

Manuscript EMBO-2016-95959

The complete structure of the chloroplast 70S ribosome in complex with translation factor pY

Philipp Bieri, Marc Leibundgut, Martin Saurer, Daniel Boehringer and Nenad Ban

Corresponding author: Nenad Ban, Swiss Federal Institute of Technology, ETH Zurich

Review timeline:

Submission date:	25 October 2016
Editorial Decision:	17 November 2016
Revision received:	24 November 2016
Accepted:	28 November 2016

Editor: Anne Nielsen

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.)

1st Editorial Decision

17 November 2016

Thank you for submitting your manuscript for consideration by the EMBO Journal. It has now been seen by two referees whose comments are shown below.

As you will see from the reports, both referees highlight the importance and quality of your findings and consequently support publication of your manuscript in The EMBO Journal, pending satisfactory revision.

For the revised manuscript I would particularly ask you to focus your efforts on the following points:

- > elaborate on the introduction and discussion as suggested by ref #1, although I will leave it up to you to decide if you keep the section discussing the relationship between chloroplast and apicoplast ribosomes.
- > please follow the suggestion from ref #1 to include any biochemical data you may have on the presence of factor pY in the ribosome preps used.
- > as ref #2 points out, related work from Bhushan and colleagues on the chloroplast large subunit was published while your study was under consideration here and I would ask you to acknowledge and discuss that other study in your manuscript.

Given the referees' positive recommendations, I would like to invite you to submit a revised version of the manuscript, addressing the comments of both reviewers. I should add that it is EMBO Journal policy to allow only a single round of revision, and acceptance of your manuscript will therefore depend on the completeness of your responses in this revised version.

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For

more details on our Transparent Editorial Process, please visit our website:
http://emboj.emboPress.org/about#Transparent_Process

We generally allow three months as standard revision time. As a matter of policy, competing manuscripts published during this period will not negatively impact on our assessment of the conceptual advance presented by your study. However, we request that you contact the editor as soon as possible upon publication of any related work, to discuss how to proceed. Should you foresee a problem in meeting this three-month deadline, please let us know in advance and we may be able to grant an extension.

Thank you for the opportunity to consider your work for publication. I look forward to your revision.

REFEREE REPORTS

Referee #1:

The manuscript entitled "the complete structure of the chloroplast 70S ribosome in complex with translation factor pY" authored by Bieri et al. describes the cryo-EM structure of the chloroplast, a plant cell organelle of endosymbiotic origin with its own transcription and translation machinery. Because gene regulation in chloroplasts is mainly done at the level of translation, so that it can rapidly adapt to changes in temperature and light levels, it is of particular importance to understand the mechanisms of translation regulation of this unique organelle. The structure of the ribosome and of factor pY are among the first brick towards this fundamental goal.

I have no major criticism towards this manuscript and would recommend it to be published with the minor revisions outlined below.

- 1- Most comparisons are made with the bacterial ribosome and some wording should probably be changed in this regard. The manuscript regularly says that the chloroplast ribosome, compared to the bacterial 70S is: "highly reorganised" "acquired new proteins", has "truncated helices", as if the chloroplast had evolved from the bacterial one, which is fundamentally not true. Both ribosomes have evolved from an ancestral bacterial ribosome, which is mentioned only at the very end of the manuscript. Of course, the bacterial ribosome probably resembles the ancestral 70S much closer than that of the chloroplast, but this discrepancy should be made clear early in the manuscript.
- 2- Following the first point, the manuscript would probably be enriched and broaden its scope by including more detailed descriptions of the evolutionary divergences between the two ribosome families and when they might have arisen on the evolutionary tree.
- 3- The 4.5S rRNA is described to be homologous to the 3'end of the bacterial 23S. A more thorough description of this would be helpful. Has it arisen from a split from the 23S and how much has it diverged? Figures comparing the 2D and 3D structures of the bacterial 23S 3'end vs the chloroplast 4.5S would help in this regard.
- 4- An important point raised in the manuscript is that initiation does not occur via Shine-Dalgarno-like interactions. The authors cite work proposing alternative initiation mechanisms but do not list or discuss them at all. This weakens their following description of the structural elements that could play a role in this process. The manuscript would benefit from a more thorough description here.
- 5- The authors claim to have identified and modeled factor pY in their density. No other method than prior knowledge of pY involvement and atomic modeling have been used. The factor has not been identified biochemically in the ribosome preparation. although there is little doubt that their interpretation is correct, additional proofs would strengthen the claim, either biochemical or from the modeling, such as evidence for correct secondary structure assignment and sequence registration. FSC Resolution estimation at the location of each new protein and RNA segments identified would also be helpful for this. This information could be added as a supplementary table, similarly to recent papers in the field (Hussain et al. 2014; Llacer et al., 2015).
- 6- A paragraph about the relationship with apicoplast ribosomes seem to only be there as a placeholder. It should either be removed entirely, or provide a bona fide description of the evolutionary and structural relationships between those and the chloroplast ribosomes and what specific features of the chloroplast ribosome give insights into the apicoplast one.

