Sample: Molecular Biology - TERRA and Telomere Elongation

TERRA expression triggers telomere elongation

Abstract

Telomeres are the regions at the end of chromosomes that protect them from degradation. Telomeric DNA damage was shown to induce cellular senescence and programmed cell death (apoptosis). Telomere shortening is associated with aging. On the other hand, activity of the enzyme that extends telomeres (telomerase) was found in almost all human tumours. The absence of telomerase activity in most human cells is a necessary condition for maintaining normal tissue homeostasis.

For years, telomeres were considered to be transcriptionally silent. That is why the recent finding of telomeric repeat-containing RNA (TERRA) transcribed at chromosome ends stunned the scientific world. It is now shown that TERRA molecules play a crucial role in telomere maintenance and regulation of telomerase activity. Cusanelli et al. suggested that TERRA expression may represent a signal induced by short telomeres to trigger telomerase recruitment and subsequent telomere elongation. Altered expression of TERRA is associated with genome instability, which can lead to cellular malignization or senescence. Since biological functions of TERRA are still unclear, finding out the molecular mechanisms of TERRA biogenesis and functioning will require a significant effort. It will advance our understanding of telomere-related diseases, cancer and aging.

Telomeres are the ends of eukaryotic chromosomes, which are associated with proteins to protect the DNA ends from being recognized as double-strand breaks (DSBs). The end replication problem with following processing by exonucleases is the mechanism of telomeres shortening at each cell division. Loss of telomere length below a critical threshold leads to cellular senescence, apoptosis, or genome instability. Telomerase can solve these problems. It is a reverse transcriptase that contains integral RNA moiety. This RNA will serve as a template for lengthening telomeric 3' overhangs via reverse transcription into telomeric DNA repeats (Feuerhahn et al., 2010). In Saccharomyces cerevisiae (budding yeast), telomerase is

constitutively expressed, and its activity is differentially regulated to extend the shortest telomeres (Cusanelli et al., 2013). Most of the human somatic cells are telomerase-negative(Feuerhahn et al., 2010).

Telomeres are heterochromatic structures and were considered to be transcriptionally silenced. However, recent studies have demonstrated that telomeres are transcribed into TElomeric Repeat-containing RNA (TERRA). A lot of scientific groups are trying to define the functions of TERRA molecules. Their investigations allowed to suggest that TERRA molecules regulate telomere structural maintenance and heterochromatin formation, inhibit telomerase activity, promote telomere capping after DNA replication. However, biological functions of TERRA are still unclear (Feuerhahn et al., 2010).

Alterations in TERRA levels were observed in cancer cells and Immunodeficiency, Centromeric region instability, Facial anomalies (ICF) syndrome cells. A number of research groups have tried to elucidate the possible function of TERRA in malignant growth. However, the data obtained are controversial. First, the levels of TERRA molecules were reported to be decreased in human cancers compared with normal tissues. Later, elevated levels of TERRA were detected in stomach, lung, colon, and ovary cancers compared with normal tissues. Besides, the transcription of some telomeres into TERRA has been inhibited, while the transcription of others has been enhanced, resulting in decreased or increased levels of TERRA transcripts that originated from different telomeres in cancers compared with normal tissues. A possible explanation for such discrepancy could be the difference between TERRA levels in various cancer types, and the difference between tumours and cancer cell lines (Cusanelli & Chartrand, 2014).

Disturbances of TERRA expression have been associated with cellular senescence. It is a powerful tumour suppressor mechanism. However, results obtained from different research groups are conflicting. Caslini et al. demonstrated a decrease in TERRA expression level in human primary fibroblasts throughout the passages. It was correlated with a decrease in H3K4 methylation, which refers to transcriptionally active telomeres. In contrast to their results, in the study conducted by Thijssen et al., no difference in TERRA levels was observed in late versus early passage human primary fibroblasts. It was correlated with the repressed state of telomeres during senescence. In mutant budding yeast with lack of telomerase activity, TERRA levels are

elevated. Balk et al. showed that TERRA-DNA hybrids could help to maintain telomere length via telomere recombination. It was shown to delay the entry into senescence. Disturbances in levels of TERRA expression in cancer and senescent cells can result in genomic instability, which contributes to malignant process (Cusanelli & Chartrand, 2014).

TERRA levels are altered in another disorder named Immunodeficiency, Centromeric region instability, Facial anomalies (ICF) syndrome. DNA methyltransferase 3b (DNMT3b) carries out DNA methylation of promoters during mammalian embryogenesis. This rare disorder is often fatal for children due to the onset of immunodeficiency symptoms. Mutations in DNMT3b gene lead to ICF, which is inherited in autosomal recessive manner. ICF is characterized by hypomethylated subtelomeres, significantly shorter telomeres, premature senescence in fibroblasts, and abnormally high TERRA levels (Sagie, 2014). Initially, elevated levels of TERRA were assumed to cause telomeres shortening, but further experiments demonstrate that telomerase elongates highly transcribed telomeres. Overexpression of human telomerase (hTERT) in ICF cells extends short telomeres, but the hypomethylated state of subtelomeric DNA does not change. Thus, interactions between telomere length, subtelomeric DNA methylation and TERRA expression are rather complex, and further investigation will shed light on this aspect of telomere biology (Cusanelli & Chartrand, 2014).

