Granule formation of elongator-specific methionine transfer RNA caused by X-ray irradiation in a non-small lung carcinoma cell line (NCI-H1299)

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Summary

We herein report the subcellular distribution of methionine transfer RNA (tRNA\textsubscript{Met}), which has two forms of initiator tRNA\textsubscript{Met} (Meti) and elongator tRNA\textsubscript{Met} (Mete). Meti induces translation initiation, and Mete is responsible for the elongation of the peptide chain. A recent report showed that the formation of tRNA\textsubscript{Met} granules occurs following heat shock stress. We analyzed the subcellular distribution change in tRNA\textsubscript{Met} using ionizing irradiation. The images acquired using confocal microscopy showed the presence of Mete granules—but not Meti granules—in the cytoplasm. Furthermore, while the Mete granules co-localized with T-cell intracellular antigen-1, they did not co-localize to typical stress granules nor processing body specific markers.

Keywords

tRNA, stress, granule, TIA-1, X-ray, irradiation

Introduction

The modulation of gene expression plays a central role in the stress response. It is subjected to careful control in a multi-step process, such as transcriptional and translational levels. RNA turnover is one of the most essential mechanisms of post-transcriptional regulation. For example, messenger RNAs (mRNAs) that are not engaged in translation can aggregate into cytoplasmic granules, referred to as processing bodies (PBs) and stress granules (SGs), which play a role in translational control.

SGs are non-membranous cytoplasmic foci formed in response to various stress stimuli, typically following the stress-induced phosphorylation of eukaryotic initiator factor 2 (eIF2) (Kedersha et al., 1999). eIF2 is an essential component of the translation-initiator eIF2-Meti-GTP ternary complex (Kedersha et al., 2013). When the alpha subunit of eIF2 is phosphorylated at residue Ser51, the levels of the active ternary complex decrease dramatically (Jackson et al., 2010), and translation initiation is regulated. Stress granules contain non-translating mRNAs, translation initiation components, and many additional proteins resulting from translational inhibition.

Unlike SGs, however, PBs are present even under non-stress conditions (Mahboubi and Stochaj, 2014). PBs are responsible for RNA degradation and translational repression by proteins and microRNAs as well as temporary ribonucleoprotein storage (Parker and Sheth, 2007). The number and size of PBs vary in response to glucose deprivation, osmotic stress, and exposure to ultraviolet light (Teixeira et al., 2005).

SGs and PBs share some protein and mRNA components but also contain a number of unique markers specific to each structure. SGs are distinguished from PBs by the small ribosomal subunits eIF3, eIF4AI, eIF4B, G3BP-1, and PABP-1, while PBs are distinguished by the presence of the mRNA decapping enzyme DCP1a, the 5-3’ exonuclease Xrn1, and the scaffolding proteins hedis/GE-1 (Kedersha and Anderson, 2007).

Transfer RNAs (tRNAs) are transcribed by RNA polymerase III and respond to several kinds of stress (Thompson et al., 2008; Hopper et al., 2010). Recent studies have reported tRNA granule formation in the nucleus of human cells under heat stress condition (Miyagawa et al., 2012). tRNAs that are re-

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preparation of the cells

The human non-small cell lung carcinoma NCI-H1299 cell line was used in this study (ATCC No. CRL-5803). H1299 cells were grown in RPMI-1640 medium (Wako Pure Chemicals, Tokyo, Japan) containing 10% fetal bovine serum (FBS) (BioWest, Nuaillé, France) and an antibiotic-antimycotic solution (GIBCO, Grand Island, NY, USA) at 37°C under an atmosphere of 5% CO₂ to 70%–80% confluence.

Materials and methods

Preparation of the cells

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X-ray irradiation of the cells

The cells (1.0 × 10⁵ cells/mL) were seeded onto a microscope cover slip (Matsunami Glass, Osaka, Japan). After an overnight incubation, they were irradiated with an X-ray generator (Pantak HF 350; Shimadzu, Kyoto, Japan) operating at 200 kV - 20 mA with a 0.5-mm Cu and 1-mm Al filter at a dose rate of 0.35 Gy/min.

Analysis of the nuclear localization of tRNA by fluorescence in-situ hybridization (FISH) using DIG-labeled RNA probes and immunofluorescence (IF) staining

The tRNA mixture was extracted from Escherichia coli using phenol and chloroform and then precipitated with ethanol. At 2 h after irradiation, the cells were fixed with a 4% paraformaldehyde phosphate buffer solution (Wako Pure Chemicals) for 20 min at room temperature. Fixed cells were rinsed with 1x phosphate-buffered saline (PBS) and permeabilized in 1x PBS containing 0.5% Triton X-100 for 10 min on ice. The cells were incubated in a prehybridization solution (2x SSC, 1x Denhardt's solution, 50% formamide, 10 mM EDTA, 100 microgram/mL yeast tRNA, and 0.01% Tween 20) at 55°C for 2 h.

