



ELSEVIER

Contents lists available at ScienceDirect

Data in Brief

journal homepage: www.elsevier.com/locate/dib

Data Article

Supporting data on prion protein translocation mechanism revealed by pulling force studies

Theresa Kriegler^a, Sven Lang^b, Luigi Notari^c, Tara Hessa^{a,*}^a Department of Biochemistry and Biophysics Arrhenius Laboratories of Natural Sciences, Stockholm University, Svante Arrhenius väg 16C, SE-10691 Stockholm, Sweden^b Department of Medical Biochemistry and Molecular Biology, Saarland University, Homburg, Germany^c Department of Clinical Neuroscience Therapeutic Immune Design Unit CMM, L8:02 Karolinska Institutet, Sweden

ARTICLE INFO

Article history:

Received 10 June 2020

Revised 20 June 2020

Accepted 22 June 2020

Available online 27 June 2020

Keywords:

Prion protein

XBP1-arrest peptide

Cotranslational folding

Pulling force

semi-permeabilized cells

ABSTRACT

The Prion protein (PrP) is a highly conserved cell surface glycoprotein. To enter the secretory pathway, the PrP precursor relies on the Sec61 complex and multiple accessory factors all gathering at the membrane of the Endoplasmic reticulum (ER). PrP topogenesis results in the formation of different PrP isoforms. Aside from the typical secretory variant (SecPrP) different pathognomonic, membrane-embedded variants (NtmPrP and CtmPrP) that are associated with neurodegenerative diseases can be found [1]. In this article, we provide supportive data related to "Prion Protein Translocation Mechanism Revealed by Pulling Force Studies" (Kriegler et al., May 2020)[2], where we utilize Xbp1 arrest peptide (AP)-mediated ribosomal stalling to study the co-translational folding experienced by PrP during its insertion into the ER. We measure translocation efficiency and characterize the force exerted on PrP nascent chain so called "pulling force profile". Here, we describe the method of AP-mediated ribosomal stalling assay together with additional experimental data to the main article. Furthermore, we describe the combination of AP-mediated ribosomal stalling and semi-permeabilized HeLa cells (SPCs) as ER membrane source. Using this experimental set-up one can directly determine the contribution of a specific membrane component, e.g. subunits

DOI of original article: [10.1016/j.jmb.2020.05.022](https://doi.org/10.1016/j.jmb.2020.05.022)

* Corresponding author.

E-mail address: tara.hessa@dbb.su.se (T. Hessa).<https://doi.org/10.1016/j.dib.2020.105931>2352-3409/© 2020 Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license. (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

of the ER protein translocase, as pulling factor exerting force on the PrP nascent chain. The data presented here covers (a) the SDS-PAGE gel images visualized by autoradiography, (b) quantification of the different populations of PrP species observed in the AP-mediated ribosomal stalling method, and (c) calculation formulas of the pulling force profiles measured in SPCs in comparison to dog pancreas microsomes as ER membrane donor. Finally, Western Blot analysis and quantification of siRNA knockdown levels compared to control conditions of various translocation components are shown.

© 2020 Published by Elsevier Inc.

This is an open access article under the CC BY-NC-ND license. (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

Specifications Table

Subject	Biochemistry
Specific subject area	Protein targeting and translocation into the Endoplasmic Reticulum
Type of data	Image Chart Graph Figure
How data were acquired	Data was acquired by using: Phosphorimager Fuji FLA-9000 equipped with ImageReader software version 1.0 (Fujifilm Corporation) for autoradiography images Azure biosystems c600 imaging system equipped with cSeries Capture Software, version 1.9.8.0403 for Western Blots and Coomassie-stained gels
Data format	Raw Analysed
Parameters for data collection	<i>In vitro</i> transcription/translation in presence or absence of dog pancreatic rough microsomes or in presence of semi-permeabilized cells prepared from HeLa cells subjected to siRNA mediated knockdown or control siRNA
Description of data collection	<i>In vitro</i> reactions were analysed by SDS-PAGE and autoradiography, the scanned images (Fuji FLA-9000) were quantified by Fiji (ImageJ) open source software version 2.0.0-rc65/1.51 u) and EasyQuant (in-house developed quantification software based on QtiPlot) and plotted into graphs. After 96 h siRNA treatment HeLa cells were prepared to semi-permeabilized cells and used for <i>in vitro</i> protein synthesis. Knockdown efficiency was analysed by Western blot and quantified as above.
Data source location	Institution: Stockholm University Department of Biochemistry and Biophysics City/Town/Region: Stockholm, Country: Sweden
Data accessibility	With the article Separate Excel file provided
Related research article	“Prion Protein Translocation Mechanism Revealed by Pulling Force Studies” Theresa Kriegler, Sven Lang, Luigi Notari, Tara Hessa Journal of Molecular Biology, 2020, DOI 10.1016/j.jmb.2020.05.022

1. Value of the data

- The data will advance our understanding of PrP biology highlighting the importance of early biosynthesis steps and factors involved in its translocation and formation of abnormal isoforms causing neurodegeneration.

