Complementary and divergent functions of zebrafish Tango1 and Ctage5 in tissue development and homeostasis

Eric Clark and Brian Link

Corresponding author(s): Brian Link, Medical College of Wisconsin

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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

RE: Manuscript #E20-11-0745
TITLE: "Complementary and divergent functions of zebrafish Tango1 and Ctage5 in tissue development and homeostasis"

Dear Dr. Link,

Two expert reviewers have evaluated your manuscript, and I am happy to report that they are very supportive. Reviewer #1 communicated to me that they had already endorsed publication of this work at another journal and remain enthusiastic. Reviewer #2 has a number of suggestions about wording and interpretation, but is otherwise positive.

If you return a revised manuscript that addresses the suggestions of Reviewer #2, I will evaluate the changes myself. I look forward to seeing the resubmission.

Best regards,
Ben Glick

Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Link,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript requires minor revisions before it can be published in Molecular Biology of the Cell, as described in the Monitoring Editor's decision letter above and the reviewer comments (if any) below.

A reminder: Please do not contact the Monitoring Editor directly regarding your manuscript. If you have any questions regarding the review process or the decision, please contact the MBoC Editorial Office (mboc@ascb.org).

When submitting your revision include a rebuttal letter that details, point-by-point, how the Monitoring Editor's and reviewers' comments have been addressed. (The file type for this letter must be "rebuttal letter"; do not include your response to the Monitoring Editor and reviewers in a "cover letter.") Please bear in mind that your rebuttal letter will be published with your paper if it is accepted, unless you have opted out of publishing the review history.

Authors are allowed 180 days to submit a revision. If this time period is inadequate, please contact us immediately at mboc@ascb.org.

In preparing your revised manuscript, please follow the instruction in the Information for Authors (www.molbiolcell.org/info-for-authors). In particular, to prepare for the possible acceptance of your revised manuscript, submit final, publication-quality figures with your revision as described.

To submit the rebuttal letter, revised version, and figures, please use this link (please enable

cookies, or cut and paste URL): Link Not Available

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Thank you for submitting your manuscript to Molecular Biology of the Cell. Please do not hesitate to contact this office if you have any questions.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

Reviewer #1 (Remarks to the Author):

I congratulate the authors for generating the mutants in tango1 and stage 5. Their data are important for strengthening the proposals on these genes that are based on cell culture assays. Their tools will be in high demand and therefore an important resource for the scientific community.

Reviewer #2 (Remarks to the Author):

In this study, the authors describe the generation and phenotypic characterization of Tango1 and Ctage5 mutants in zebrafish, and their double mutant combination. Tango1 and Ctage5 belong to a family of proteins implicated in secretory traffic. If they function as very specific or rather general cargo receptors, or as ER exit site structural organizing proteins, or both, are heavily contested matters in the field. The specific roles of the different domains of the proteins and the degree of functional overlap among Tango1, Ctage5 and other family members are also unclear. In that context, this genetic study provides important information. Nonetheless, the nature of the alleles generated and the functional overlap between Tango1 and Ctage5, which this study importantly confirms, preclude definitive answers to those questions. In this regard, the authors make a rigorously honest discussion of limitations and different possible interpretations of their results, including that differences in phenotype may be due to divergent expression pattern rather than divergent function. Overall, this is an interesting and carefully conducted study. I have the following suggestions for improvement:

1. The authors generate a Ctage5 mutant by deleting the PRD domain and show about 50% transcript remains. Because of the modular nature of the protein, this is likely a hypomorphic mutation, not a null. Calling it a "Ctage5 deletion" is misleading, both in the physical sense (it is a partial deletion) and functional sense (there is no guarantee that this completely eliminates Ctage5

function). The Tango1 mutant is also a partial deletion of the encoding locus, in this case of the SH3 domain-encoding part, eliminating the Tango1l isoform (the SH3 containing one) and leaving untouched the Tango1s isoform (the one that does not contain SH3 and is, thus, most similar to Ctage5). Again, calling this a "Tango1 deletion" is misleading. It is a Tango1l deletion or SH3 deletion, even though in this case, it could be safely assumed to be a null, but for Tango1l, not for all Tango1.

2. Last sentence of abstract: "Together, our results suggest that Ctage5 and Tango1 have overlapping, but also divergent roles in tissue development and homeostasis."

I would say the results, even if the Ctage5 mutation is hypomorphic, demonstrate, rather than just suggest, that Ctage5 and Tango1l (for which the allele generated can be safely considered a null), have at least some overlapping function, as shown by the increased severity of several phenotypes in double mutants. Because this is a Tango1l null, this is not just (or not only) a cooperative interaction, or "complementary function" in the title, in the weak sense that Ctage5 and Tango1l may interact physically and cooperate, but strict functional redundancy must exist too. Otherwise, no phenotypic enhancement would be seen in a double mutant.

As for divergent roles, the results indeed suggest divergent roles but cannot demonstrate them, as their results could be consistent with different expression patterns and, in one extreme possibility, no divergent function at all, as the authors rightly discuss.

