Journal of Cell Science

Growth conditions inducing G1 cell cycle arrest enhance lipid production in the oleaginous yeast *Lipomyces starkeyi*

Yasutaka Morimoto, Shigeaki Saitoh and Yuko Takayama
DOI: 10.1242/jcs.259996

Editor: David Glover

Review timeline
Original submission: 8 March 2022
Editorial decision: 23 May 2022
First revision received: 13 June 2022
Accepted: 11 July 2022

Original submission

First decision letter

MS ID#: JOCES/2022/259996

MS TITLE: Rapamycin and a nitrogen-depleted medium induce G1 phase arrest and enhance lipid production in the oleaginous yeast *Lipomyces starkeyi*

AUTHORS: Yasutaka Morimoto, Shigeaki Saitoh, and Yuko Takayama
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 reviewer raises a number of substantial criticisms that prevent me from accepting the paper at this stage. They suggest, however, that a revised version might prove acceptable, if you can address their concerns. If you think that you can deal satisfactorily with the criticisms on revision, I would be pleased to see a revised manuscript. We would then return it to the reviewer.

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

In this study, Morimoto et al. investigated the cell-cycle dependency of accumulation of triacylglycerol (TAG)

in Lipomyces starkeyi. They found that L. starkeyi accumulated TAG when cultured in nitrogen-depleted medium or treated with nocodazole or rapamycin but not when treated with hydroxyurea. From these results the authors concluded that L. starkeyi does not accumulate TAG in the S phase, but begins to accumulate TAG in the G2/M phase and the accumulation of TAG is enriched in the G1 phase. Overall, the experiment was well designed and executed and the results support the main conclusion, which provides an important contribution to the rapidly growing field of the production of TAG by oleaginous yeasts. Some points described below may improve this manuscript.

Comments for the author

Major point
(1) In Fig. 2 and 4, the authors need to perform multiple comparisons instead of Student's t-test in the statistical treatment.

Minor points
(2) If possible, please explain why cells cultured in the 0.3% glucose-containing medium accumulates more TAG for 36 h than those in the 5% glucose-containing medium.
(3) The duration of treatment of HU or nocodazole (3 hours) is considered shorter than the doubling time of L. starkeyi in YPD medium, but is it enough time to synchronize the cell cycle? Did the longer treatment affect the viability of L. starkeyi?
(4) If possible, please explain why nocodazole treatment would cause G1 synchronization only when pre-treated with rapamycin.
(5) In the legend of Fig. 3, it would be better to write the difference between “white diamond” and “white circle (difference in initial concentration).
(6) In Fig. 5, what results suggest that the amount of TAG in G1 cells is reduced in S phase? Is there any evidence that the accumulated TAG is distributed almost equally in the daughter cells?
(7) Were cells diluted and sonicated before counting cell concentration by a particle counter, CDA-1000?

First revision

Author response to reviewers' comments

Reviewer 1 Comments for the Author:
Major point
(1) In Fig. 2 and 4, the authors need to perform multiple comparisons instead of Student's t-test in the statistical treatment.

Thank you for this useful comment. We reanalyzed the data presented in Figs. 2 and 4 by multiple comparisons of Dunnett contrasts. The Fig. 2 and Fig. 4 legends were changed from “Student’s *t* test” to “Dunnett contrasts”. In Fig. 2, the 5% glucose sample at 96 h showed no significant differences compared to N medium. The same results were also obtained using Tukey contrasts. Therefore, we changed the sentence as follows. “when the glucose concentration was increased up to 5%, the lipid amounts were decreased at 72 h, but increased to a level comparable to that in regular N medium containing 0.3% sucrose at 96 h, …”.

Minor points
(2) If possible, please explain why cells cultured in the 0.3% glucose-containing medium accumulates more TAG for 36 h than those in the 5% glucose-containing medium.

As the reviewer pointed out, cells cultured in the 0.3% glucose N medium accumulated more TAG than those in the 5% glucose N medium at 72 h (Fig. 2 shows TAG data of 72 or 96 h, therefore we assume that by 36 h, you meant 72 h.). We suspect that the presence of a high concentration of glucose slows TAG accumulation, and therefore the TAG levels became similar between 0.3% and 0.5% glucose media at a later time point (96 h). Consistently, at earlier time points (24 and 48 h), the TAG levels were found to be higher in 0.3% glucose N medium than in 5% glucose N medium. To

explain this point more clearly, we add the following sentences. “when the glucose concentration was increased up to 5%, lipid amounts were decreased at 72 h, but increased to a level comparable to that in regular N medium containing 0.3% sucrose at 96 h, suggesting that TAG accumulates faster under low glucose conditions (Fig. 2, 5% Glu).
Consistently, at earlier time points (24 and 48 h), the levels of accumulated TAG were higher in 0.3% glucose N medium than in 5% glucose N medium; at 24 and 48 h, the TAG amounts were 1.0 ± 0.3 mg/10^8 cells and 1.8 ± 0.2 mg/10^8 cells in 0.3% glucose N medium, whereas they were 0.6 ± 0.1 mg/10^8 cells and 1.3 ± 0.3 mg/10^8 cells in 5% glucose N medium.”

