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Research paper

Heat shock increases lifetime of a small RNA and induces its accumulation in cells

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ABSTRACT

4.5SH and 4.5SI RNA are two abundant small non-coding RNAs specific for several related rodent families including Muridae. These RNAs have a number of common characteristics such as the short length (about 100 nt), transcription by RNA polymerase III, and origin from Short Interspersed Elements (SINEs). However, their stabilities in cells substantially differ: the half-life of 4.5SH RNA is about 20 min, while that of 4.5SI RNA is 22 h. Here we studied the influence of cell stress such as heat shock or viral infection on these two RNAs. We found that the level of 4.5SI RNA did not change in stressed cells; whereas heat shock increased the abundance of 4.5SH RNA 3.2–10.5 times in different cell lines; and viral infection, 5 times. Due to the significant difference in the turnover rates of these two RNAs, a similar activation of their transcription by heat shock increases the level of the short-lived 4.5SH RNA and has minor effect on the level of the long-lived 4.5SI RNA. In addition, the accumulation of 4.5SH RNA results not only from the induction of its transcription but also from a substantial retardation of its decay. To our knowledge, it is the first example of a short-lived non-coding RNA whose elongated lifetime contributes significantly to its accumulation in stressed cells.

1. Introduction

The study of small cellular RNAs began with the discovery of tRNA and 5S ribosomal RNA. Their role in translation of mRNA was soon confirmed. A number of new small RNAs (70–300 nt) were found in eukaryotic cells in the 1970-s (Weinberg and Penman, 1968; Ro-Choi and Busch, 1974; Zieve and Penman, 1976). It became clear that they play an important role in pre-mRNA splicing (U1, U2, U4, U5, U6, U11, and U12 RNAs), pre-rRNA processing (U3 and U14 RNAs) and modification (C/D box RNAs and H/ACA box RNAs), as well as protein secretion (7SL RNA), transcription regulation (7SK RNA), and the initiation of DNA replication (Y RNAs) (Makarova Iu and Kramerov, 2007; Cooper et al., 2009; Diribarne and Bensaude, 2009; Moazed, 2009; Krude, 2010). The discovery of numerous microRNAs about 22 nt-long caused a surge of studies, which established that these RNAs regulate the gene expression using mRNA silencing (Bartel, 2004).

Among the first discovered and sequenced small RNAs were 4.5SI and 4.5SH RNAs (Ro-Choi et al., 1972; Harada and Kato, 1980). Each is about 100 nt in length, but their nucleotide sequences only slightly resemble each other. These RNAs are synthesized by RNA polymerase III (pol III), which generates short similar sequences: (i) internal promoter consisting of A and B boxes (each 11 nt long), and (ii) 3–4 U nucleotide residues on the 3′-end of the RNA which result from transcription of the terminator (TTTTTT) (Leinwand et al., 1982; Reddy et al., 1983). Both RNAs are transcribed in various tissues and are localized predominantly in the nucleus; however, their functions remain unclear. Nevertheless, it was recently reported that 4.5SH RNA could interact with antisense B1 sequence (see below) in mRNA molecules and retain them in the nucleus (Ishida et al., 2015).

Contrary to most abundant small RNAs, 4.5SH RNA has rapid turnover in mouse cells (Schoeniger and Jelinek, 1986). The half-life of 4.5SH RNA is only 18 min, whereas $t_{1/2}$ of 4.5SI RNA is 22 h (Koval et al., 2012). Our experiments showed that the complementary interaction between 5′- and 3′- end regions (16 nt) of 4.5SI RNA contributed to its stability in cells, whereas lack of such complementarity in 4.5SH RNA caused its rapid decay (Koval et al., 2012, 2015).

