Repression of Global Protein Synthesis by Eif1a-Like Genes That Are Expressed Specifically in the Two-Cell Embryos and the Transient Zscan4-Positive State of Embryonic Stem Cells

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Abstract

Mouse embryonic stem (ES) cells are prototypical stem cells that remain undifferentiated in culture for long periods, yet maintain the ability to differentiate into essentially all cell types. Previously, we have reported that ES cells oscillate between two distinct states, which can be distinguished by the transient expression of Zscan4 genes originally identified for its specific expression in mouse two-cell stage embryos. Here, we report that the nascent protein synthesis is globally repressed in the Zscan4-positive state of ES cells, which is mediated by the transient expression of newly identified eukaryotic translation initiation factor 1A (Eif1a)-like genes.

Eif1a-like genes, clustered on Chromosome 12, show the high sequence similarity to Eifa1 and consist of 10 genes (Eif1al1 – Eif1al10) and 9 pseudogenes (Eif1al-ps1 – Eif1al-ps9). The analysis of the expressed sequence tag database showed that Eif1a-like genes are expressed mostly in the two-cell stage mouse embryos. Microarray analyses and quantitative real-time polymerase chain reaction analyses show that Eif1a-like genes are expressed specifically in the Zscan4-positive state of ES cells. These results indicate a novel mechanism to repress protein synthesis by Eif1a-like genes and a unique mode of protein synthesis regulation in ES cells, which undergo a transient and reversible repression of global protein synthesis in the Zscan4-positive state.

Key words: Eif1a-like genes; global translational repression; embryonic stem cell; two-cell stage embryo; Zscan4

1. Introduction

Zinc finger and SCAN domain-containing 4 (Zscan4) genes are originally identified as genes that are specifically expressed in the two-cell stage of mouse pre-implantation embryos (Falco et al., 2007). The two-cell stage is critical for mouse development, as this is the time when the dramatic epigenetic changes occur concomitantly with the transcriptional activation of the maternally derived genome and paternally derived genome for the first time (called zygotic genome activation, ZGA). Although human genome contains only one copy of ZSCAN4, mouse genome contains nine copies of genes (Zscan4a, Zscan4b, Zscan4c, Zscan4d, Zscan4e, Zscan4f, and three pseudogenes). Zscan4d is expressed predominantly in the two-cell stage mouse embryos, whereas Zscan4c is expressed predominantly in the mouse embryonic stem (ES) cells. We here collectively call them Zscan4.

The expression of Zscan4 in mouse ES cells is transient and reversible, resulting in the unique expression pattern with expression in only 1–5% of ES cells at
one time in culture. Further analysis reveals that ES cells oscillate between the Zsca4-negative (Zscan4−) state and Zscan4-positive (Zscan4+) state, in which Zscan4 functions to extend telomeres and to enhance genomic stability. A recent study shows that this transient state is also marked with the expression of endogenous retroviruses, such as MuERV-L, which are also highly expressed in the two-cell stage mouse embryos. Additionally, Zscan4 has also been shown to further enhance the efficiency of generating mouse induced pluripotent stem cells and to increase their quality.

Through the global expression analysis, we have noticed that a eukaryotic translation initiation factor (Eif1a) is highly up-regulated in the Zscan4− state of ES cells compared with the Zscan4+ state. Studies using a yeast model have shown that, together with Eif1a, Eif1 plays an important role in the identification of translation initiation codon in eukaryotes. Eif1a is identified as a mouse eukaryotic elongation factor Tu in erythroleukemic cells. It was also identified as a translation initiation factor eIF-4C, a gene expressed transiently in the two-cell stage of the pre-implantation mouse embryos. Although Eif1a has been used as a specific marker for ZGA in the two-cell stage embryos, its function in either ZGA or two-cell embryos is not clear.