The DNA templates for transcription of the initiator tRNA⁹ (Met) and elongator tRNA⁹ (Mete) RNA probes were prepared by PCR (Mete forward primer: 5'-AGC AGA GTG GCG CAG CGG-3', Meti reverse primer: 5'-CCA AGC TCT AAT ACG ACT CAC TAT AGG AGC AGA GGA TGG TTT CGA TCC-3'; Mete forward primer: 5'-GCC TCG TTA GCG CAG TAG GT-3', Mete reverse primer: 5'-CCA AGC TCT AAT ACG ACT CAC TAT AGC CCG TGT GAG GAT CGA ACT C-3'). Meti and Mete RNA probes were prepared using a DIG RNA labeling kit (Roche, Basel, Switzerland) in accordance with the manufacturer's protocols. Prehybridized cells were incubated in a hybridization solution (prehybridization solution plus 5% dextran sulfate) containing the DIG-labeled RNA probe at 55°C overnight. The cells were washed twice with prewarmed wash buffer (2x SSC, 50% formamide, 0.01% Tween-20) at 55°C for 30 min. Excess probes were digested with 10 µg/mL RNase A in NTET buffer at 37 degrees Celsius for 30 min. The cells were washed twice with buffer (2x SSC, 0.01% Tween-20) at 55°C for 1 h. The cells were then blocked with a blocking buffer (10% FBS, 1x PBS, 0.01% Tween-20) at room temperature for 1 h and incubated with an anti-DIG antibody (1 ng/mL in 50% glycerol solution, 1:50 dilution; 11214667001; Roche), anti-Dcp1a antibody (1:800 dilution, ab47811; Abcam, Cambridge, UK), or anti-PABP antibody (1:300 dilution, ab21060; Abcam) diluted in the blocking buffer for 1.5 h. Unbound antibodies were removed by washing 3 times in PBST (1x PBS, 0.2% Tween-20) for 15 min. The cells were incubated with Alexa-conjugated secondary antibodies (Invitrogen, Carlsbad, CA, USA) for 1 h. After washing, the cells were counterstained with 4', 6-diamidino-2-phenylindole (DAPI).

Image and statistical analyses of the fluorescence intensity

All of the imaging experiments were performed using a FLUOVIEW FV1200 laser scanning microscope and FV-OSR system (Olympus, Tokyo, Japan).
tRNA granule caused by X-ray radiation stress

We studied the subcellular distribution of initiator methionine-tRNA (Meti) and elongator methionine-tRNA (Mete) under 0.5-Gy X-ray irradiation using the fluorescence in-situ hybridization (FISH) and immunofluorescence (IF) method. The formation of Mete—but not Meti—granules was detected (Fig. 1). Although both Meti and Mete transport methionine to the ribosome, they play distinct roles in the translation process. Meti is a translation initiation factor that forms a specific complex with the initiation factor eIF2-alpha, while Mete is a translation elongation factor that forms a ternary complex with elongation factor eEF1 (Petrushenko et al., 2002).

Results

Formation of type-specific tRNA granules under X-ray irradiation

We studied the subcellular distribution of initiator methionine-tRNA (Meti) and elongator methionine-tRNA (Mete) under 0.5-Gy X-ray irradiation using the fluorescence in-situ hybridization (FISH) and immunofluorescence (IF) method. The formation of Mete—but not Meti—granules was detected (Fig. 1). Although both Meti and Mete transport methionine to the ribosome, they play distinct roles in the translation process. Meti is a translation initiation factor that forms a specific complex with the initiation factor eIF2-alpha, while Mete is a translation elongation factor that forms a ternary complex with elongation factor eEF1 (Petrushenko et al., 2002).

Co-localization of Mete granules

We investigated the co-localization of Mete granules with several kinds of stress marker proteins using the FISH and IF methods. First, we visualized polyadenylate-binding protein 1 (PABP-1), which is exclusively found in SGs (Anderson and Kedersha, 2006). PABP-1 is an RNA-binding protein that binds to the polyadenine tail at the 3' end of mRNAs (Brook and Gray, 2012). Our results showed that PABP-1 was distributed evenly throughout the cytoplasm without aggregation and did not co-localize with the Mete granules (Fig. 2), indicating the absence of SGs in the cytoplasm. Some reports have indicated that X-ray irradiation does not induce the formation of SGs because it is more likely to induce apoptosis through the stress-activated p38 and JNK MAPK pathways (Arimoto et al., 2008; Takahashi et al., 2013). Our results were consistent with these previous reports.