4.5SI and 4.5SH RNAs were discovered in mouse and rat cells, but they were not found in the human cells (Harada et al., 1979; Busch et al., 1982). Later it was established that 4.5SI RNA is only present in rodents of three related families: Muridae (mice, rats, gerbils), Cricetidae (hamsters, voles), and Spalacidae (mole rats, root rats, zokors) (Gogolevskaya and Kramerov, 2002; Gogolevskaya et al., 2010). 4.5SH RNA is found in the same rodents, as well as jerboas and birch mice (Dipodidae) (Gogolevskaya et al., 2005). The genes that code these two RNAs are organized differently in the genome. In mice, 4.5SI RNA is transcribed from three genes located on chromosome 6 and spaced 40 kb apart (Gogolevskaya and Kramerov, 2010). The

Abbreviations: EMC, encephalomyocarditis; HSP, heat shock protein; KAC, Krebs ascites carcinoma; PFU, plaque-forming unit; PKR, protein kinase R; Pol, RNA polymerase; SINE, Short Interspersed Element.

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number of 4.5SH genes is much higher (700–800) in the genomes of all rodents studied; each 4.5SH gene is a part of a 4–5 kb tandemly repeated unit (Schoeniger and Jelinek, 1986; Gogolevskaya et al., 2005). Interestingly, both RNAs are evolutionarily related to Short Interspersed Elements (SINEs): it would appear that 4.5SI RNA genes originated from SINE B2 (Serdobova and Kramerov, 1998), whereas 4.5SH RNA genes were derived from a copy of an ancient SINE pB1 (Krayev et al., 1982; Quentin, 1994). SINEs are non-autonomic mobile genetic elements that are typical of most multicellular eukaryotes (Vassetzky and Kramerov, 2013). Their lengths range from 100 to 500 bp and their genomic copy numbers can reach a million. The relationship between SINEs and the genes of 4.5SI and 4.5SH RNAs is not unique: other examples of evolutionary linkage between SINEs and genes of small noncoding RNAs (BC1, BC200, snaR) have been reported (Martignetti and Brosius, 1993; Kim et al., 1994; Parrott et al., 2011).

It is well established that cells (*in vivo* or *ex vivo*) respond biochemically to heat shock and other stresses (Velichko et al., 2013). In heat stressed cells transcription of most of the genes ceases (DiDomenico et al., 1982; Vazquez et al., 1993), splicing of pre-mRNA stops (Yost and Lindquist, 1986; Vogel et al., 1995), translation of many mRNAs ends, and these mRNAs together with initiation translation factors accumulate in so called stress granules (Buchan and Parker, 2009). A small increase in temperature can cause protein unfolding, entanglement, and unspecific aggregation in cells. The main response to heat stress is the synthesis of heat shock proteins (HSPs), their number in human may approach 100 (Richter et al., 2010). Chaperones that play a critical role in protein folding, intracellular trafficking of proteins, and coping with proteins denatured by heat, are the most important HSPs for cells to overcome the consequences of heat shock.

A discrete specific field connected with SINEs transcripts emerged in studies of cell response to heat shock and other stresses. It was found that heat shock leads to an increase in the level of RNAs transcribed from such SINEs as B1 and B2 (mice, hamsters), Alu (human), C (rabbit) and Bm 1 (silkworm) (Fornace and Mitchell, 1986; Liu et al., 1995; Li et al., 1999; Kimura et al., 2001). It is caused by a significant increase of SINE transcription by pol III. Viral infection is also considered a type of cell stress. Interestingly, transcription of SINEs Alu, B1 and B2 significantly increases during infection of cells by different viruses (herpes simplex virus, adenovirus, minute virus of mice) (Jang and Latchman, 1992; Russanova et al., 1995; Williams et al., 2004).

The group of Schmid (Chu et al., 1998; Rubin et al., 2002) showed that increased level of Alu RNA after cellular heat shock stimulates protein synthesis by inhibiting PKR, the eIF2 kinase that is regulated by double-stranded RNA. It was proposed that such stimulation of protein synthesis contributes to the survival of stressed cells. Later the group of Goodrich and Kugel made an amazing discovery: the RNAs transcribed by pol III from SINEs such as B2 and Alu can bind to RNA pol III polymerase II (pol II) and effectively inhibit it (Espinoza et al., 2004, 2007; Mariner et al., 2008). The authors suggested that the high level of B2 or Alu RNA observed during heat shock suppresses the activity of pol II and the synthesis of mRNA, characteristic for cellular response to stress (Allen et al., 2004; Mariner et al., 2008). The genes of HSPs somehow avoid such inhibition of transcription and, on the contrary, increase their expression. Consequently, the synthesis of heat shock chaperones contributes to cell recovery from stress.

