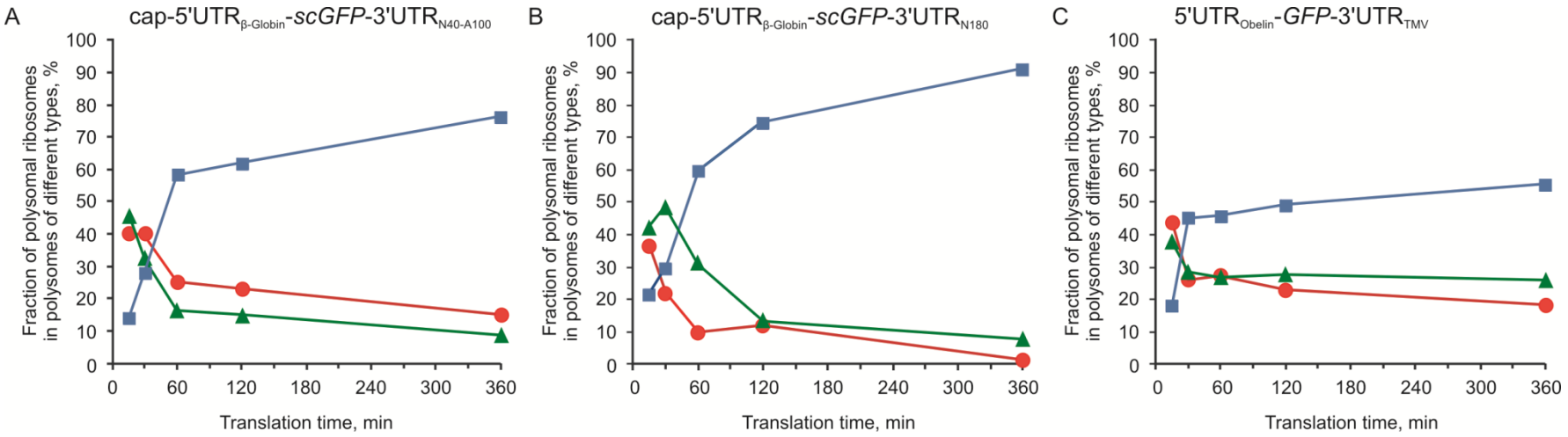
SUPPLEMENTARY DATA

Supplementary Table S1. **Summary of the quantitative analysis of tomograms obtained at different time points of translation in cell-free systems with the mRNAs used.** Number of ribosomes found in polysomes of distinct conformational types was counted and summarized for tomograms of each time point.

