THE EMBO JOURNAL

Architecture of the active post-translational Sec translocon

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DOI: 10.15252/embj.2020105643

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Review Timeline:

Submission Date:	18th May 20
Editorial Decision:	1st Jul 20
Revision Received:	21st Sep 20
Editorial Decision:	16th Oct 20
Revision Received:	20th Oct 20
Accepted:	23rd Oct 20

Editor: Elisabetta Argenzio

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. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

1st Editorial Decision 1st Jul 2020

Thank you for submitting your manuscript entitled "Architecture of the active post-translational Sec translocon" [EMBOJ-2020-105643] to The EMBO Journal. Please accept my sincerest apologies for the delay in getting back with our decision. Your study has been sent to three reviewers for evaluation, whose reports are enclosed below.

As you can see, referee #2 and #3 find the study interesting and support its publication after addressing some minor issues. Reviewer #1 appreciates the quality of the data but stresses the limited conceptual advance and insight provided. Given these opinions, we asked each referee to comment the other referees' reports. The outcome of this discussion is that while the study does not change our understanding of how the translocon works, it provides a complete picture of an important complex.

Given the overall interest of your study, I am pleased to invite submission of a revised manuscript as indicated in the reports attached herein. In addition, your study was also seen by an external advisor, who strongly recommended to increase the resolution of the complex.

I would like to point it out that addressing the advisor's and the referees' points in a conclusive manner would be essential for publication in The EMBO Journal.

I should also add that it is our policy to allow only a single round of major revision. Therefore, acceptance of your manuscript will depend on the completeness of your responses in this revised version.

We generally grant three months as standard revision time. As we are aware that many laboratories cannot function at full capacity owing to the COVID-19 pandemic, we may relax this deadline. Also, we have decided to apply our 'scooping protection policy' to the time span required for you to fully revise your manuscript and address the experimental issues highlighted herein. Nevertheless, please inform us as soon as a paper with related content published elsewhere.

Before submitting your revision, primary datasets (and computer code, where appropriate) produced in this study need to be deposited in an appropriate public database (see http://msb.embopress.org/authorguide#dataavailability). Please remember to provide a reviewer password if the datasets are not yet public. Include a "Data availability" section even if there are no primary datasets produced in the study.

I thank you for the opportunity to consider this manuscript and look forward to your revision.

Referee #1:

This study reports two findings. The first finding is a moderate-resolution cryo-EM structure of the heptameric Sec translocon engaged by a signal sequence in detergent solution. The EM map allowed docking of earlier higher resolution empty Sec translocon structural models, plausible

assignment of a putative signal sequence helix, and plausible assignment of the two Sec62 transmembrane domains. The second finding is a crystal structure of the N-terminal cytosolic domain of Sec62 and characterization of its previously established interaction with a peptide from Sec63. The structure was docked into the EM map, and the details of the interaction deduced by HDX-MS and validated by site-specific mutagenesis.

The data are solid, the technical quality of the paper is high, and the interpretation is generally sound. However, the conceptual advance and new insights into the biology of Sec translocon function is rather modest. The position of the signal sequence was quite strongly established by a combination of site-specific crosslinking experiments and structural work on the co-translational Sec61 and post-translational SecY systems. While seeing some moderate-resolution density in the expected place is satisfying, it does not advance our understanding of translocation or translocon function appreciably. Similarly, the crystal structure of the cytosolic domain and finer details of its (known) interaction with the C-terminus of Sec63 are solid new information, but provide minimal biological insight.

For these reasons, the study in this referee's opinion is not sufficiently advanced to warrant publication in EMBO. A more specialised journal is more appropriate.

Minor comments:

The position of the putative second transmembrane helix of Sec62 might represent an artefact of detergent solubilization because as currently positioned, it does not cross the plane of the membrane. This should be noted. Such artefacts, or even worse artefacts such as complete flipping of a protein (PMID 32327568), are sometimes seen in detergent.

In the abstract, it says "a crystal structure of the Sec62 subunit" but perhaps better to be explicit and say "a crystal structure of the Sec62 cytosolic domain" or equivalent.

Referee #2:

The manuscript by Weng et al. describes the structure of a post-translational Sec complex bound to the prepro-alpha factor signal sequence. The study builds on recent structures of apo post-translational Sec complexes and offers several notable advances: 1) the presence of signal sequence within Sec61 suggests a translocation mechanism that is consistent with other models derived from co-translational Sec61 structures; 2) the resolvability of Sec62 TM helices in the presence of signal sequence is suggestive of its role in recognizing and stabilizing translocation substrates; 3) the crystal structure of the Sec62 cytosolic domain enables its modeling into the Sec complex and clarifies its role during post-translational translocation; and 4) elegant biochemical data are presented that rationalizes the essential nature of the interaction between Sec62 and Sec63. These advances enable the authors to construct the most complete model of the post-translational Sec complex to date. The work is high quality and the significance of the structure is meritorious of publication.