Table 1

Variant of the Xbp1-derived arrest peptide (AP) relevant to the study.

Name	Sequence
Wildtype sequence	DPVPYQPPFLCQWGRHQPSWKPLMN
AP-PrP	DPVPYQPPFLCQWGRHQVAWKPLMN
AP-Prl	DPVPYQPPFLCQWGRHQCAWKPLMN
NF AP	DPVPYQPPFLCQAIDVQPSGKPLMN
Stop mutant	DPVPYQPPFLCQWGRHQCAWKPLM**

- The data offers an *in vitro* based method for measuring co-translational protein folding combined with cellular manipulations of the membrane protein décor.
- The data provide new insights into the components of the ER translocation machinery and its accessory factors being involved in the co-translational translocation of secretory proteins containing charged and intrinsically disordered domains.
- Researchers in the field of protein translocation, folding, and processing at the endoplasmic reticulum and protein trafficking can benefit from these data.
- The approach presented here can be applied to study the biogenesis of any secretory or membrane protein of interest in any cell line amenable to semi-permeabilization. The method can be further extended using other cell line manipulations to study protein biogenesis under various conditions, such as during ER stress.
- Data comparison with similar studies will advance and enrich the field of secretory and membrane protein folding and trafficking.

2. Data description

The data presented here support the use of the arrest peptide-mediated stalling assay *in vitro* [3,4] in rabbit reticulocyte lysate as a method to study the co-translational protein translocation of the prion protein (PrP) into the ER (Fig. 1). Separation of samples by SDS-PAGE and detection by autoradiography allows the quantification of the relevant protein populations. Calculating the fraction of full length protein, f(FL), and plotting it against the corresponding construct length results in “force profiles” as shown in Figs. 2 and 3. Fig. 2 presents data confirming that this assay can also be used in combination with semi-permeabilized cells (SPCs) [5–8] instead of the traditionally used rough microsomes (RMs). Western blot data (Fig. 3) validate that SPCs prepared from cells treated with siRNAs against ER translocon components are efficiently depleted of the targeted components. These knock-downs can lead to changes in the observed force profile as we demonstrate on Prolactin (Prl) protein as a positive control. Therefore, these data reinforce the analysis of PrP translocation events discussed in detail in the manuscript by Kriegler et al. [2].

3. Experimental design, materials, and methods

3.1. Plasmids and constructs

All Prion protein (PrP) constructs with the Xbp1-derived arrest peptide (AP) were based on the hamster PrP sequence in SP64 vector and is described in detail in [2]. The Prolactin (Prl) constructs have been previously described [4]. All the different variants of the arrest peptide described below are shown in Table 1. The wildtype sequence [9] was modified to increase the stalling effect requiring a stronger force on the nascent chain to resume translation. The strength of the AP was tuned to the strength of the pulling events being studied in order to capture the widest possible range of pulling events, while limiting the fraction of pulling-independent full length protein formed due to imperfect stalling of the AP. Mutations were based on structural