Next, we examined the localization of T-cell intracellular antigen-1 (TIA-1). TIA-1 is a useful SG marker but not SG-specific and may also be associated with PBs in unstressed cells (Kedersha and Anderson, 2007). We visualized the localization of TIA-1 and found co-localization between the localization of Mete granules and TIA-1 foci (Fig. 3).

Finally, we examined the localization of mRNA-decapping enzyme 1A (DCP1A), which is a PB-specific marker (Eystathioy et al., 2003). The decapping and decay of mRNA occur in cytoplasmic processing bodies (Sheth and Parker, 2003). mRNA is decapped by a decapping complex that includes DCP1A and is then degraded by the exonuclease Xrn1 through the 5'–3' mRNA decay pathway (Chen and Shyu, 2011; Adjibade and Mazroui, 2014). Our results showed no co-localization between the localization of Mete granules and PBs (Fig. 4), although PBs were observed in both control and irradiated cells.
Fig. 2. An analysis of the localization of PABP-1 and Mete under 0.5-Gy X-ray irradiation. The gray arrow indicates the Mete (green) granule. The PABP-1 (Red) was distributed evenly throughout the cytoplasm without aggregation, even under 0.5-Gy X-ray irradiation. All scale bars indicate 10 µm.

Fig. 3. An analysis of the localization of TIA-1 and Mete under 0.5-Gy X-ray irradiation. The gray arrow indicates the Mete (green) granule. The orange arrow indicates the TIA-1 (Red) granule. The merged image showed good co-localization between the location of Mete granules and TIA-1 foci. All scale bars indicate 10 µm.

Fig. 4. An analysis of the localization of DCP1A and Mete under 0.5-Gy X-ray irradiation. The gray arrow indicates the Mete (green) granule. There was no co-localization of Mete granules and DCP1A (white), which is a PB-specific marker. PBs were also observed under the control (0 Gy) conditions (data is not shown). All scale bars indicate 10 µm.
Discussion

Miyagawa et al. showed the formation of tRNA granules in the nucleus of heat-stressed human cells (Miyagawa et al., 2012). Under heat stress conditions, both Meti and Mete are translocated into the nucleus and form granules that overlap with nuclear stress bodies, which are characterized by the marker protein heat shock transcription factor-1.

In contrast, the present study found that only Mete formed granules that co-localized with cytoplasmic TIA-1 granules. Our results suggest that cytoplasmic Meti distribution may not have been affected by X-ray radiation. Since the initiation step is a critical point in translation, our results suggest that X-rays may have relatively little effect in terms of translation.

TIA-1 is found predominantly in SGs, but a small fraction is also detected in PBs (Eulalio et al., 2007). However, our results indicated that TIA-1-Mete granules did not co-localize with SG- or PB-specific markers. Cytoplasmic TIA-1-positive foci that do not contain other SG-defining components, which are similar to our observations, have been reported for poliovirus infection (White and Lloyd, 2011).

TIA-1 consists of approximately 375 amino acids and contains 3 N-terminal RNA-recognition motifs (RRMs) and a C-terminal glutamine-rich prion-related domain (PRD) (Waris et al., 2014). TIA-1 plays a role in several RNA turnover pathways, including alternative heterogeneous nuclear RNA splicing and the regulation of mRNA translation (Zhang et al., 2005). TIA-1 binds mRNA through its 3 RRMs, and the interaction of the histone acetyltransferase-terminal PRD structure promotes granular formation (Gilks et al., 2004). External stress induces the formation of temporary cytoplasmic granules that contain non-translating mRNA various proteins. We might have detected Mete attached to mRNAs in the TIA-1-positive foci identified in the present study, which would explain their granular-like appearance.

Even now, the effects of X-ray radiation on the human body at the cellular level are still largely unknown. We believe that our detection of TIA-1-Mete granules will result in some controversy regarding the effects of ionizing radiation. Given the findings in the present study, the presence of Mete granules should be investigated further from the aspect of temporal sequences, component composition, and molecular interactions.

Acknowledgments

This work was supported by a Grant-in-Aid for Young Scientists (B) to Ryu MIYAGAWA (Japan Society for the Promotion of Science, KAKENHI Grant number: 26830096). The authors sincerely thank Prof. M. HATAKEYAMA, who gave his permission for us to use the required equipment.

Author contributions

AK and RM analyzed and interpreted the experimental data. AK was a major contributor in writing this report. RM conceived this report and participated in its design and coordination. RM revised this report critically for important intellectual content. All of the authors read and approved the final manuscript.

Received February 8, 2016; revised version accepted April 8, 2016

Reference


Lett 584, 310-317.


