Reorganization of the DNA replication landscape during adipogenesis is closely linked with adipogenic gene expression

Takuya Hayakawa, Asahi Yamamoto, Taiki Yoneda, Sakino Hori, Nanami Okochi, Kazuhiro Kagotani, Katsuzumi Okumura and Shin-ichiro Takebayashi
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MS TITLE: Reorganization of the DNA replication landscape during mitotic clonal expansion is closely linked with adipogenic gene expression

AUTHORS: Takuya Hayakawa, Asahi Yamamoto, Taiki Yoneda, Sakino Hori, Nanami Okochi, Kazuhiro Kagotani, Katsuzumi Okumura, and Shin-ichiro Takebayashi
ARTICLE TYPE: Research Article

We have now reached a decision on the above manuscript.

To see the reviewers' reports and a copy of this decision letter, please go to: https://submit-jcs.biologists.org and click on the 'Manuscripts with Decisions' queue in the Author Area. (Corresponding author only has access to reviews.)

As you will see, the reviewers gave favourable reports but raised some critical points that will require amendments to your manuscript. I hope that you will be able to carry these out because I would like to be able to accept your paper.

Please ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion.

I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. Please attend to all of the reviewers' comments. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

This manuscript by Hakayaka et al. studied the changes in the DNA replication landscape during the mitotic clonal expansion (MCE) while mouse 3T3-L1 cells differentiate into adipocytes, using

methods including single-cell replication timing analysis, the method previously developed in their group. Consequently they have identified coordinated changes between Late-to-Early replication timing shifts and (transcriptional activation of adipogenesis-associated genes. Their data demonstrate the changes in the replication timing precede the induction of adipose-specific genes, suggesting that reorganization of replication timing may contribute to the differentiation. To support this idea they also observed cells that underwent two rounds of DNA replication during MCE had a higher tendency to differentiate into lipid droplet-accumulating adipocytes than cells that underwent only one round of DNA replication during MCE.

It was previously shown that replication timing shifts could lead to changes in histone modifications and spatial genome organization (e.g., Klein et al. 2021). However, understanding of the relationship between DNA replication timing and gene expression is limited. Importantly, this manuscript further suggests that replication timing shifts could affect cellular differentiation programs, not just gene expression. Although the exact mechanism is yet to be studied, this manuscript will gain our understanding significantly.

The manuscript is written in a clear and concise manner, and I believe it's appropriate for publication on the JCS, with minor text and figure revisions.

Comments for the author

The manuscript is written in a clear and concise manner, and I believe it's appropriate for publication on the JCS, with minor text and figure revisions.

General point
I would prefer to appeal novelties and strengths of the research more explicitly in the text. However, it would be a personal preference.

Specific points
- Fig 2B. Readers will appreciate it if the locations with significant RT changes are marked (e.g., 75 Mb).
- There's no reference to Fig 2C in the main text.
- Page 6, lines 136-137. The authors state "We found a clear tendency of these adipogenic genes being associated with shifts towards earlier RT". However, this cannot be easily read from Fig 4A. Wouldn't it be better to add a heatmap based on RT changes?
- In Discussion: Are there any other explanations for higher lipid accumulation in cells that underwent two rounds of DNA replication?

Reviewer 2

Advance summary and potential significance to field

REVIEWER's COMMENTS In the MS entitled "Reorganization of the DNA replication landscape during mitotic clonal expansion is closely linked with adipogenic gene expression" by Takuya Hayakawa and collaborators, the Authors address the connections between changes in replication timing and reprogramming of transcription that occur during differentiation of cultured mouse fibroblasts into adipocytes.

It is well established that regions of the genome that are transcriptionally active tend to replicate earlier than those that are transcriptionally less active, or else silent. Also, shifts in replication timing accompanying changes in transcriptional status have been well documented. Although the replication timing of a locus can be envisaged as an epigenetic mark per se, it remains poorly understood how changes in replication timing of specific chromosomal loci couple temporally to transcriptional activation (or silencing) and remodelling of epigenetic marks (DNA, chromatin) of the target loci. Regarding the massive alterations in replication timing and, concurrently, of transcriptional landscapes that accompany the set-up of differentiation programmes, the role played by intervening rounds of DNA replication between the initial differentiation stimulus and the final differentiated status remains obscure. As hypothesized, these rounds of replication (dubbed

mitotic clonal expansions/MCE) might be critically required for the progressive, adaptive shifts in replication timing of key loci involved in cellular differentiation.

In this piece of research, the Authors addressed some of these issues using high-end technology and expertise, and an established model of differentiation of cultured mouse fibroblasts (3T3) into adipocytes after an initial hormonal stimulus.