In this study, we have identified a cluster of Eif1a-like genes on mouse Chromosome 12 and revealed that, not Eif1a on Chromosome 18, but Eif1a-like genes are expressed specifically in the two-cell stage mouse embryos and in the Zscan4+ state of mouse ES cells. We also found that the nascent protein translation is globally repressed in the Zscan4+ state of ES cells, which can be explained by the specific expression of Eif1a-like genes in these cells, as we have also shown that the over-expression of Eif1a-like genes can indeed repress the global protein synthesis. Together, the findings from this study demonstrate a new mechanism by which global protein translation can be regulated in the ES cells.

2. Materials and methods

2.1. Mouse embryonic stem cell culture and transfection

Mouse ES cell lines, V6.5, derived from F1 hybrid mouse strain C57BL/6 × 129/Sv (Thermo Scientific, Rockford, IL, USA), and MC1, derived from 129S6/SVeVTac mouse strain (Transgenic Core Laboratory, Johns Hopkins University School of Medicine, Baltimore, MD, USA), were used in this study. ES cells were maintained on 0.1% gelatin-coated culture plates in complete ES cell media consisting of Dulbecco’s modified Eagle’s medium (DMEM; Gibco Invitrogen, Grand Island, NY, USA) supplemented with 15% fetal bovine serum (FBS) (Atlanta Biologicals, Lawrenceville, GA, USA), 1000 U/ml leukaemia inhibitory factor (LIF; ESGRO, Chemicon Millipore, Billerica, MA, USA), 1 mM sodium pyruvate, 0.1 mM non-essential amino acids, 2 mM GlutaMAX, 0.1 mM β-mercaptoethanol, 100 U/ml penicillin, and 100 μg/ml streptomycin (all media supplements were from Gibco Invitrogen unless otherwise stated). ES cell culture medium was changed daily, and cells were passed every 3 days. ES cells were transfected using Effectene (Qiagen, Valencia, CA, USA) following the manufacturer’s protocol.

2.2. Vectors and DNA Constructs

3 × FLAG tag were added to the 5′-prime end of mouse Eif1a (NM_0101120.5), Eif1a6l (U106343), and Eif1a9 (U285359) and cloned into the pPyCAG-BstXI-IP vector (a generous gift from Dr Hitoshi Niwa, RIKEN Center for Developmental Biology, Japan, http://www.cdb.riken.jp/pcs/protocol/vector/vector_top.html) at Xhol and NotI sites.

2.3. Nascent protein synthesis labelling

Click-IT L-azidohomoalanine (AHA) Alexa Fluor 488 Protein Synthesis Assay Kit (Invitrogen Life Sciences) was used to label newly synthesized protein following the manufacturer’s protocol. Cells were plated on 12-well plates and allowed to adhere overnight. Cells were cultured in methionine-free complete ES cell media for 30 min, labelled in AHA-containing methionine-free complete ES cell media for 1 h, and subsequently fixed for immunofluorescence studies or protein extracted using RIPA lysis buffer (Thermo Scientific). Tetramethylrhodamine (TAMRA) detection of AHA-labelled protein samples were performed using the Click-IT TAMRA Protein Analysis Detection Kit (Invitrogen Life Sciences) following the manufacturer’s protocol.

2.4. Immunofluorescence

Cells were fixed with 4% paraformaldehyde (Electron Microscopy Sciences, Hatfield, PA, USA) for 20 min and permeabilized with 0.5% Triton X-100 (Sigma-Aldrich, St. Louis, MO, USA) in phosphate buffered saline (PBS) for 15 min at room temperature. Cells were blocked for 30 min at room temperature in PBS containing 10% FBS, 10% goat serum (Gibco Invitrogen), and 1% donkey serum (Sigma-Aldrich). The cells were incubated in primary antibodies overnight at 4°C followed by secondary antibodies for 2 h at room temperature and lastly stained with 4′,6-diamidino-2-phenylindole, dihydrochloride (DAPI) (Roche, Indianapolis, IN, USA). Custom-made polyclonal rabbit anti-Zscan4 antibodies (GenScript, Piscataway, NJ, USA) were generated against the C-terminal
epitope Zscan4 as described previously.\textsuperscript{6} Anti-FLAG M2 (F1804; Sigma), mouse anti-Zscan4 (Abnova, Taipei, Taiwan), anti-Eif1α (Abcam, Cambridge, MA, USA), anti-eIF2α phosphoserine 51 (Abcam), anti-histone H3 phosphoserine 10 (Abcam), anti-actin (Sigma), and fluorescent Alexa Fluor secondary (Invitrogen) antibodies were purchased. Fluorescence was visualized and captured using a Nikon Eclipse TE300 inverted microscope (Nikon, Melville, NY, USA).