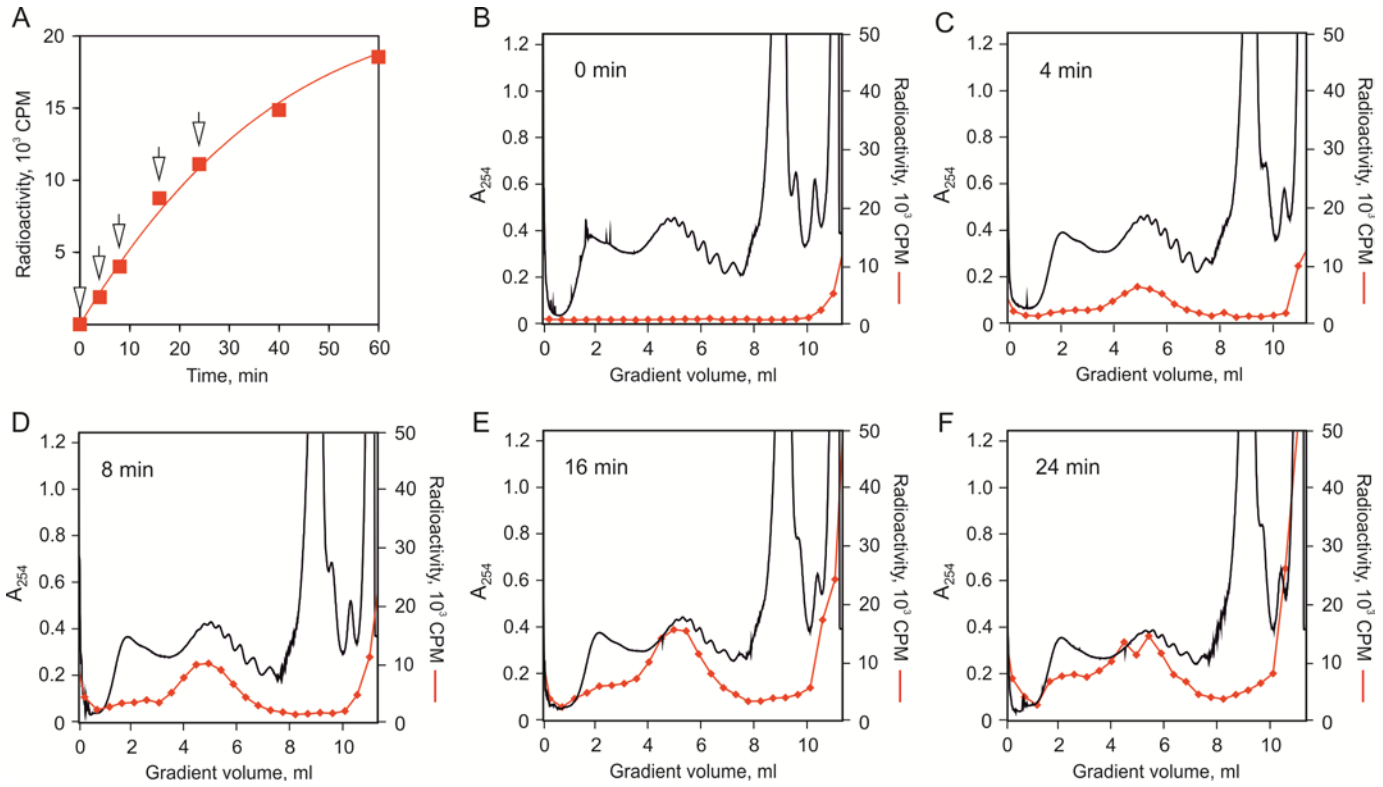
Translation time, min	Number of tomograms	Total number of polysomes	Number of polysomal ribosomes			
			Total, in all polysomes	in circular polysomes	in linear polysomes	in helical polysomes
Cap-5'UTR$_{\beta Globin}$-scGFP-3'UTR$_{(N)40-(A)100}$						
15	6	162	874	**352**	**399**	**123**
30	4	127	838	**333**	**271**	**234**
60	4	72	561	**142**	**92**	**327**
120	5	104	918	**211**	**139**	**568**
360	5	33	494	**74**	**43**	**377**
Cap-5'UTR$_{\beta Globin}$-scGFP-3'UTR$_{(N)180}$						
15	5	128	637	**233**	**268**	**136**
30	3	79	549	**121**	**266**	**162**
60	4	88	730	**71**	**224**	**435**
120	4	60	619	**75**	**82**	**462**
360	7	19	316	**4**	**24**	**288**
5'UTR$_{Obe}$-GFP-3'UTR$_{TMV}$						
15	4	134	821	**360**	**312**	**149**
30	3	74	576	**151**	**165**	**260**
60	4	109	853	**234**	**229**	**390**
120	3	51	674	**155**	**187**	**332**
360	3	34	304	**56**	**79**	**169**



