SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Bioinformatic analysis

Content of reads in genic features and size distribution analysis. To calculate the number of reads mapping to individual genic features (CDSs, 5' UTRs, 3' UTRs, introns, intercistronic regions, intergenic regions), only reads unambiguously assigned to a given feature were taken into account. From the set of annotated UTRs of transcripts the longest form was used, based on data presented in (7). The number of reads mapping to genic features was determined using BEDTools v2.27.0 package and is expressed as the percentage of all mapped reads in the mtRNA-Seq or mtRibo-Seq library. For the five dicistronic transcripts (*rpl5-cob, rpl2-tatC, nad3-rps12, nad4L-atp4* and *rps3-rpl16)* identified in *Arabidopsis* mitochondria, the 5' UTR reads were counted only for the upstream cistron and the 3' UTR ones - for the downstream cistron. The distribution of ribosome footprint lengths was calculated for reads mapping to CDS regions only using STAR or BEDTools software.

Identification of mtRibo-Seq-sRNAs in non-coding genic regions. Potential sRNA sequences (mtRibo-Seq-sRNAs) were identified among mtRibo-Seq reads mapping to non-coding genic regions (5' UTRs, 3' UTRs, introns, intercistronic regions) using sRNA miner (18). The default software settings (min. reads per end 40 and sharpness of end 0.75) were used. The software allowed the detection of mtRibo-Seq-sRNAs with both sharp ends or only one end. The 5' ends of mtRibo-Seq-sRNAs could differ slightly in length between the wild-type and mutants and therefore only reads for the shortest form were counted for quantitative mtRibo-Seq-sRNA reads comparisons between the wild-type and *rps10*. The number of mtRibo-Seq-sRNAs reads was determined by featureCounts (assuming fracOverlap = 0.8) counting only reads mapping to a given feature in ≥80%.

Determination of the number of reads mapping to spliced and unspliced mRNA junctions. mtRNA-Seq or mtRibo-Seq reads encompassing all possible junctions: 5' exon - 3' exon, 5' exon - intron and intron - 3' exon (50 nt on each side of a junction) were counted using featureCounts with fracOverlap = 1, with or without the MetaFeature option allowing for calculating the reads in two features together or calculating the reads separately for either feature, respectively. The number of spliced reads at each intron was calculated by deducting the sum of reads mapped to both exons separately (without MetaFeature option) from the

number of reads mapped to spliced 5’ exon - 3’ exon (with MetaFeature option). The number of unspliced reads mapped at the exon-intron junctions was calculated by deducting the sum of reads mapped separately to exon and to intron (without MetaFeatures option) from the number of reads mapped to exon - intron junction (with MetaFeature option). The percentage of spliced reads was then calculated by dividing the number of reads spanning each exon - exon junction (spliced) by the sum of spliced (exon - exon) and unspliced (5’ exon - intron or intron - 3’ exon) reads. The percentage of unspliced reads at each intron was calculated by dividing the number of reads spanning each unspliced junction (5’ exon - intron or intron - 3’exon) by the sum of spliced (exon - exon) and unspliced (5’ exon - intron or intron -3’ exon) reads. The amount of spliced and unspliced mRNAs from genes with *trans*-spliced introns cannot be determined with confidence, so junctions containing introns of those type were excluded from analysis.