2.5. Sodium dodecyl sulphate polyacrylamide gel electrophoresis and western blot analysis

Protein samples were prepared by lysing mouse ES cells in RIPA buffer (Thermo Scientific) following manufacturer's protocol. Protein concentration was determined using the Micro BCA Protein Assay Kit (Thermo Scientific). 20 μg of protein samples were loaded onto NuPAGE 4–12% Bis-Tris gel (Novex Life Technologies, Carlsbad, CA) and ran in MOPS sodium dodecyl sulphate running buffer (Invitrogen) at 200 V for 50 min. Protein transfer was performed using the Bio-Rad transfer system at 80 V for 3 h at 4°C onto polyvinylidene difluoride (PVDF) membrane (GE Healthcare, Buckinghamshire, UK). Membranes were blocked in Tris-buffered saline (BioSource, Chevy Chase, MD, USA) containing 0.05% Tween 20 (Bio-Rad) and 5% milk. Primary antibodies were incubated at 4°C overnight. Enhanced chemi-luminescence solutions (Pierce Thermo Scientific) were applied to the membranes and were exposed against Kodak X-Omat AR2 film (Perkin Elmer).

2.6. Quantitative real-time PCR

RNA samples were isolated from cells using the RNeasy mini kit (Qiagen). One microgram of total RNA was used for cDNA synthesis using the high capacity cDNA reverse transcription kit (Invitrogen). Quantitative polymerase chain reaction (PCR) analysis was performed in triplicates with the SYBR green master mix and run on 7900HT system (Applied Biosystems). All procedures above were performed following the respective manufacturer’s protocols. Primers used for PCR reactions were: wild type Eif1a forward, 5'–GGCCATCGTGAGCTGAAGTA–3'; reverse, 5'–TGAGTTTCCTAGCTGAGA–3'; Eif1al6 forward, 5'–CCAACAAATTGGCCATCTCCG–3'; reverse, 5'–GCATT GACAAAAACACATGC–3'; Eif1al9 forward, 5'–CAA TGAGGTAGTTGTTAAGAGCA–3'; reverse, 5'–GAAA TGGTTCACACAAAAAGGACA–3'; Gapdh forward, 5'–CC CAAATGTGCCGCTCGTG–3'; reverse, 5'–GCGTCCTCCA CCACCTTCT–3'. All measurements were normalized to Gapdh loading control and relative expression of Eif1α, and variant expression were compared with untreated control samples using the ΔΔ Ct method.\textsuperscript{17}

3. Results

3.1. Nascent protein synthesis is globally repressed in the Zscan4\textsuperscript{+} state of mouse ES cells

Zscan4, a specific marker for two-cell embryo, has been shown in previous studies to be transiently expressed in mouse ES cells.\textsuperscript{4,6} Mouse ES cells oscillate between Zscan4\textsuperscript{+} and Zscan4\textsuperscript{−} states. Eif1α, a translation initiation factor, is found to be co-upregulated with Zscan4 in two-cell embryo\textsuperscript{18–20} and was also found to be one of the top up-regulated genes in cells enriched for Zscan4 (pZscan4-Emerald).\textsuperscript{10} In earlier studies, we have also noticed that, at the RNA levels, the expression of Zscan4 is often accompanied with the expression of Eif1α.\textsuperscript{21} Because Eif1α is a known translation initiation factor, we hypothesized that the high expression of Eif1α in the Zscan4\textsuperscript{+} state may play a role in the protein synthesis in these cells.

