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Human Long Telomeres and Epigenetic Marks

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ABSTRACT

We have read with interest the article "Telomere length regulates TERRA levels through increased trimethylation of telomeric H3K9 and HP1α" by Arnoult and colleagues [1]. This study focuses on human telomeric chromatin structure using different techniques like Chromatin Immunoprecipitation (ChIP), cytolocalization or RT-qPCR. However, it has been performed without taking into consideration the presence of Interstitial Telomeric Sequences (ITSs) in the human genome. Some of the conclusions of the article are undoubtedly clear but there are others that might be explained in alternative ways, considering the existence of ITSs. Following, we mention some comments that arise from this interesting article.

Keywords: Telomeres; TERRA; Epigenetics; Human

1. Introduction

First, we would like to point out that there is a misunderstanding in the introduction section, when the authors comment on Telomere Position Effect (TPE) in *Saccharomyces cerevisiae*. Native TPE in *Saccharomyces cerevisiae* was first discovered in 1997 [2] and not in 1999 [3,4], as Arnoult and colleagues suggested. The 1997 publication showed that the yeast retrotransposon Ty5-1 was repressed by the telomeric SIR silencing complex, which constitutes one of the earliest demonstration of mobile elements heterochromatic regulation in eukaryotes based on the analysis of heterochromatin mutants [2,5].

Secondly, referring to the well-performed experiments presented by Arnoult and colleagues, we would like to mention that humans, as many other organisms, contain ITSs. In *Arabidopis thaliana*, ITSs, as an average, exhibit heterochromatic features and have been shown to be transcribed [6,7]. Similarly, human ITSs are most probably transcribed, at least at certain levels, and are bound by TRF2 [8-11]. Therefore, human TERRA transcripts might be generated from telomeres and from ITSs.

Arnoult and colleagues demonstrated that the increase of telomere length, by means of ectopic expression of either the hTERT catalytic subunit of telomerase or both, hTERT and hTR subunits, caused a reduction of telomeric TERRA levels. In turn, the telomeric TERRA transcripts contained more telomeric sequences. They showed that the reduction of telomeric TERRA transcripts was coincident with an increase of TRF2 loading at the longer telomeres. In addition, they ascribed the lower levels of telomeric TERRA transcripts to an increase of heterochromatic marks at telomeres (H3K9me3 and HP1α) and demonstrated that the levels of telomeric TERRA, of the association of H3K9me3 with telomeric sequences and of the association of HP1α with TRF2 correlate through the cell cycle (Figure 7 [1]). All these conclusions were essentially based on 1) the study of telomeric TERRA levels by RT-qPCR of subtelomeric regions in different cell types, including cells containing siRNAs for SUV39H1 or HP1α, 2) ChIP experiments using antibodies against H3K9me3 followed by dot-blot hybridization with a telomeric probe or by subtelomeric qPCR, 3) the cytolocalization of TERRA transcripts, TRF2 and HP1α and 4) the study of the cell cycle dependent cellular distribution of these marks.

Following, we comment some of the experiments shown by Arnoult and colleagues:

1) Arnoult and colleagues demonstrated by RT-qPCR that the levels of subtelomeric transcripts are lower in cells with elongated telomeres than in cells with normal telomeres, which indicated that the levels of telomeric TERRA transcripts are lower in cells with elongated telomeres (Figures 1 and 2 [1]).

2) Arnoult and colleagues showed by ChIP and qPCR that changes of telomeric TERRA levels in cells with

longer telomeres cannot be attributed to changes at the level of TERRA promoter chromatin (Supplementary Figure 3 [1]).

3) Arnoult and colleagues also showed by Northern-blot and hybridization with a telomeric probe that the levels of TERRA transcripts of similar size, including short RNA molecules, are higher in cells with elongated telomeres than in normal cells, which, in principle, is in contradiction with the results obtained by RT-qPCR mentioned above (Figure 6a [1]). They explained this contradiction arguing that the Northern-blot should not be considered quantitative because a concentration of 2.2 M of formaldehyde was used in the experiment (Figure 6a [1]). However, if the Northern-blot were considered quantitative, we should conclude that TERRA molecules originnated from ITSs are more abundant in cells with elongated telomeres.