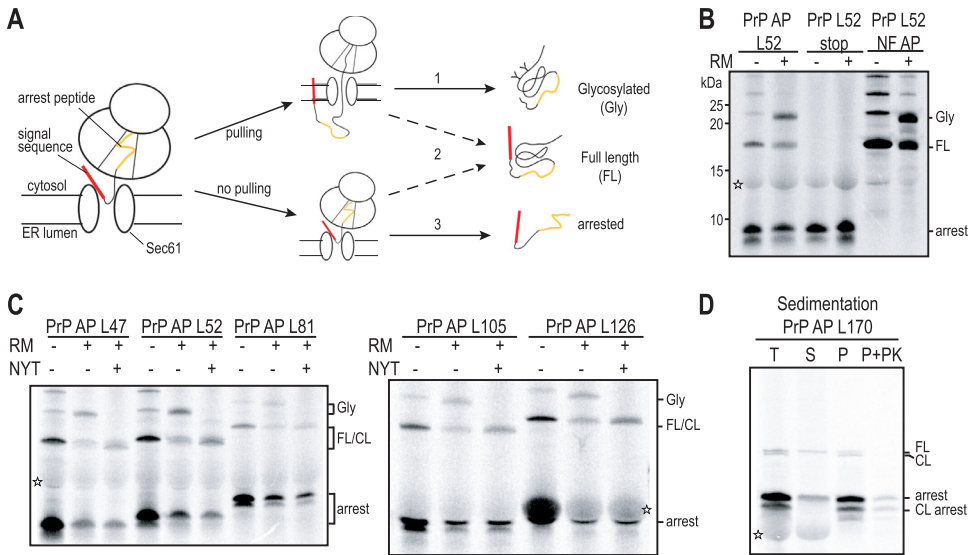


Fig. 1. The arrest peptide mediated stalling assay as a tool to study translocation of the Prion protein (PrP) in the ER. (A) Schematic representation of the assay. Constructs encoding an arrest peptide (AP) are expressed *in vitro* in the presence of an ER membrane. As translation by the ribosome reaches the last amino acid of the AP (yellow), translation is stalled due to interactions of the AP with the ribosomal exit tunnel that prevent incorporation of the next amino acid at the peptidyl-transferase center. However, “pulling” on the nascent chain (*i.e.* via chain tension which can result from protein-protein interactions or folding events) overcomes this stalling by loosening the interactions between the AP and the ribosomal exit tunnel, allowing translation to resume (upper panel). In (1) protein is fully translated, translocated and processed: the signal sequence (red) is cleaved off and glycosylation (Gly, indicated by the “Y”) completed. In (2) is the situation that arises due to imperfect stalling efficiency causing some protein to be released in the cytosol and thus and escape processing even in the absence of a specific pulling event. The signal sequence remains and the full length protein is not glycosylated (FL). In the absence of pulling on the nascent chain the majority of the protein remains stalled (lower panel) leading to (3) where RNase digestion at the end of the experiment releases this short protein fraction referred to as “arrested”.

(B) Autoradiograph showing the position of the different protein populations. Different PrP arrest peptide constructs (PrP AP) of an arrested length of 52 amino acids (L52) were expressed *in vitro* in rabbit reticulocyte lysate in the presence or absence of rough microsomes (RMs) for 30 min at 30 °C, labeled by the presence of ³⁵S-methionine. The protein populations described in (A) were separated by SDS-PAGE and detected by autoradiography. The identity of the different protein species was confirmed by analyzing control constructs (Table 1) where either: the last amino acid of the AP is substituted with a stop codon (PrP Stop) yielding a product equivalent in size to the arrested; or 5 amino acids relevant for stalling are mutated (PrP NF AP) resulting in a non-functional AP and therefore proteins equivalent to FL and Gly. Of note, while the FL signal is equivalent to the PrP molecules not transported into the ER, the Gly signal only occurs in presence of RM and indicates the population of transported PrP molecules that are glycosylated once entering the ER lumen. The higher molecular weight bands above FL in absence of RMs (-RMs) are mono- and poly-ubiquitinated forms of PrP separated by roughly 8 kDa each, which corresponds to the size of one ubiquitin. The star indicates the running position of hemoglobin that is present in large quantities in the lysate and causes distortion of nearby bands on the gel. (C) Confirmation of the glycosylation (Gly) of full length species. Representation of PrP AP constructs at arrested lengths of 47, 52, 81, 105 or 126 amino acids (L47-L126) expressed as in (B) in the presence or absence of RMs and an Asn-Tyr-Thr (NYT) tripeptide that competitively inhibits N-linked glycosylation by the oligosaccharyl-transferase complex. Populations are indicated as in (A) and (B) with the addition of CL. In presence of the RM and glycosylation inhibitor NYT cleavage of the signal sequence becomes evident as the transported full-length protein lacking the signal sequence (CL) runs slightly lower compared to the FL population not transported and hence not being processed.

(D) Proteinase K accessibility of different populations in the ER membrane. Protein samples were subjected to sedimentation on a sucrose cushion after coupled transcription/translation for 30 min. After the pellet fraction was suspended and divided, one half was subjected to proteinase K (PK) treatment for 60 min on ice. Total (T), supernatant (S), untreated pellet (P) and PK treated pellet (P+PK) fractions were analyzed. The presented construct has an arrested length of 170 amino acids and does not carry any glycosylation sites but cleavage of the signal sequence (CL) is apparent in both the full length (FL) and the arrested population. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