7- Figure S1 shows negative stain images of ribosomes from the 80S fraction and the chloroplast fraction. These images are not very informative since it is difficult to ascertain the identity of the ribosomes from them. 2D class averages would better help in that respect, or a clearer description and quantification of the presence of 80S in the 3D classification (Fig S2).

Referee #2:

Bieri et al. present an interesting and comprehensive structural analysis of the 70S ribosome from chloroplasts using state-of-the-art cryo-EM technology. Although the chloroplast 70S ribosome is related to the well-studied bacterial 70S ribosome there are important changes that the authors have elucidated in structural terms. The described changes at the mRNA entry and exit sites and the polypeptide tunnel are of functional interest. Furthermore, the structure includes the regulatory translation factor pY.

Specific points:

1. Very recently, an independent cryo-EM structure of the large subunit of the spinach chloroplast ribosome was published by Ahmed et al. and should be compared to the present structure.
2. The discussion of 4.5S rRNA is to some extent misleading (e.g. in the abstract). Whereas it is an rRNA unique to plastids formally, sequence homology and the presented structural analysis shows that it resembles the 3' part of bacterial 23S rRNA. Therefore, it constitutes an example for fragmentation of the LSU rRNA but 4.5S rRNA is not an additional component.

1st Revision - authors' response

24 November 2016

Response to Referees

We thank the referees for their positive feedback about our work and the constructive criticisms of the manuscript. We have now revised the manuscript according to their suggestions.

Referee #1

1- Most comparisons are made with the bacterial ribosome and some wording should probably be changed in this regard. The manuscript regularly says that the chloroplast ribosome, compared to the bacterial 70S is: "highly reorganised" "acquired new proteins", has "truncated helices", as if the chloroplast had evolved from the bacterial one, which is fundamentally not true. Both ribosomes have evolved from an ancestral bacterial ribosome, which is mentioned only at the very end of the manuscript. Of course, the bacterial ribosome probably resembles the ancestral 70S much closer than that of the chloroplast, but this discrepancy should be made clear early in the manuscript.

Chloroplast and bacterial 70S ribosomes share a common ancestor, whose exact composition and architecture is unknown. Therefore, indeed the structural comparison of the chloroplast with bacterial ribosomes can be misleading. To avoid this misunderstanding we have carefully checked the wording throughout the manuscript and added the following clarifying statement in the introduction:

“Although the chloroplast and the bacterial 70S ribosomes share a common ancestor, they have diverged considerably from each other as evident from proteomic analysis (Yamaguchi and Subramanian, 2000, 2003; Yamaguchi et al., 2000) and structural characterization at low resolution (Manuell et al., 2007; Sharma et al., 2007).“

However, the bacterial 70S ribosome from *Escherichia coli* is the most similar structurally characterized ribosome and therefore, it was used in our studies for structural comparisons.

2- Following the first point, the manuscript would probably be enriched and broaden its scope by including more detailed descriptions of the evolutionary divergences between the two ribosome families and when they might have arisen on the evolutionary tree.