The Authors found that: 1) genome-wide, hundreds of loci change replication timing (from late-to-early (LtoE) or early to late (EtoL)) during the first and the second MCE, before full differentiation is reached; 2) the first and second MCEs are not exactly redundant, with some chromosome segments changing replication time exclusively during the 1st or 2nd MCE; 3) occurrence of two replication rounds before full differentiation are more effective than just one at producing mature adipocytes; 4) shifts in replication timing of specific loci involved in adipogenesis may either follow, precede, or be concurrent with alterations in transcriptional activity; the time-lag is however short.

In all, these results are novel and welcome. The results are, however, mostly descriptive and correlative as they stand; this is the major caveat with this research. Despite this, the data are relevant (and hard to obtain at this level of expertise) and shall provide a solid ground for subsequent, more experimentally driven studies. These studies should aim at establishing causal relationships, namely between transcriptional activation of adipogenesis-related genes and their shift in replication timing. To such end, targeted promoter-crippling approaches using gene-editing technology could be used.

This Reviewer finds the data of interest and relevance to a broad, non-expert audience.

Comments for the author

REMARKS to AUTHORS (minor):

1. Overall, the text reads well and is written in a clear, concise style.
Some typos have been detected: cf lines 78, 189.
In line 160 the word “rate” is not appropriate should be replaced by either percentage/frequency/ or fraction.

2. In Discussion, line 201: “..are largely consistent with A and B compartments”; the nature of these compartments should be explained in advance not afterwards. The neophyte reader may not be acquainted to this classification of TADs, to which the Authors provided original contributions in the past.

3. In lines 189-191: “Taking these observations into consideration, the (we?) observed that LtoE switching of adipogenic genes may significantly impact chromatin structure and transcriptional activation of these genes” . This sentence shall be rephrased; the Authors have not addressed chromatin structure issues in this research!! As it stands, this is considered an overstatement.

4. In Legend to Figure 5 “Some gene loci show EtoL switching after transcriptional activation”. It should be LtoE switching, right??

5. In Acknowledgements the name of the Institution should be also stated, not just the Department.

First revision

Author response to reviewers' comments

Reviewer 1 Advance Summary and Potential Significance to Field:
This manuscript by Hakayaka et al. studied the changes in the DNA replication landscape during the mitotic clonal expansion (MCE) while mouse 3T3-L1 cells differentiate into adipocytes, using

methods including single-cell replication timing analysis, the method previously developed in their group. Consequently, they have identified coordinated changes between Late-to-Early replication timing shifts and (transcriptional activation of adipogenesis- associated genes. Their data demonstrate the changes in the replication timing precede the induction of adipose-specific genes, suggesting that reorganization of replication timing may contribute to the differentiation. To support this idea, they also observed cells that underwent two rounds of DNA replication during MCE had a higher tendency to differentiate into lipid droplet-accumulating adipocytes than cells that underwent only one round of DNA replication during MCE.

It was previously shown that replication timing shifts could lead to changes in histone modifications and spatial genome organization (e.g., Klein et al. 2021). However, understanding of the relationship between DNA replication timing and gene expression is limited. Importantly, this manuscript further suggests that replication timing shifts could affect cellular differentiation programs, not just gene expression. Although the exact mechanism is yet to be studied, this manuscript will gain our understanding significantly.

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General point

I would prefer to appeal novelties and strengths of the research more explicitly in the text. However, it would be a personal preference.

Thank you for your suggestion. We added a new paragraph in the beginning of the discussion section as follows.

(Lines 167-175)

“In the present study, we carefully examined the genome-wide temporal order of DNA replication during MCE by E/L Repli-seq and scRepli-seq approaches and identified a number of RT changes. We found that (1) each round of MCE is qualitatively different in terms of replication landscape. (2) RT switching from late to early in the second round of MCE is linked to transcriptional induction of adipogenic genes in early adipogenesis and with full transcriptional activation in late adipogenesis. (3) Differentiation potential of cells is linked with how many DNA replication cycles cells undergo during MCE. Thus, our study revealed a previously unrecognized dynamic RT regulation during MCE and provides a basis for understanding the biological significance of MCE”.

Specific points

- Fig 2B. Readers will appreciate it if the locations with significant RT changes are marked (e.g., 75 Mb).

According to the suggestion, a significant RT switching region is marked.

- There's no reference to Fig 2C in the main text.

Figure 2C is now referred in the text.

- Page 6, lines 136-137. The authors state "We found a clear tendency of these adipogenic genes being associated with shifts towards earlier RT". However, this cannot be easily read from Fig 4A. Wouldn't it be better to add a heatmap based on RT changes?

According to the suggestion, a new heat map was added to Figure 4A.

- In Discussion: Are there any other explanations for higher lipid accumulation in cells that underwent two rounds of DNA replication?

We added a statement in the discussion section that genes undergoing RT changes during MCE2 includes those directly involved in lipid metabolism. At this point, we have no other reasonable explanation than the possibility we discussed, because this is the first report that investigated the relationship between the number of DNA replication cycles and higher lipid droplet accumulation and our current knowledge is too limited to make a meaningful discussion.