Other comments:

A. Second paragraph of introduction: "The simplified protein diversity of the Ctage5/Tango1 in zebrafish compared to humans allows investigation of their complimentary and divergent functions."

In humans, apart from Ctage5, Tango1 and alternative transcripts from the same MIA2 and MIA3 loci, there are more family members encoded from MIA, OTOR and six additional CTAGE genes. It would be interesting to mention if these are present as well in zebrafish.

B. Third paragraph of introduction: "Drosophila studies have showed secretion of multiple extracellular proteins including... "

In Drosophila, where a single Tango1 exists (without alternative splicing or other MIA/CTAGE genes), studies have shown retention of every secreted protein analyzed and disruption of ERES morphology in every tissue upon Tango1 loss. The reason is still argued (general receptor function vs clogging by few specific cargos vs general ERES structural role), but all secretion is affected.

C. First paragraph of results: "Primers designed to measure tango1s mRNA did not reveal a change between wild-type and tango1l mutant embryos, indicating that Tango1s protein could still be made (Fig. S2)."

Suggesting, rather than indicating, as protein level was not directly tested".

Also, according to S2, the primers would measure both Tango1s and Tango1l, which means Tango1s is upregulated in absence of Tango1l if I understand correctly, as no change is seen in the mutant lacking Tango1l. Is that right?

D. "Our results suggest that collagen II trafficking is not affected with ctage5 deletion, indicating Tango1 has the essential role in Collagen II trafficking and craniofacial development."

However, the Ctage5 mutant enhances the craniofacial development phenotype of Tango1 mutant, so Ctage5 is involved in craniofacial development as well. Maybe less than Tango1l for functional or expression reasons, or the Ctage5 mutant is hypomorphic and does not show the whole extent of the involvement, but it is involved as well.

E. Third paragraph discussion: "While our studies addressed the shared and unique functions between Ctage5 and Tango1 with respect to trafficking and cellular homeostasis, the experiments in zebrafish also demonstrated that Tango1 plays an important role in maintaining ER-Golgi morphology, consistent with in vitro observations (Bard et al., 2006; Rios-Barrerra et al., 2017; Reynolds et al., 2019)."

These are studies in flies, so not in vitro. Also reported in Liu et al., 2017.

F. In abstract and throughout text, but correct in title: complEmentary.

We thank the Reviewers for their comments and time in evaluating our manuscript. Below we have responded point-by-point to each comment. Our responses are in BOLD font. In addition, we included a 'track-changes' version of our manuscript to more easily see our edits and updates.

Reviewer #1 (Remarks to the Author):

I congratulate the authors for generating the mutants in tango1 and ctage 5. Their data are important for strengthening the proposals on these genes that are based on cell culture assays. Their tools will be in high demand and therefore an important resource for the scientific community.

- **We thank the Reviewer for their comments. Indeed, we look forward to sharing the mutants zebrafish with the scientific community.**

Reviewer #2 (Remarks to the Author):

In this study, the authors describe the generation and phenotypic characterization of Tango1 and Ctage5 mutants in zebrafish, and their double mutant combination. Tango1 and Ctage5 belong to a family of proteins implicated in secretory traffic. If they function as very specific or rather general cargo receptors, or as ER exit site structural organizing proteins, or both, are heavily contested matters in the field. The specific roles of the different domains of the proteins and the degree of functional overlap among Tango1, Ctage5 and other family members are also unclear. In that context, this genetic study provides important information. Nonetheless, the nature of the alleles generated and the functional overlap between Tango1 and Ctage5, which this study importantly confirms, preclude definitive answers to those questions. In this regard, the authors make a rigorously honest discussion of limitations and different possible interpretations of their results, including that differences in phenotype may be due to divergent expression pattern rather than divergent function. Overall, this is an interesting and carefully conducted study. I have the following suggestions for improvement:

1. The authors generate a Ctage5 mutant by deleting the PRD domain and show about 50% transcript remains. Because of the modular nature of the protein, this is likely a hypomorphic mutation, not a null. Calling it a "Ctage5 deletion" is misleading, both in the physical sense (it is a partial deletion) and functional sense (there is no guarantee that this completely eliminates Ctage5 function). The Tango1 mutant is also a partial deletion of the encoding locus, in this case of the SH3 domain-encoding part, eliminating the Tango1l isoform (the SH3 containing one) and leaving untouched the Tango1s isoform (the one that does not contain SH3 and is, thus, most similar to Ctage5). Again, calling this a "Tango1 deletion" is misleading. It is a Tango1l deletion or SH3 deletion, even though in this case, it could be safely assumed to be a null, but for Tango1l, not for all Tango1.

The Reviewer makes a valid point regarding our language related to the mutant alleles we have generated. We have made changes throughout the paper to be more clear. In general, we have replaced descriptions of "Ctage deletion", with "Ctage5 mutant allele", and referred to "Tango1

deletions" as "Tango1l deletions". We thank the Reviewer for acknowledging the fact that we transparently discussed these matters in the Introduciton and Discussion sections.

2. Last sentence of abstract: "Together, our results suggest that Ctage5 and Tango1 have overlapping, but also divergent roles in tissue development and homeostasis."