To test this notion, we first examined the nascent protein synthesis in mouse ES cells using the AHA assay. To our surprise, the dramatic repression of global protein synthesis was observed in \textasciitilde 95% of Zscan4-positive cells when compared with surrounding Zscan4-negative ES cells (Fig. 1Ai–iii). To further validate this result, we established a stable mouse ES cell line in which the puromycin-resistant gene is driven by the Zscan4c promoter, thus allowing the enrichment of cells in the Zscan4\textsuperscript{+} state by adding puromycin (Fig. 1B). The AHA assay on these cells confirmed the global repression of protein synthesis in the cells enriched by the Zscan4\textsuperscript{+} state (Fig. 1C). As expected, levels of proteins detected by antibodies against Eif1α increased in \textasciitilde 85% of the Zscan4-enriched ES cells, despite the repression of global protein synthesis (Fig. 1Ai–vi). The immunohistochemistry result was further supported by the western blot analysis of ES cells enriched with the Zscan4\textsuperscript{+} state (Fig. 1C).

It is known that the high expression of proteins could cause the repression of global protein synthesis by the stress-induced response in cells.\textsuperscript{22} To test this possibility, we carried out immunostaining of ES cells with antibodies against Zscan4 and phosphorylated Eif2α at serine 51 (a marker for stress-induced global protein synthesis repression).\textsuperscript{23,24} Co-expression of Zscan4 and phospho-Eif2α (Ser51) was not observed, indicating that the global protein repression observed in endogenously Zscan4-enriched ES cells was not due to stress-induced response (Fig. 2A). Another condition in which cells are known to undergo global protein synthesis repression is during mitosis (M phase) of the cell cycle.\textsuperscript{25,26} To check whether the Zscan4\textsuperscript{−} cells are in the G2/M phase, ES cells were fixed and immunostained with antibodies against Zscan4 and histone H3 phosphoserine 10 (H3S10ph; a marker of the late G2 and M phase).\textsuperscript{27–29} Cells in late G2 and prophase cells
showed more speckled staining of H3S10ph, whereas cells undergoing metaphase had more intense and compacted staining H3S10ph. Consistent with previous studies cells in the metaphase showed a decrease in protein synthesis as measured by AHA labelling (Fig. 2Bi–iv). However, co-staining of H3S10ph and Zscan4 antibodies was not observed, indicating that Zscan4⁺ cells repressed protein synthesis through a different mechanism. Together, these data demonstrate that the protein synthesis repression observed in Zscan4⁺ state cells does not occur through stress-induced or mitosis-induced mechanisms and suggests an alternative mechanism that is responsible for global protein synthesis repression observed in these cells.

3.2. Identification of Eif1a-like genes that are predominantly expressed in two-cell embryos and the Zscan4⁺ state of ES cells

Results thus far suggest that Eif1a plays a role in repressing global protein synthesis, which would be a novel and unexpected function of Eif1a, if confirmed. To gain more insights into the Eif1a gene, we looked into all oligonucleotide probes used to target different regions of the Eif1a gene in the DNA microarray. Interestingly, we found that not all probes against Eif1a showed an increase in expression in Zscan4⁺ state ES cells compared with the Zscan4⁻ state. Probes that targeted a part of the protein-coding region showed a 10.5-fold increase in the Zscan4⁺ state of ES cells compared with the Zscan4⁻ state, whereas

![Figure 1](image-url)
probes that targeted the 3′-untranslated region (UTR) or 5′-UTR of Eif1a did not show a change in expression (see more detailed analysis below). This suggested the presence of more than one Eif1a genes.