This is indeed an interesting topic, however, more extensive discussion of the evolutionary divergence between the bacterial and the plastid ribosome families will probably be better covered

in a review. Nevertheless, we expanded the description of plastid evolution in the introduction following referee's suggestion.

3- The 4.5S rRNA is described to be homologous to the 3' end of the bacterial 23S. A more thorough description of this would be helpful. Has it arisen from a split from the 23S and how much has it diverged? Figures comparing the 2D and 3D structures of the bacterial 23S 3' end vs the chloroplast 4.5S would help in this regard.

The 4.5S rRNA element evolved through the fragmentation of the 3' tail of the 23S rRNA by the insertion of a spacer element (115 nts in spinach) in the chloroplast genome. After transcription, this spacer element has to be removed from the primary transcript and the 23S rRNA and the 4.5S rRNA are integrated as independent rRNA elements into the chloroplast large ribosomal subunit. The chloroplast 4.5S rRNA and the 3' tail of the bacterial 23S rRNA show sequence (55% sequence identity) and structural homology, although the 4.5S rRNA has a plastid-specific insertion and the newly formed tail interactions are stabilized by plastid-specific extensions of ribosomal proteins. Therefore, we would like to state that the 4.5S rRNA evolved through fragmentation to an independent rRNA element of the chloroplast ribosomal subunit.

As pointed by both referees, the description of the 4.5S rRNA was not consistent throughout the manuscript and a detailed comparison with the 3' tail of the bacterial 23S rRNA was lacking. Therefore, we checked the wording throughout the manuscript and added a more detailed description of the differences. As further recommended by the referee, we added the secondary structure diagram and structural model of the 3' tail of the bacterial 23S rRNA for a better comparison.

Because it might be helpful for other researchers using our model for structural and biochemical analysis, we have now included the complete secondary structure diagrams of the chloroplast 23S, 16S, 5S and 4.5S rRNA in the Appendix.

4- An important point raised in the manuscript is that initiation does not occur via Shine-Dalgarno-like interactions. The authors cite work proposing alternative initiation mechanisms but do not list or discuss them at all. This weakens their following description of the structural elements that could play a role in this process. The manuscript would benefit from a more thorough description here.

We have extended the description of the proposed translation initiation mechanism by highlighting the cis-elements on the 5'-UTRs of plastid mRNAs that are probably the key regulatory elements of plastid mRNA translation initiation:

“Considering that translation initiation in chloroplasts does not rely on Shine-Dalgarno (SD) like interactions between the mRNA and the anti-SD sequence of the plastid 16S rRNA and considering that two thirds of all transcripts lack a SD-like sequence (Drechsel and Bock, 2011; Hirose et al., 1998; Ruf and Kossel, 1988), alternative mechanisms for plastid translation initiation have been proposed (Sugiura, 2014; Zerges, 2000), in which cis-elements in the 5'-UTRs of plastid mRNAs are proposed to be the major determinants of correct translation initiation in plastids. Nuclear-encoded trans-acting factors, which are partially regulated by abiotic factors as light or temperature specifically bind to these cis-elements and enable efficient translation initiation either by rearranging the structure of the mRNA 5'-UTR or by mediating the interaction between the mRNA and structural elements of the small ribosomal subunit.”

5- The authors claim to have identified and modeled factor pY in their density. No other method than prior knowledge of pY involvement and atomic modeling have been used. The factor has not been identified biochemically in the ribosome preparation. although there is little doubt that their interpretation is correct, additional proofs would strengthen the claim, either biochemical or from the modeling, such as evidence for correct secondary structure assignment and sequence registration. FSC Resolution estimation at the location of each new protein and RNA segments identified would also be helpful for this. This information could be added as a supplementary table, similarly to recent papers in the field (Hussain et al. 2014; Llacer et al., 2015).

The binding of the chloroplast translation factor pY (PSRP1) to the chloroplast ribosome under cold conditions was previously detected by mass spectrometry analysis by Yamaguchi and colleagues.