Reviewer 2 Advance Summary and Potential Significance to Field:

In the MS entitled “”Reorganization of the DNA replication landscape during mitotic clonal expansion is closely linked with adipogenic gene expression”” by Takuya Hayakawa and collaborators, the Authors address the connections between changes in replication timing and reprogramming of transcription that occur during differentiation of cultured mouse fibroblasts into adipocytes.It is well established that regions of the genome that are transcriptionally active tend to replicate earlier than those that are transcriptionally less active, or else silent. Also, shifts in replication timing accompanying changes in transcriptional status have been well documented. Although the replication timing of a locus can be envisaged as an epigenetic mark per se, it remains poorly understood how changes in replication timing of specific chromosomal loci couple temporally to transcriptional activation (or silencing) and remodelling of epigenetic marks (DNA, chromatin) of the target loci. Regarding the massive alterations in replication timing and, concurrently, of transcriptional landscapes that accompany the set-up of differentiation programmes, the role played by intervening rounds of DNA replication between the initial differentiation stimulus and the final differentiated status remains obscure.

As hypothesized, these rounds of replication (dubbed mitotic clonal expansions/MCE) might be critically required for the progressive, adaptive shifts in replication timing of key loci involved in cellular differentiation. In this piece of research, the Authors addressed some of these issues using high-end technology and expertise, and an established model of differentiation of cultured mouse fibroblasts (3T3) into adipocytes after an initial hormonal stimulus. The Authors found that: 1) genome-wide, hundreds of loci change replication timing (from late-to-early (LtoE) or early to late (EtoL)) during the first and the second MCE, before full differentiation is reached; 2) the first and second MCEs are not exactly redundant, with some chromosome segments changing replication time exclusively during the 1st or 2nd MCE; 3) occurrence of two replication rounds before full differentiation are more effective than just one at producing mature adipocytes; 4) shifts in replication timing of specific loci involved in adipogenesis may either follow, precede, or be concurrent with alterations in transcriptional activity; the time-lag is however short. In all, these results are novel and welcome. The results are, however, mostly descriptive and correlative as they stand; this is the major caveat with this research. Despite this, the data are relevant (and hard to obtain at this level of expertise) and shall provide a solid ground for subsequent, more experimentally driven studies. These studies should aim at establishing causal relationships, namely between transcriptional activation of adipogenesis- related genes and their shift in replication timing. To such end, targeted promoter-crippling approaches using gene-editing technology could be used. This Reviewer finds the data of interest and relevance to a broad, non-expert, audience.

Reviewer 2 Comments for the Author:
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In line 160 the word “rate” is not appropriate should be replaced by either percentage/frequency/ or fraction.

These were corrected accordingly.

2. In Discussion, line 201: “..are largely consistent with A and B compartments”; the nature of these compartments should be explained in advance, not afterwards. The neophyte reader may not

be acquainted to this classification of TADs, to which the Authors provided original contributions in the past.

According to the suggestion, we rewrote this paragraph as follows.

(Lines 210-221)
"The genome is divided into two nuclear compartments called A/B compartments and each compartment consists of multiple topologically associating domains, as revealed by the chromosome conformation capture technique, Hi-C (Lieberman-Aiden et al., 2009). A and B compartments are associated with active and inactive chromatin, respectively. B compartments are characterized as preferentially interacting with the nuclear periphery, which favors transcriptionally inactive chromatin formation. This is supported by the observation that forced tethering of active genes to the nuclear periphery induces transcriptional silencing (Finlan et al., 2008; Kumaran and Spector, 2008; Reddy et al., 2008). RT profiles can be used to predict 3-dimensional genome organization in the nuclear space because early and late RT domains are largely consistent with A and B compartments (Ryba et al., 2010). Reorganization of RT domains during cell differentiation is accompanied by changes in the A/B compartments (Miura et al., 2019; Takebayashi et al., 2012)".

3. In lines 189-191: "Taking these observations into consideration, the (we?) observed that LtoE switching of adipogenic genes may significantly impact chromatin structure and transcriptional activation of these genes" . This sentence shall be rephrased; the Authors have not addressed chromatin structure issues in this research!! As it stands, this is considered an overstatement.

The sentence was rephrased as follows.

(Lines 199-200)
"it is possible that LtoE switching of adipogenic genes may significantly impact chromatin structure and transcriptional activation of these genes".

4. In Legend to Figure 5 "Some gene loci show EtoL switching after transcriptional activation". It should be LtoE switching, right??

Thank you for pointing out this error.
This was corrected.

5. In Acknowledgements the name of the Institution should be also stated, not just the Department.

This was corrected.

Second decision letter

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MS TITLE: Reorganization of the DNA replication landscape during adipogenesis is closely linked with adipogenic gene expression

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ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Journal of Cell Science, pending standard ethics checks.