For my review, I have chosen a study carried out by Cusanelli research group (Cusanelli et al., 2013). They conducted a line of brilliant experiments with the use of modern molecular biology approaches. It helped the scientists to answer a number of questions and to propose a model of TERRA expression and their functions at telomeres. The studies were conducted in the yeast. Genes, molecular and biochemical processes are highly conserved within eukaryotes. Thus, investigations conducted in the yeast are very helpful in studying the fundamental biology of all eukaryotes.

Cusanelli et al. (2013) studied TERRA functions in wild-type yeast, which was quite challenging due to low levels of TERRA in wild-type yeast. In budding yeast, TERRA molecules are actively degraded by the exonucleases. Thus, the functions of TERRA transcripts in yeast cells are still not defined. Cusanelli et al. used RNA fluorescence in situ hybridization (FISH) and live-cell imaging analysis (with the help of MS2-GFP system) for TERRA visualization. The scientists

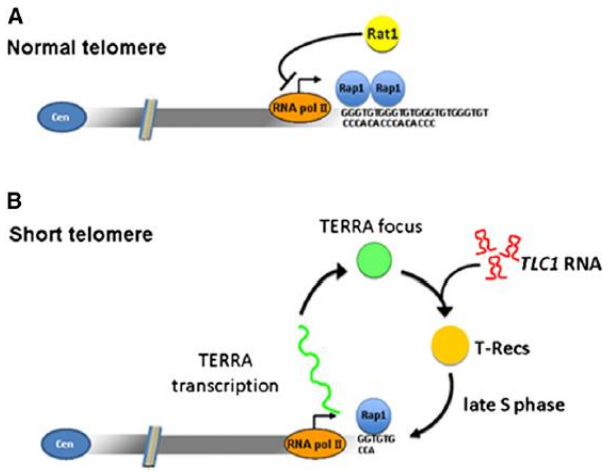
revealed that TERRA molecules were transcribed in a small population of yeast cells. TERRA transcripts were localized at the nuclear periphery, and 96% of TERRA expressing cells contained only a single TERRA focus. Thus, most likely, TERRA expression is regulated by a cis-regulatory element. These findings helped the scientists to hypothesize that TERRA transcription could be induced at short telomeres. To check this hypothesis, the level of TERRA synthesis was measured by RT-qPCR in telomerase negative cells with short telomeres. The level of TERRA transcription in these cells was found to be elevated. Furthermore, the expression of TLC1 (budding yeast telomerase RNA molecule) in mutant cells decreased the level of TERRA transcription as compared to the wild-type cells' level. That is why the scientists suggested that lack of telomerase was crucial for high levels of TERRA transcription. This suggestion was confirmed in a live-cell assay with telomerase-negative TERRA-MS2 cells: the quantity of yeast cells with TERRA transcription was increased, as well as the number of TERRA foci per cell.

Since elevated levels of TERRA expression were observed in cells with short telomeres, Cusanelli et al. used an inducible short telomere assay to investigate the influence of telomere length on induction of TERRA transcription. The scientists revealed that TERRA levels increased after telomere shortening and decreased during telomere extension. Besides, only short telomeres actively transcribed into TERRA. The levels of TERRA synthesis from normal-sized telomeres were stable and were not affected by the transcription of short telomeres. Thus, the scientists confirmed their hypothesis about the initiation of TERRA transcription at each chromosome with the help of cis-regulatory elements and by telomere length-dependent mechanisms.

Both chromatin immunoprecipitation (ChIP) experiments and live-cell imaging demonstrated that TERRA molecules were transiently bound with their telomeres during S-phase of the cell cycle only. Since telomerase activity peaks during late S-phase, scientists suggested that TERRA transcripts may colocalize with telomerase on their telomeres. TERRA-MS2-GFP immunoprecipitates were enriched in TLC1 RNA. Moreover, 93% of TERRA foci were colocalized with a TLC1 RNA. Thus, it is very likely that TERRA-TLC1 clusters are formed in S-phase of budding yeast cell cycle in vivo. Furthermore, the factors involved in the recruitment of telomerase at telomeres are also engaged in the telomeric localization of TERRA.

Iglesias et al. (2011) showed that the yeast telomere-binding protein Rap1 recruited proteins Sir and Rif to repress TERRA expression. That is why, Cusanelli et al. suggested, short telomeres will be able to recruit a smaller number of Rap proteins; consequently, the level of repression of TERRA transcription will be lower. A large quantity of repressive proteins at extended telomeres will greatly inhibit the level of TERRA expression. Elevated levels of TERRA transcription in cancer cells and autosomal-recessive immunodeficiency, centromeric region instability, and facial anomalies syndrome (ICF) cells are in agreement with the results shown by Cusanelli et al. All these cells contain short telomeres.

Based on the obtained results, Cusanelli et al. speculate that TERRA is a scaffold for TERRA-telomerase complex formation (T-Rec). The scientists have proposed their model of TERRA expression and functions at telomeres:



1. Polymerase II transcribes telomere repeats into TERRA molecules, which are then accumulated in a focus.
2. TLC1 RNA recruitment by TERRA molecules results in T-Recs (telomerase recruitment clusters) formation.
3. In late S-phase, T-Recs are bound to the telomeres from which TERRA molecules were transcripted. Consequently, telomeres will be lengthened.

Thus, TERRA expression can be triggered in response to telomeres shortening in order to extend their length (Cusanelli et al., 2013).

Reference list

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