A few minor comments should be addressed prior to publication:

• It would be helpful to indicate the N->C directionality of the signal sequence in Fig. 2.
• The fitting of Sec62 into the EM reconstruction in Fig. 3C does not look very convincing. Closeup views of secondary structure fits, for example of the N-terminal helical bundle, would be warranted.

• It's hard for me to appreciate the structural comparisons shown in Figs. 2C-E and EV4. The blue arrows are rather small and the text labels are grainy. I wonder if videos of morphing between structures would make the differences clearer.
• A couple of references to nonexistent figure panels on page 10: Fig. 2F and 2G.
• The last few lines of the caption for Figure EV3 have been duplicated.

Referee #3:

This manuscript presents a cryo-EM structure (at 4.7-4.8 A resolution) of the posttranslational Sec heptameric complex with a signal sequence inserted into the lateral gate, yielding a state wherein the gate opening is wider than in previous structures with the plug domain relocated from the subcentral postion to a position just below the constriction ring. Of specific interest are the positions of the two helices of Sec62 that flank the signal sequence. An important novelty of the manuscript is that the crystal structure of a soluble Sec62 domain is presented, and fitted into the densities of the cryoEM map. Further, sites of interaction with the C-terminus of Sec63 were mapped. Overall, this work present a more complete structural insight of the posttranslational Sec complex, beyond what was earlier presented, in particular with respect to the Sec62-63 interaction and structure. Further, the work results in an interesting hypothesis how the {trade mark, serif} domains of Sec62 may stabilize the less hydrophobic signal sequences of posttranslational secretory substrates.

Point-by-point response

Referee #1:

This study reports two findings. The first finding is a moderate-resolution cryo-EM structure of the heptameric Sec translocon engaged by a signal sequence in detergent solution. The EM map allowed docking of earlier higher resolution empty Sec translocon structural models, plausible assignment of a putative signal sequence helix, and plausible assignment of the two Sec62 transmembrane domains. The second finding is a crystal structure of the N-terminal cytosolic domain of Sec62 and characterization of its previously established interaction with a peptide from Sec63. The structure was docked into the EM map, and the details of the interaction deduced by HDX-MS and validated by site-specific mutagenesis.

The data are solid, the technical quality of the paper is high, and the interpretation is generally sound. However, the conceptual advance and new insights into the biology of Sec translocon function is rather modest. The position of the signal sequence was quite strongly established by a combination of site-specific crosslinking experiments and structural work on the co-translational Sec61 and post-translational SecY systems. While seeing some moderate-resolution density in the expected place is satisfying, it does not advance our understanding of translocation or translocon function appreciably. Similarly, the crystal structure of the cytosolic domain and finer details of its (known) interaction with the C-terminus of Sec63 are solid new information, but provide minimal biological insight.

For these reasons, the study in this referee's opinion is not sufficiently advanced to warrant publication in EMBO. A more specialised journal is more appropriate.

Response:
We agree with the reviewer that we do not provide a surprising conceptual advance. Yet, we feel that actually showing how the signal sequence interaction with the heptameric complex compares to that of the related Sec complexes is of high interest, even if it is not fundamentally different. Moreover, the signal sequences engaging in the post-translational translocation mode are less hydrophobic when compared to those engaging the Sec complex in the cotranslational mode. In addition, it was shown before that the ground state of the heptameric complex is wider open and thus different from the Sec61 and SecY complexes. Therefore, observing that the active state also deviates from the canonical behavior represents novel insights into the conformational dynamics of this important complex. Finally, by providing the X-ray structure of the globular domain of Sec62 and combining it with the cryo-EM data we can now provide a near complete architectural model of the posttranslocational translocon.

Minor comments:

The position of the putative second transmembrane helix of Sec62 might represent an artefact of detergent solubilization because as currently positioned, it does not cross the plane of the membrane. This should be noted. Such artefacts, or even worse artefacts such as complete flipping of a protein (PMID 32327568), are sometimes seen in detergent.

Response:
We agree with the reviewer that the density for the second helix is not clearly crossing the complete bilayer, which we believe is a result of its apparent flexibility. Since we cannot rule out that this may be caused by the presence of detergent, we noted the possibility of a detergent driven artefact in the discussion in main text as requested.
It reads now: “On the other hand, it should be noted that in our map the density for the Sec62 TM2 does not span completely across the membrane, which might be caused by the presence of detergent.”

In the abstract, it says "a crystal structure of the Sec62 subunit" but perhaps better to be explicit and say "a crystal structure of the Sec62 cytosolic domain" or equivalent.

Response:
We agree with the reviewer and adjusted the abstract as requested.

Referee #2:

The manuscript by Weng et al. describes the structure of a post-translational Sec complex bound to the prepro-alpha factor signal sequence. The study builds on recent structures of apo post-translational Sec complexes and offers several notable advances: 1) the presence of signal sequence within Sec61 suggests a translocation mechanism that is consistent with other models derived from co-translational Sec61 structures; 2) the resolvability of Sec62 TM helices in the presence of signal sequence is suggestive of its role in recognizing and stabilizing translocation substrates; 3) the crystal structure of the Sec62 cytosolic domain enables its modeling into the Sec complex and clarifies its role during post-translational translocation; and 4) elegant biochemical data are presented that rationalizes the essential nature of the interaction between Sec62 and Sec63. These advances enable the authors to construct the most complete model of the post-translational Sec complex to date. The work is high quality and the significance of the structure is meritorious of publication.