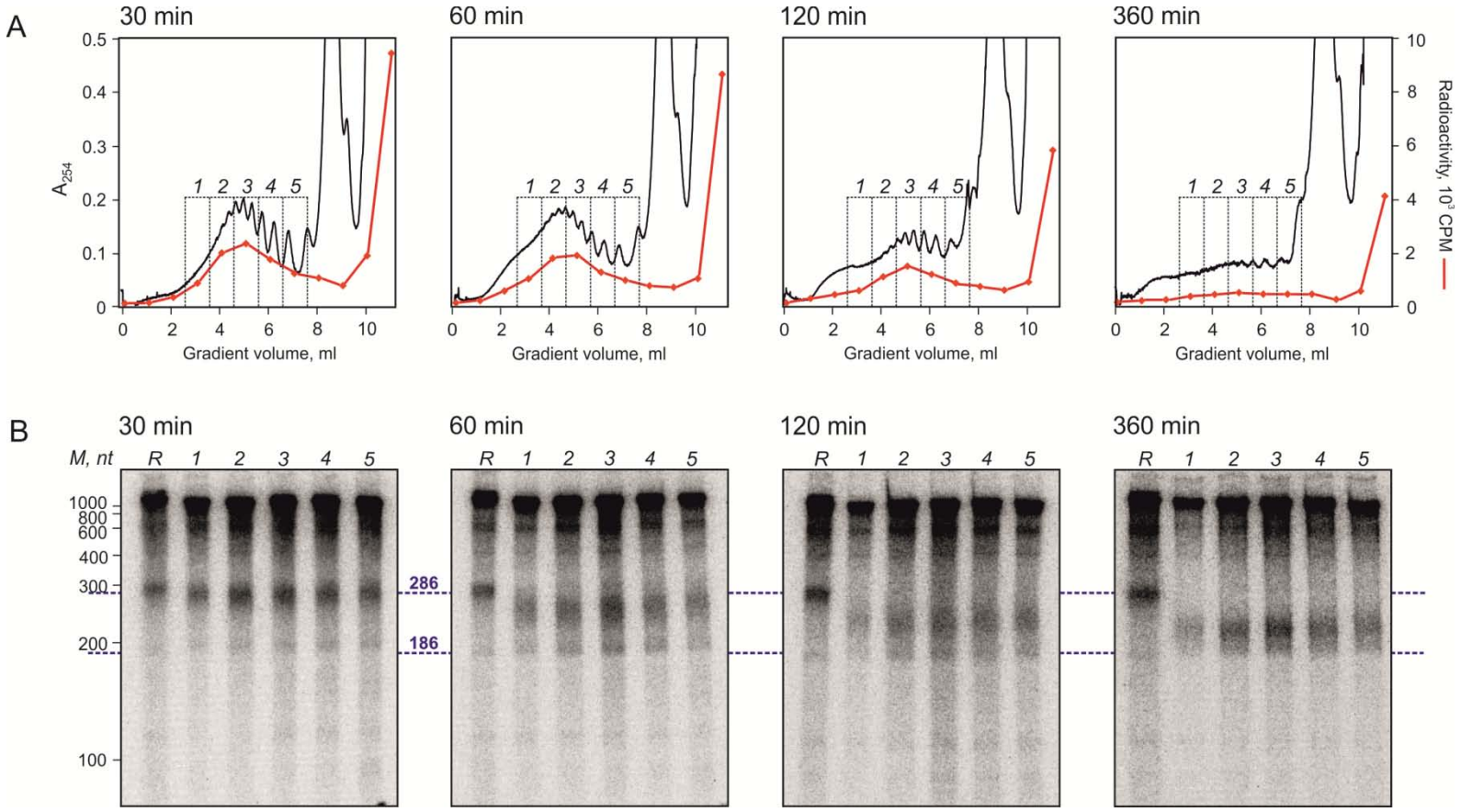
Supplementary Figure S1. Reconstructed cryo-electron tomogram of polyribosomes formed in cell-free translation system programmed with uncapped non-polyadenylated GFP-encoding mRNA, 750 nt coding sequence (construct No. 2, see Materials and Methods) after 60 min translation. The reconstructed polyribosomes were colored depending on their conformations revealed by cryo-ET analysis (see ref. 21, 29 for methodological details). Thus, basing on the general topology of polysomal mRNAs and the peculiarities of mRNA path along a polyribosome, the following colors were assigned to different conformational types and their variants: ***red*** – circular topology (closed loop); ***magenta*** – circular with 3D bulge; ***green*** – linear topology, usually planar zigzag shape; ***blue*** – linear with 3D bulge; ***dark violet*** – 3D helices. Arrows point to polyribosomes with checked circular topology of their mRNA path; arrowheads point to polyribosomes with proved linear topology of their mRNA. Trisomes ***(yellow)*** were not attributed to any conformational type, monosomes are not shown.