Therefore, we have not included any further biochemical data indicating the binding of pY in the initially submitted manuscript. Following referee's request for biochemical data proving the identification of translation factor pY, we separated the chloroplast ribosome sample on a SDS-PAGE gel and analysed bands within a certain molecular weight range with spectrometry by an in-house mass spectrometry facility (Functional Genomic Centre Zurich). Thereby, we could confirm chloroplast pY as component of our ribosome sample. Furthermore, we show in an additional figure in the Appendix the comparison of the predicted and the built model of plastid pY and a zoom in of the local resolution plot for the density for pY. Altogether, this should be sufficient to convince the readers of the presence and the identity of chloroplast translation factor pY.

6- A paragraph about the relationship with apicoplast ribosomes seem to only be there as a placeholder. It should either be removed entirely, or provide a bona fide description of the evolutionary and structural relationships between those and the chloroplast ribosomes and what specific features of the chloroplast ribosome give insights into the apicoplast one.

So far, only little information is available about the composition of apicoplast ribosomes, which are mainly based on sequence comparisons and discussed in the cited review (Habib et al. 2016). The plastid-specific ribosomal components of these ribosomes are unknown and mass spectrometry analysis is required to gain a concrete picture of their composition. Therefore, we point to the evolutionary relationship between the chloroplasts in algae and plants and the apicoplasts in human parasites, which is in our opinion an interesting example of the complex evolutionary tree of plastids and of which maybe many readers are not aware of, without providing structural comparison. Therefore, we are of the opinion that this section will be of interest and would therefore suggest that it is retained. To be more specific, we now mention the number of missing ribosomal proteins with bacterial homologs for the human parasites *Plasmodium falciparum* and *Toxoplasma gondii*.

7- Figure S1 shows negative stain images of ribosomes from the 80S fraction and the chloroplast fraction. These images are not very informative since it is difficult to ascertain the identity of the ribosomes from them. 2D class averages would better help in that respect, or a clearer description and quantification of the presence of 80S in the 3D classification (Fig S2).

We have replaced the negative stain images of the sucrose gradient peaks by a representative selection of 2D class averages indicating the number of assigned particles to a group of class averages (80S, 70S, 50S large, and 30S small subunit and bad particles). Due to the difference in size, the class averages of 80S particles could be distinguished clearly from 70S class averages. Therefore, we could remove the major fraction of 80S particles already at the 2D classification step. The 80S ribosome did not show up as a 3D reconstruction during the 3D classification and the few remaining 80S particle images were probably assigned to the 3D reconstruction showing a distorted 70S ribosome and these particles were removed from further calculation.

Referee #2

1. Very recently, an independent cryo-EM structure of the large subunit of the spinach chloroplast ribosome was published by Ahmed et al. and should be compared to the present structure.

We now reference the above mentioned publication in the introduction, however we do not provide more detailed discussion of the similarities with our findings since the atomic coordinates of their model and the cryo-EM map are not yet available on the PDB- and the EMDB-databases. Based on the description in their publication, we did not discover any major discrepancies between the two features of the large ribosomal subunit they describe and the equivalent portion of our density in the context of the entire chloroplast ribosome.

2. The discussion of 4.5S rRNA is to some extent misleading (e.g. in the abstract). Whereas it is an rRNA unique to plastids formally, sequence homology and the presented structural analysis shows that it resembles the 3' part of bacterial 23S rRNA. Therefore, it constitutes an example for fragmentation of the LSU rRNA but 4.5S rRNA is not an additional component.

The 4.5S rRNA element evolved through the fragmentation of the 3' tail of the 23S rRNA by the insertion of a spacer element (115 nts in spinach) in the chloroplast genome. After transcription, this

spacer element has to be removed from the primary transcript and the 23S rRNA and the 4.5S rRNA are integrated as independent rRNA elements into the chloroplast large ribosomal subunit. The chloroplast 4.5S rRNA and the 3' tail of the bacterial 23S rRNA show sequence (55% sequence identity) and structural homology, although the 4.5S rRNA has a plastid-specific insertion and the newly formed tail interactions are stabilized by plastid-specific extensions of ribosomal proteins. Therefore, we would like to state that the 4.5S rRNA evolved through fragmentation to an independent rRNA element of the chloroplast ribosomal subunit.