4) Arnoult and colleagues found by cytolocalization that the number of TERRA and TRF2 foci in normal cells were very high, which indicates that a portion of them localize at internal loci (Figure 5b [1]). In addition, not all the TERRA foci were found coincident with TRF2 in normal cells. In contrast, the major foci detected in cells with elongated telomeres were more intense than in normal cells and contained both, TERRA and TRF2 (Figure 5b [1]). These results are in agreement with their proposal that elongated telomeres contain longer telomeric TERRA transcripts with more telomeric sequences and more TRF2 proteins than normal cells. In principle, TRF2 proteins should be sequestered to the elongated telomeres from other genomic loci. Therefore, TRF2 molecules could migrate from ITSs to telomeres in cells with elongated telomeres causing a change in the chromatin structure of ITSs.

5) Arnoult and colleagues demonstrated by cytolocalization that HP1α associates with some, but not all, TRF2 foci with very low intensity in cells with longer telomeres (Figure 7e [1]). They concluded from this result that longer telomeres were loaded with HP1α. However, the TRF2 foci associated with HP1α might also correspond to ITSs.

6) Arnoult and colleagues also showed by ChIP and dot-blot hybridization with a telomeric probe that telomeric sequences in cells with elongated telomeres are enriched in H3K9me3 with regard to normal cells (Figure 3 [1]). They concluded from this result that longer telomeres are loaded with H3K9me3. However, this increase of H3K9me3 at telomeric sequences might also be due to the loading of H3K9me3 at ITSs.

7) Arnoult and colleagues studied, by RT-qPCR of subtelomeric sequences, the levels of telomeric TERRA transcripts in normal cells and in cells with elongated telomeres after the introduction of SUV39H1 or of HP1α siRNAs. The introduction of these siRNAs leaded to decreased levels of H3K9me3 or HP1α in both kinds of cells. Arnoult and colleagues showed that the levels of telomeric TERRA transcripts in cells with elongated telomeres containing these siRNAs were similar to those found in their parental normal cells containing the same siRNAs (Figure 4 [1]). They also showed that in some normal cells containing the siRNAs, the levels of TERRA transcripts at certain telomeres were higher than in the same cells without the siRNAs (Figure 4b [1]). Therefore, H3K9me3 and HP1α are involved in the regulation of telomeric TERRA levels in cells with elongated telomeres and might also regulate TERRA levels in normal cells. In our opinion, further experiments should establish that this regulation is exerted directly by binding of H3K9me3 and HP1α at telomeres and not indirectly by their binding at ITSs or even at other genomic loci.

In summary, Arnoult and colleagues have elegantly demonstrated that elongated telomeres lead to lower levels of telomeric TERRA transcripts containing longer tracts of telomeric sequences, and higher levels of telomeric TRF2. These conclusions are very robust. However, some of the experiments presented by Arnoult and colleagues might be explained in alternative ways taking into consideration the presence of ITSs. For example, the increased number of telomeric TRF2 molecules present at longer telomeres could be sequestered from ITSs, which, in turn, would lead to higher levels of TERRA transcripts from ITSs and to the recruitment of heterochromatic marks (H3K9me3 and HP1α) at ITSs, and not at telomeres, in a cell cycle regulated manner. In this context, the lower levels of telomeric TERRA transcripts found at cells with elongated telomeres might be caused by alternative features.

We do not favor the heterochromatinization of long telomeres versus ITSs or vice versa. However, we believe that the influence of ITSs should be ruled out in telomeric chromatin structure studies to strongly support conclusions [6]. Two different approaches have been designed to study the chromatin structure of telomeres independently of ITSs in another model system [12-14]. These approaches could be adapted to other organisms including humans [12,15].

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