Response:
We are happy about this overall very positive validation.

A few minor comments should be addressed prior to publication:

• It would be helpful to indicate the N->C directionality of the signal sequence in Fig. 2.

Response:
We agree and changed the Fig. 2 as suggested.

• The fitting of Sec62 into the EM reconstruction in Fig. 3C does not look very convincing. Closeup views of secondary structure fits, for example of the N-terminal helical bundle, would be warranted.

Response:

We apologize for the lack of clarity regarding the Sec62 fitting. Unfortunately, the domain is so flexible that we could not improve the resolution beyond 10-15 Å. Therefore, it is not possible to provide a fit on the basis of resolved secondary structure, and the exact position of this domain in the density, which shows the overall correct dimensions, must remain somewhat arbitrary due to its high degree of flexibility. However, we picked a position that allows the globular domain to connect properly to the following transmembrane helix. We mention this now more clearly in the manuscript and adjusted the panel in Fig. 3 C illustrating the fitting by showing a density lowpass filtered at 15 Å.
In the results part it reads now: "The dimension of Sec62-N agreed overall with the EM density at the given local resolution of about 15 Å, and we positioned it accordingly into the engaged state adjacent to the front side of Sec61α in order to allow for connectivity with the putative TMs of Sec62 (**Fig. 3C**). The apparent flexibility of this domain as indicated by the limited resolution did not allow for secondary structure-based fitting and more exact positioning."

• It's hard for me to appreciate the structural comparisons shown in Figs. 2C-E and EV4. The blue arrows are rather small and the text labels are grainy. I wonder if videos of morphing between structures would make the differences clearer.

Response:
We agree with the reviewer and (i) changed the color of the arrows from blue to black making the Figures clearer and (ii) provide a video showing morphs between the structures in different conformations.

• A couple of references to nonexistent figure panels on page 10: Fig. 2F and 2G.

Response:
We thank the reviewer for pointing out these mistakes which we corrected accordingly.

• The last few lines of the caption for Figure EV3 have been duplicated.

Response:
We thank the reviewer for pointing out these mistakes which we corrected accordingly.

Referee #3:

This manuscript presents a cryo-EM structure (at 4.7-4.8 A resolution) of the posttranslational Sec heptameric complex with a signal sequence inserted into the lateral gate, yielding a state wherein the gate opening is wider than in previous structures with the plug domain relocated from the subcentral postion to a position just below the constriction ring. Of specific interest are the positions of the two helices of Sec62 that flank the signal sequence. An important novelty of the manuscript is that the crystal structure of a soluble Sec62 domain is presented, and fitted into the densities of the cryoEM map. Further, sites of interaction with the C-terminus of Sec63 were mapped. Overall, this work present a more complete structural insight of the posttranslational Sec complex, beyond what was earlier presented, in particular with respect to the Sec62-63 interaction and structure. Further, the work results in an interesting hypothesis how the domains

of Sec62 may stabilize the less hydrophobic signal sequences of posttranslational secretory substrates.

Response:
We are happy about this overall very positive validation.

1st Revision - Editorial Decision 16th Oct 2020

Thank you for submitting your revised manuscript. The study has been seen by two of the original referees, whose comments are shown below.

As you can see, the reviewers find that their criticisms have been sufficiently addressed.

However, there are a few editorial issues concerning the text and the figures that I need you to address before we can officially accept your manuscript.

Referee #2:

The revised manuscript by Weng et al. offers moderately improved reconstructions (initial 4.7-4.8 Å; revised 4.3-4.4 Å) and presentation quality. The authors also addressed my concern about fitting of the cytosolic Sec62 domain by clarifying that its local resolution is substantially lower (10-15 Å) than the rest of the complex. Fitting its crystal structure as a rigid body and using TM1 as an anchoring position is therefore an acceptable approach. The local resolution heat map in Appendix Figure S1, however, does not indicate a poorly resolved Sec62 that the authors describe in the text and it would be important to address this apparent discrepancy. Overall, the manuscript has been improved and I have no further suggestions.

Referee #3:

No further comments, the authors have to my opinion adequately addressed the points made. For the review, I refer to my initial review

2nd Authors' Response to Reviewers 20th Oct 2020

Authors made the requested editorial changes.

2nd Revision - Editorial Decision 23rd Oct 2020

I am pleased to inform you that your manuscript has been accepted for publication in The EMBO Journal.

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Roland Beckmann
Journal Submitted to: EMBO Journnal
Manuscript Number: EMBOJ-2020-105643

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

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.
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- ➔ 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 χ2 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.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

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

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Is the variance similar between the groups that are being statistically compared?	NA

C- Reagents

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.	Yes,the yeast strain was mentioned in the Materials and methods section.

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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
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F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for '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	Yes, all the data had been deposited into the relevant databases and the accession codes are provided in the data availability section.
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. 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

22. 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