Thorough analysis of NIA Mouse Gene Index and Mouse Genome Sequence (9 and 10 mm) identified Eif1a (Chromosome 18), Eif1ax (Chromosome X), and a cluster of 19 Eif1a-like genes on mouse Chromosome 12. Based on their protein-coding capacity, we noted 10 Eif1a-like genes (named here Eif1al1–Eif1al10) and nine pseudogenes (named here Eif1al-ps1–Eif1al-ps9; Fig. 3A). The Eif1a-like genes could be distinguished from each other by their mRNA sequences (Fig. 3B) and protein sequences (Fig. 4A) when the sequences were aligned using the Clustal OMEGA program. However, the protein sequences between the coding region of Eif1a (Chromosome 18) and the Eif1a-like genes were highly conserved, especially in the N-terminal half of the protein sequences (Supplementary Fig. S1), which is the region to which the Eif1a antibody (ab38976, Abcam) we used was raised against. Considering the predominant expression of Eif1a-like genes in the Zscan4+ state of ES cells, the results shown in Fig. 1Aiv–vi and C required re-

Figure 2. Global nascent protein synthesis observed in Zscan4+ state cells not due to stress-induced response or cells being blocked in the mitosis stage of cell cycle. (A) Mouse ES cells were co-immunostained with antibodies against Zscan4 (i) and Eif2α phosphoserine 51 antibodies (ii) and visualized using Alexa Fluor 568 and 488, respectively. DNA was visualized by 4′,6-diamidino-2-phenylindole, dihydrochloride (DAPI) staining (iii). White arrows indicate the position of Zscan4+ state cells. Pictures were taken at ×40 magnification using a Nikon Eclipse TE300 microscope (scale bar indicates 50 mm). White arrows indicate Zscan4-positive cells. (B) To determine global protein synthesis, mouse ES cells were cultured in methionine-free ES cell media for 30 min, labelled with AHA, a methionine analogue, for 1 h and visualized with Alexa Fluor 488 alkyne through Click-iT reaction (i) and co-stained with histone H3 phosphoserine 10 (H3S10ph) antibody (ii). DNA was visualized by DAPI staining (iv and viii). White arrows indicate the position of H3S10ph stained cells in i–iv. To investigate whether cells under Zscan4+ state are blocked in the mitotic phase of the cell cycle, mouse ES cells were co-immunostained with Zscan4 (v) and H3S10ph (vi) antibodies. Pictures were taken at ×40 magnification using a Nikon Eclipse TE300 microscope (scale bar indicates 50 mm).
interpretation, as the enhanced immunofluorescence signal detected by the Eif1a antibody in cells enriched with the Zscan4\(^+\) state was of Eif1a-like genes and not Eif1a.

The analysis of expressed sequence tag (EST) frequency in the NIA Mouse Gene Index\(^31\) showed that Eif1a-like genes were expressed mostly in two-cell embryos and to some extent in leukaemia erythroblasts (Fig. 3A; Supplementary Table S1). With the current genomic information available, Eif1a-like genes clusters were not identified in human or rat genomes. However, due to the highly repetitive nature of this region, there is potential for identification of this cluster in future genome assemblies. Currently, this Eif1a-like gene cluster can only be confirmed in the mouse. Interestingly, 5 of 19 genes and pseudogenes from this mouse Chromosome 12 cluster (Eif1a-ps3, Eif1al9, Eif1al2, Eif1al-ps6, and Eif1al-ps7) were found to have synteny to human Chromosome 1 pseudogene EIF1AXP1 (ENSG00000236698) using the Multi-VISTA browser.\(^36\)