Indeed, the description of the 4.5S rRNA was misleading in some of our statements and, therefore, we have now revised the manuscript to clarify that the 4.5S has evolved through fragmentation and is incorporated as a separated rRNA element. Furthermore, we specify the similarities and the differences to the 3' tail of the 23S rRNA in more detail.

We changed the sentence about the 4.5S rRNA element in the abstract:

“The complete structure reveals the features of the 4.5S rRNA, which probably evolved by the fragmentation of the 23S rRNA, and all five plastid-specific ribosomal proteins (PSRPs).”

2nd Editorial Decision

28 November 2016

Thank you for submitting a revised version of your manuscript. I have now read the revised text file and your response to the referee concerns and I am pleased to inform you that your study has been accepted for publication in The EMBO Journal.

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Nenad Ban

Journal Submitted to: EMBO Journal

Manuscript Number:

Reporting Checklist For Life Sciences Articles (Rev. July 2015)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures**1. Data****The data shown in figures should satisfy the following conditions:**

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if $n < 5$, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions**Each figure caption should contain the following information, for each panel where they are relevant:**

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/ varied/ perturbed in a controlled manner.
- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
 - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ^2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
 - are tests one-sided or two-sided?
 - are there adjustments for multiple comparisons?
 - exact statistical test results, e.g., P values = x but not P values < x;
 - definition of 'center values' as median or average;
 - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

Please ensure that the answers to the following questions are reported in the manuscript itself. We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

In the pink boxes below, provide the page number(s) of the manuscript draft or figure legend(s) where the information can be located. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable).

B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	NA
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	NA
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	NA
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	NA
For animal studies, include a statement about randomization even if no randomization was used.	NA
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	NA
4.b. For animal studies, include a statement about blinding even if no blinding was done	NA
5. For every figure, are statistical tests justified as appropriate?	NA
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	NA
Is there an estimate of variation within each group of data?	NA
Is the variance similar between the groups that are being statistically compared?	NA

C- Reagents**USEFUL LINKS FOR COMPLETING THIS FORM**<http://www.antibodypedia.com><http://1degreebio.org><http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-repo><http://grants.nih.gov/grants/olaw/olaw.htm><http://www.mrc.ac.uk/Ourresearch/Ethicsresearchguidance/Useofanimals/index.htm><http://ClinicalTrials.gov><http://www.consort-statement.org><http://www.consort-statement.org/checklists/view/32-consort/66-title><http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tun><http://datadryad.org><http://figshare.com><http://www.ncbi.nlm.nih.gov/gap><http://www.ebi.ac.uk/ega><http://biomodels.net/><http://biomodels.net/miriam/><http://ijb.biochem.sun.ac.za>http://oba.od.nih.gov/biosecurity/biosecurity_documents.html<http://www.selectagents.gov/>

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	NA
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	NA

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	NA
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	NA
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	NA

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	NA
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	NA
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	NA
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	NA

F- Data Accessibility

18. Provide accession codes for deposited data. See author guidelines, under 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	The 3.4 Å cryo-EM map of the chloroplast 70S ribosome, the 3.2 Å cryo-EM map of the 50S subunit and the 3.6 Å cryo-EM map of the 30S subunit will be deposited in the Electron Microscopy Databank (EMDB). The refined coordinates of the complete chloroplast 70S ribosome as well as of the 50S subunit and of the 30S subunit will be deposited in the protein databank (PDB).
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	NA
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	NA
21. As far as possible, primary and referenced data should be formally cited in a Data Availability section. Please state whether you have included this section. Examples: Primary Data Wetmore KM, Deutschbauer AM, Price MN, Arkin AP (2012). Comparison of gene expression and mutant fitness in <i>Shewanella oneidensis</i> MR-1. Gene Expression Omnibus GSE39462 Referenced Data Huang J, Brown AF, Lei M (2012). Crystal structure of the TRBD domain of TERT and the CR4/5 of TR. Protein Data Bank 4O26 AP-MS analysis of human histone deacetylase interactions in CEM-T cells (2013). PRIDE PXD000208	NA
22. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	NA

G- Dual use research of concern

23. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	NA
---	----