Supplementary Figure S2. Dynamics of conformational changes of polyribosomes during multi-round translation in the long-term cell-free translation systems programmed with three mRNAs (indicated in the Figure) studied in this work. The distribution of polysomal ribosomes between different types of polyribosomes – circular (red circles), linear (green triangles) and 3D helical (blue squares) – depending on the time passed after translation start is shown. The percentage of ribosomes in the polysomes of each type are plotted for each time-point; the total number of polysomal ribosomes at this point being 100% (based on the data of Supplementary **Table S1**) .



Supplementary Figure S3. Analysis of translational activity of polyribosomes**.**
After CECF system translation of *5'UTR$_{Omega}$-Luc-3'UTR$_{TMV}$* mRNA (construct No. 4) during 2 hours at 25°C, the translation reaction was switched over to batch format, and radioactive amino acids ([^{14}C]Ser and [^{14}C]Phe) were added (up to 40 µM and 60 µM, respectively). Translation reaction was continued at 25°C for 1 hour more, and the synthesis was controlled by measuring the radioactivity incorporation in the TCA-precipitable product (A). 25 µl aliquots were taken at 0, 4, 8, 16 and 24 minutes after addition of the radioactive amino acids (indicated with arrows) and subjected to zonal centrifugation in 15-50% sucrose gradient using SW-41 rotor at 37000 rpm during 2 hours at 4°C. The incorporation of the radioactive amino acids into nascent polypeptides (TCA-insoluble product) was determined in fractions of the sucrose gradient. The ratio between radioactivity (nascent peptide) and A_{254} (ribosomes) reflects relative translational activity of polysomes of different sizes (occupancy).



Supplementary Figure S4. Control of the integrity of the 3'-end of polyribosomal mRNA during translation in a wheat germ cell-free system. See the next page for details of the experiment.
(**A**). Sedimentation profiles of the system analyzed at the indicated time of translation. Fractions used for the PAGE-analysis are shown by frames 1-5.
(**B**) Radioautographs of the gels. The lanes were loaded with the RNAse H products of: R - initial mRNA, 1-5 – RNA from the gradient fractions 1-5. Positions of the mRNA markers (Low Range, Fermentas) are indicated on the left side. The dotted lines indicate position of 3'-fragment of original intact mRNA with full length polyA tail (~100 nt), designated “286”, and 3'-fragment of non-adenylated mRNA, designated “186”. Positions of markers and reference fragments were determined in parallel lanes with non-radioactive mRNAs and ethidium bromide staining (not shown).

Supplementary Figure S4 (continued).
The mRNA *cap-5'UTR*$_{\beta Globin}$*-scGFP-3'UTR*$_{(N)40-(A)100}$ (construct No.1) was prepared by *in vitro* transcription in the presence of α-[^{32}P]UTP and cap-analog and further polyadenylated as described (25). CECF translation system was performed with the [^{32}P]-labeled mRNA, aliquots were taken at different time points, and the polyribosomes were separated by sedimentation in 15-45% sucrose gradient (2 hrs at 37 000 rpm in SW41 rotor at 4°C). Radioactivity in fractions was measured (Cherenkov) and the RNA was recovered from gradient fractions by phenol-chloroform extraction and ethanol precipitation; purified RNA was incubated with RNase H and DNA oligonucleotide complementary to the region 146-166 nt upstream of stop-codon. In this way two fragment of mRNA was generated, the shorter one contained 146 nt of coding sequence, 40 nt of the 3'UTR sequence, and polyA sequence (about 100 nt). The products of RNase H cleavage were analyzed by 4% urea-PAGE and radioactivity in gels was detected by radioautography with PhosphoImage plate.