Next, we looked at the microarray data with the new annotations of the 60-mer probes for Eif1a and Eif1a-like genes. Thus, the probe that targeted a protein-coding region can detect both Eif1a and Eif1a-like genes, showing that a 10.5-fold increase in the Zscan4\(^+\) state of ES cells compared with the Zscan4\(^-\) state, whereas probes that targeted the 3'UTR of Figure 3. A cluster of Eif1a-like genes is newly identified. (A) Schematic diagram of Chromosome 12 Eif1a-like genes. Eif1a-like genes were initially found by transcript assembly from the EST database (NIA Mouse Gene Index, http://lgsun.grc.nia.nih.gov/geneindex/).\(^31\) Subsequently, the full set of these genes was discovered by aligning known sequences (Eif1a, Eif1ax, and Eif1a like) to the genome (10 mm) using the BLAT software (http://genome.ucsc.edu/cgi-bin/hgBlat), and then displayed within the UCSC Genome Browser. Genome targets were extracted and repeatedly aligned until no new alignment was found. Forward-strand genes are shown by coloured boxes above the genome line, and reverse-strand genes are shown below the line. The colour of each box corresponds to the number of ESTs that matched these genes in the UCSC Genome Browser. (B) Dendrogram of similarity between mRNA sequences of Eif1a, Eif1ax, and Eif1a-like genes. Multiple sequence alignment was done by CLUSTALW (http://www.genome.jp/tools/clustalw), and the dendrogram was plotted using TreeView (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html). The colour of each circle corresponds to the number of ESTs that matched to genes in the UCSC Genome Browser.
Eif1a, detecting only Eif1a, did not show a change in expression (Fig. 4B). More specifically for Eif1a-like genes, microarray probes against a conserved region on 3'-UTR (16.1-fold increase, P-value, 0.0001), coding region (25.4-fold increase, P-value < 0.0001), and 5'-UTR of Eif1a6 (23.8-fold increase, P-value < 0.0001) all showed an increase in the Zscan4⁺ state of ES cells compared with the Zscan4⁻ state (Fig. 4B).

To further validate the microarray results, we carried out quantitative real-time PCR on RNA samples extracted from cells enriched with the Zscan4⁺ state by puromycin selection using PCR primers designed specifically against the non-conserved region of the 3'-UTR of Eif1a versus Eif1a6 (U106343) and Eif1a9 (U285359). Primers against the specific Eif1a-like genes may also detect other Eif1a-like genes, because this region in the 3'-UTR among the Eif1a-like genes are highly similar to, but are different from the Eif1a sequence. The results showed that the two Eif1a-like genes tested (Eif1a6 and Eif1a9) had nearly a 100-fold increase in RNA levels in cells enriched with the Zscan4⁺ state compared with non-puromycin-selected cells (Fig. 4C). The expression of Eif1a was only slightly enhanced in cells enriched with the Zscan4⁺ state (~1.5-fold increase).
Figure 5. Over-expression of Eif1a-like genes represses global nascent protein translation. (A) mESC were transiently transfected with FLAG-tagged Eif1a (i–iv) or FLAG-Eif1a-like genes, Eif1a16 (v–viii) or Eif1a19 (ix–xii), constructs for 18 h before selection with puromycin for 24 h. Cells were then cultured in methionine-free ES cell media for 30 min and labelled with AHA for 1 h. AHA-labelled cells were visualized with Alexa Fluor 488 alkyne through Click-iT reaction (ii, vi, and x) and co-stained with FLAG antibody (i, v, and ix). DNA was visualized by DAPI staining (iv, viii, and xii). Pictures were taken at ×40 magnification using a Nikon Eclipse TE300 microscope (scale bar indicates 50 mm). White arrows indicated cells with high levels of FLAG-Eif1a or FLAG-Eif1a-like genes over-expression. (B) Cells were labelled with AHA as described above and protein harvested using RIPA buffer containing protease inhibitors. AHA-labelled samples were then detected using TAMRA alkyne via Click-iT reaction (i). Samples were run on a 4–12% Bis-Tris SDS–PAGE acrylamide gel, transferred on the PVDF membrane, and blotted with antibodies against Zscan4, Eif1a and beta-actin (ii). (C) The model of global protein synthesis repression in Zscan4+ and Zscan4− states in mouse ES cells. Diagram modified from Zalzman et al.6
Together, these results demonstrated that Eif1a-like genes, not Eif1a, were highly expressed in two-cell stage pre-implantation embryos and in the Zscan4+ state of ES cells.