As mentioned above, the genes of 4.5SH and 4.5SI RNAs are evolutionary related to SINEs B1 and B2, respectively. Therefore, we were interested to know how these two RNAs would behave after exposure of cells to heat shock. We found that the level of 4.5SH RNA (but not 4.5SI RNA) increased several-fold under the influence of heat shock in different cell lines of mice and rats. We found that this effect is determined by the activation of 4.5SH RNA genes transcription and by the considerable retardation of its decay. Viral infection of cells, another stress influence, produced similar results. Furthermore, we obtained data indicating specific character of 4.5SH RNA accumulation in cells exposed to heat shock.

2. Materials and methods

2.1. Cultivation of KAC II, Rat1, L929, NIH/3T3 and 4T1 cells

Procedures involving live animals were reviewed and approved by the Animal Care and Use Committee of The Severtsev Institute of Problems of Evolution and Ecology (Russian Academy of Sciences) where animals were housed. Krebs ascites carcinoma II cells (KAC II) (Yushok et al., 1956) were collected from a mouse tumor 7–10 days after intraperitoneal administration of 0.7 ml of previously obtained ascites. The cells were washed with PBS and suspended (10^7 cells per ml) in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum and 10 mM Hepes, pH 7.0 and gently stirred at 37 °C in a vial with a tightly closed cap for 1 h before experiment.

Rat1 (Reynolds et al., 1987), L929 (ATCC CCL-1), NIH/3T3 (ATCC CRL-1658) and 4T1 (ATCC CRL-2539) cells were grown to 70–90% confluence in 75 cm^2 flasks using DMEM with 10% fetal calf serum and then passaged to 25 cm^2 flasks for experiment.

2.2. Cell heat shock

Heat shock of the KAC II cells was performed by placing the vial into a 45 °C stirring water bath for 30 min. After that the vial was returned to 37 °C for recovery for 24 h. 5 ml aliquots of medium with KAC II cells were taken at different time points during heat shock and recovery.

Flasks with Rat1, L929, NIH/3T3 and 4T1 cells were exposed to 45 °C heat shock in a water bath for 30 min (Rat1, L929 and 4T1) or for 12 min (NIH/3T3). Recovery of the cells after heat shock was performed for different periods of time (0 to 24 h) in a 37 °C incubator.

2.3. Infection of the cells by the encephalomyocarditis (EMC) virus

KAC II cells were suspended in DMEM containing EMC (titer 2×10^9 PFU/ml) at the multiplicity of infection (MOI) 20 PFU/cell and were left at room temperature for 30 min. The cells were washed with PBS and suspended in DMEM containing 10% fetal calf serum. The cells (10^7 per ml) were gently stirred at 37 °C in a vial with a tightly closed cap for 6 h. 5 ml aliquots were taken at different time points.

2.4. Inhibition of transcription

To determine the lifetime of the RNAs the inhibitor of transcription, actinomycin D (5 μg/ml), was added to medium after 1 h of recovery. The RNA of interest was detected by Northern-blot hybridization and its level was measured with phosphoroimager (Section 2.8). To characterize the RNA lifetime in cell the value $t_{1/2}$ was used (the time of decay of half the studied RNA). The value $t_{1/2}$ (half-life) is generally applied to decay with a constant rate. Although in some of our experiments the 4.5SH RNA decay rate was changing in course of measuring the half-life, in such cases we also used $t_{1/2}$ which therefore represented the average value for the measuring period. In spite of such extended interpretation of the value $t_{1/2}$, it appeared to be quite convenient for comparing the RNA decay kinetics.

2.5. Transfection of 4T1 cells

4T1 cells were grown to 70–80% confluence in 25 cm^2 flasks using DMEM with 10% fetal calf serum. 4T1 cells were transiently transfected with 5 μg of plasmid DNA applying TurboFect *in vitro* Transfection Reagent (Fermentas, Vilnius, Lithuania) following the manufacturer's protocol.

2.6. Permeabilization of cell membranes and degradation reaction

1 volume of KAC II ascites was collected, washed with PBS, then with permeabilization buffer (150 mM sucrose, 80 mM KCl, 35 mM Hepes