(3) The duration of treatment of HU or nocodazole (3 hours) is considered shorter than the doubling time of L. starkeyi in YPD medium, but is it enough time to synchronize the cell cycle? Did the longer treatment affect the viability of L. starkeyi?

We have checked in detail the treatment times for HU and nocodazole. In HU treatment, when *L. starkeyi* cells were treated for more than 4 h, the rate of large bud cells (% of G2/M phase cells) was gradually increased. In addition, cells cultured in HU medium for 5 h failed to resume the cell cycle when cultured in fresh YPD medium. Nocodazole induced G2 arrest from a 3-h treatment. When the treatment time exceeded 4 h, restart of the cell cycle did not synchronously proceed. For these reasons, the treatment times for HU and nocodazole were 3 hours.

(4) If possible, please explain why nocodazole treatment would cause G1 synchronization only when pre-treated with rapamycin.

The original text was inadequate and misleading on this point. The exact procedure of the experiment was as follows: first, cells were treated with nocodazole for pre-synchronization at G2/M phase. The G2/M-synchronized cells were recovered in YPD medium for 30 min, and then rapamycin was added to the medium. The cells were effectively synchronized at G1 phase in these preparations. We changed the sentence as follows. “To overcome this problem, the cells were first arrested at G2/M phase with nocodazole, transferred to fresh YPD medium, and cultivated for 30 min, during which time the cells resumed cell cycle progression toward the next G1 phase, and then rapamycin was added to the medium (Fig. 4A).”

(5) In the legend of Fig. 3, it would be better to write the difference between “white diamond” and “white circle (difference in initial concentration).

Thank you for the helpful suggestion. We changed the sentence as follows. “White circles, initial concentration is 3 × 10^6 cells/mL in N medium; white diamonds, initial concentration is 1 × 10^6 cells/mL in N medium; black circles, initial concentration is 1 × 10^6 cells/mL in S medium.”

(6) In Fig. 5, what results suggest that the amount of TAG in G1 cells is reduced in S phase? Is there any evidence that the accumulated TAG is distributed almost equally in the daughter cells?

Fig. 4C shows that the amount of TAG in cells arrested with HU (S) was less than that in cells arrested in rapamycin (G1) or nocodazole (G2/M). Moreover, cell membrane synthesis is induced for early bud formation during S phase. Lipolysis is known to be essential for membrane synthesis. For these reasons, we think that the decrease in TAG occurs during S phase. In addition, BODIPY staining experiments were performed on cells arrested with HU or nocodazole, as shown below. In HU-arrested cells, small lipid droplets were present in daughter cells. And large lipid droplets were detected in the daughter cells attested in G2 phase using nocodazole. Therefore, Fig. 5 was modified to clarify that daughter cell contains a small lipid droplet at S phase and a large droplet at G2 phase.

NOTE: *We have removed unpublished data that had been provided for the referees in confidence.*

(7) Were cells diluted and sonicated before counting cell concentration by a particle counter, CDA-1000?

As pointed out, the cells were diluted and sonicated before counting the cell concentration. We

added information on the preparative steps before cell counting. “Cell concentrations were determined by a particle counter, CDA-1000 (Sysmex, Japan), after 200-fold dilution with Cellpack (Sysmex, Japan) and brief sonication with a HandySonic (Tomy Seiko, Power 4).”

Second decision letter

MS ID#: JOCES/2022/259996

MS TITLE: Growth conditions inducing G1 cell cycle arrest enhance lipid production in the oleaginous yeast *Lipomyces starkeyi*

AUTHORS: Yasutaka Morimoto, Shigeaki Saitoh, and Yuko Takayama
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.

Reviewer 1

Advance summary and potential significance to field

As previously mentioned in the review letter of the original manuscript, the authors provide an important contribution to the rapidly growing field of the production of TAG by oleaginous yeasts.

Comments for the author

The authors explained clearly about previous review’s comments with appropriate way and the revised manuscript has been improved to be more understandable to readers. I do not have any substantial amendments to suggest.