I would say the results, even if the Ctage5 mutation is hypomorphic, demonstrate, rather than just suggest, that Ctage5 and Tango1l (for which the allele generated can be safely considered a null), have at least some overlapping function, as shown by the increased severity of several phenotypes in double mutants. Because this is a Tango1l null, this is not just (or not only) a cooperative interaction, or "complementary function" in the title, in the weak sense that Ctage5 and Tango1l may interact physically and cooperate, but strict functional redundancy must exist too. Otherwise, no phenotypic enhancement would be seen in a double mutant.

We have modified the Abstract to state that complementary functions have been demonstrated.

As for divergent roles, the results indeed suggest divergent roles but cannot demonstrate them, as their results could be consistent with different expression patterns and, in one extreme possibility, no divergent function at all, as the authors rightly discuss.

We have now modified to the Abstract to state divergent roles are suggested.

Other comments:

A. Second paragraph of introduction: "The simplified protein diversity of the Ctage5/Tango1 in zebrafish compared to humans allows investigation of their complimentary and divergent functions."

In humans, apart from Ctage5, Tango1 and alternative transcripts from the same MIA2 and MIA3 loci, there are more family members encoded from MIA, OTOR and six additional CTAGE genes. It would be interesting to mention if these are present as well in zebrafish.

We feel that description of more distant family members of Ctage5 and Tango1 dilute the focus of our study. Furthermore, the less well annotated state zebrafish genome leaves questions for existence of orthologs not found. That said, OTOR is not described for zebrafish, but 8 addition Ctage members exist.

B. Third paragraph of introduction: "Drosophila studies have showed secretion of multiple extracellular proteins including... "

In Drosophila, where a single Tango1 exists (without alternative splicing or other MIA/CTAGE genes), studies have shown retention of every secreted protein analyzed and disruption of ERES morphology in every tissue upon Tango1 loss. The reason is still argued (general receptor function vs clogging by few specific cargos vs general ERES structural role), but all secretion is affected.

The Reviewer highlights an interesting controversy. We have added a sentence to state the uncertainty in mechanism: "The specific mechanisms underlying the broad disruption to ER trafficking is still being investigated"

C. First paragraph of results: "Primers designed to measure tango1s mRNA did not reveal a change between wild-type and tango1l mutant embryos, indicating that Tango1s protein could still be made (Fig. S2)."

Suggesting, rather than indicating, as protein level was not directly tested".

We have modified text : " … suggesting that Tango1s protein could still be made (Fig. S2)."

Also, according to S2, the primers would measure both Tango1s and Tango1l, which means Tango1s is upregulated in absence of Tango1l if I understand correctly, as no change is seen in the mutant lacking Tango1l. Is that right?

Actually, there is no change (up or down) in Tango1s, when Tango1l is mutated (SH3 deletion).

D. "Our results suggest that collagen II trafficking is not affected with ctage5 deletion, indicating Tango1 has the essential role in Collagen II trafficking and craniofacial development."

However, the Ctage5 mutant enhances the craniofacial development phenotype of Tango1 mutant, so Ctage5 is involved in craniofacial development as well. Maybe less than Tango1l for functional or expression reasons, or the Ctage5 mutant is hypomorphic and does not show the whole extent of the involvement, but it is involved as well.

We have modified test to read: "Our results suggest that collagen II trafficking is not affected with *ctage5* deletion alone, indicating Tango1 has a more significant role in Collagen II trafficking and craniofacial development."

E. Third paragraph discussion: "While our studies addressed the shared and unique functions between Ctage5 and Tango1 with respect to trafficking and cellular homeostasis, the experiments in zebrafish also demonstrated that Tango1 plays an important role in maintaining ER-Golgi morphology, consistent with in vitro observations (Bard et al., 2006; Rios-Barrerra et al., 2017; Reynolds et al., 2019)."

These are studies in flies, so not in vitro. Also reported in Liu et al., 2017.

Thank you for the clarification. We have updated this sentence and the citations: "While our studies addressed the shared and unique functions between Ctage5 and Tango1 with respect to trafficking and cellular homeostasis, the experiments in zebrafish also demonstrated that Tango1 plays an important role in maintaining ER-Golgi morphology,

consistent with observations by others (Bard et al., 2006; Liu et al., 2017; Rios-Barrerra et al., 2017; Reynolds et al., 2019).”

F. In abstract and throughout text, but correct in title: complEmentary.

We have made the spelling corrections throughout the manuscript

RE: Manuscript #E20-11-0745R
TITLE: "Complementary and divergent functions of zebrafish Tango1 and Ctage5 in tissue development and homeostasis"

Dear Dr. Link,

Thanks for your attention to the suggestions from the reviewers. I am pleased to accept the revised manuscript for publication.

We appreciate your sending this nice work to MBoC.

Sincerely,
Benjamin Glick
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Link:

Congratulations on the acceptance of your manuscript.

A PDF of your manuscript will be published on MBoC in Press, an early release version of the journal, within 10 days. The date your manuscript appears at www.molbiolcell.org/toc/mboc/0/0 is the official publication date. Your manuscript will also be scheduled for publication in the next available issue of MBoC.

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