3.3. Over-expression of Eif1a-like genes can repress global protein synthesis

To further determine the mechanism for global protein synthesis repression in the Zscan4+ state of ES cells, we investigated whether over-expression of Eif1a-like genes can repress global protein synthesis. We generated FLAG-tagged constructs of Eif1a (FLAG-Eif1a6), and Eif1a9 (FLAG-Eif1a9) and transfected them into ES cells, followed by the analysis of nascent protein synthesis determined via AHA labelling and immunofluorescence. Both cells transfected with either FLAG-Eif1a6 or FLAG-Eif1a9 showed reduction in AHA labelling, whereas cells transfected with the FLAG-Eif1a did not always show a consistent reduction in AHA labelling (Fig. 5A). Furthermore, using the TAMRA secondary antibody to detect the AHA-labelled proteins on sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE) gel of samples extracted from cells transfected with Eif1a-like genes, FLAG-Eif1a-like cells showed a clear reduction in AHA labelling, when compared with cells transfected with either vector alone or FLAG-Eif1a (Fig. 5B).

The western blot analysis of the SDS–PAGE gel used for the TAMRA antibody detection above showed that the levels of FLAG-Eif1a-like proteins and FLAG-Eif1a proteins were similar to detected by Eif1a and FLAG antibodies (Fig. 5B). This indicates that the protein synthesis repression observed was not due to differences in expression levels of the various constructs. Taken together, these results showed that over-expression of Eif1a-like genes were able to repress protein synthesis globally and demonstrated a mechanism by which global protein synthesis was repressed in the Zscan4+ state of ES cells.

4. Discussion

We report here a novel mechanism to repress global protein synthesis in mouse ES cells. As shown previously, mouse ES cells oscillate between the Zscan4− and Zscan4+ states: the Zscan4− state turns into the Zscan4+ state at a rate of ~3% per day, whereas the Zscan4+ state turns into the Zscan4− state at a rate of ~47% per day (Fig. 5C).6 The Zscan4+ state is transient and relatively short; however, while ES cells are in the Zscan4+ state, several dramatic changes occur, including the up-regulation of meiosis-specific genes, a rapid extension of telomeres by a telomerase-independent recombination-based mechanism. The current work now adds the global repression of nascent protein synthesis as one of the unique feature of the transient Zscan4+ state. These results indicate that ES cells undergo the short period of the repressed protein synthesis in the standard cell culture condition.

The current study also provides further evidence for the commonality between the Zscan4+ state of mouse ES cells and the two-cell stage mouse embryos. The expression of Zscan4, which is originally identified as genes expressed specifically in two-cell embryos,4 has indicated such commonality.6 Further evidence for such commonality has been provided recently: this transient state is also marked with the expression of endogenous retroviruses, such as MuERV-L, which are also expressed specifically in mouse two-cell stage embryos.7 Finding the unique expression of Eif1a-like genes both in the two-cell mouse embryos and in the Zscan4+ state of ES cells provides further connection between them. Considering the global repression of protein synthesis in the Zscan4+ state of ES cells, it would be interesting to know whether there is a novel mechanism for protein synthesis, or protein synthesis repression, during the ZGA in the development of pre-implantation embryo.

High sequence homology of Eif1a-like genes to Eif1a suggests that only the few amino acid differences to the Eif1a-like genes are responsible for global protein repression. Thus, it is highly likely that Eif1a-like genes function as a naturally occurring dominant-negative factor, which interferes with the function of Eif1a as the ubiquitous factor important for the translation machinery in eukaryotes. This may indicate the possible use of Eif1a-like genes as potent repressors of the global protein synthesis in basic and clinical applications.

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