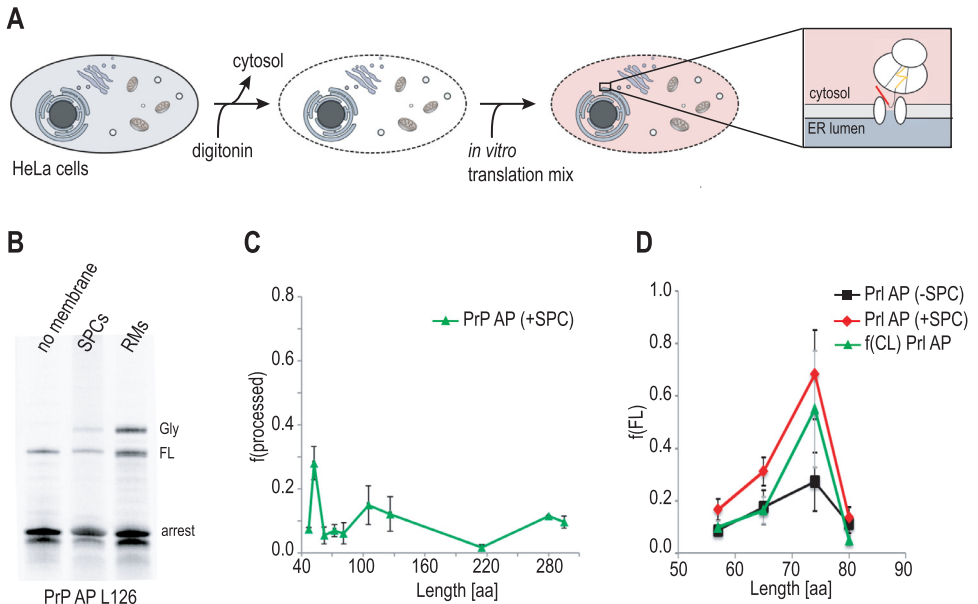


Fig. 2. Semi-permeabilized cells can be used as ER membrane donor in *in vitro* pulling force studies. (A) Schematic representation of the preparation of semi-permeabilized cells (SPCs) for pulling force studies. Short HeLa cells are subjected to mild digitonin treatment for 5 min on ice, which permeabilizes only the plasma membrane, but leaves the organelles intact. The cytosol is washed out with Hepes buffer and endogenous mRNA is removed by treatment with nuclease. These SPCs can be used alternatively to rough microsomes (RMs) in *in vitro* translation reactions to study protein translocation into the ER. (B) Autoradiograph of the protein populations observed in SPCs compared to RMs. Presented here is the PrP construct with the arrested length of 126 amino acids in the absence of any membrane, in the presence of SPCs and in the presence of RMs, demonstrating similar translocation and processing of protein into SPCs as in RMs. The different protein populations are indicated: Gly, glycosylated; FL, full length and arrest (see Fig. 1 for details). (C) Pulling force profile of PrP AP synthesized in presence of SPCs. The fraction of processed protein generated in the presence of SPCs is plotted against the corresponding construct length (length of the arrested protein product in amino acids). The fraction of processed protein $f(\text{processed})$ is calculated as the amount of processed protein (cleaved + glycosylated) divided by the total protein in all the PrP populations. The pulling force profile indicates at which points in translation PrP experiences a force-generating event like interaction with the translocation machinery. Experiments have been performed at least twice and error bars present the standard deviation between the independent experiments.

(D) Demonstration of efficient translocation in SPCs. Constructs of prolactin (Prl) encoding an arrest peptide (AP) were used as a positive control. The $f(\text{FL})$ is calculated as the quantity of full length protein and cleaved full length protein divided by total protein in all relevant populations. $f(\text{FL})$ observed in the presence (red) and absence (black) of SPCs. The $f(\text{CL})$ (green) was determined as the cleaved protein population divided by total PrP protein. As this fraction represents the properly translocated protein fraction $f(\text{CL})$ can only be calculated in the presence of membranes. Experiments have been performed three times and error bars represent the standard deviation between the independent experiments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and biochemical studies performed on the Xbp1 arrest peptide [10]. On the same basis, a non-functional variant of the AP (NF AP) was developed where relevant interactions of the AP with the ribosomal exit tunnel were disrupted eliminating arrest and serving as a control for full length protein populations. A similar control was created for the arrested protein populations by substituting a stop codon at the last position in the AP catalysing the release of the nascent chain even in absence of any pulling factor [4].

HeLa Cells (ATCC no. CCL-2 or DSM no. ACC57) were kept at 37 °C under 5% CO₂ conditions. The media was Dulbecco's modified Eagle's medium (DMEM, Gibco) with 10% fetal bovine serum (Sigma) and with final concentrations of 4 mM L-glutamine, 1 mM sodium pyruvate and 1% penicillin/streptomycin (all from Sigma) (See also [2]).

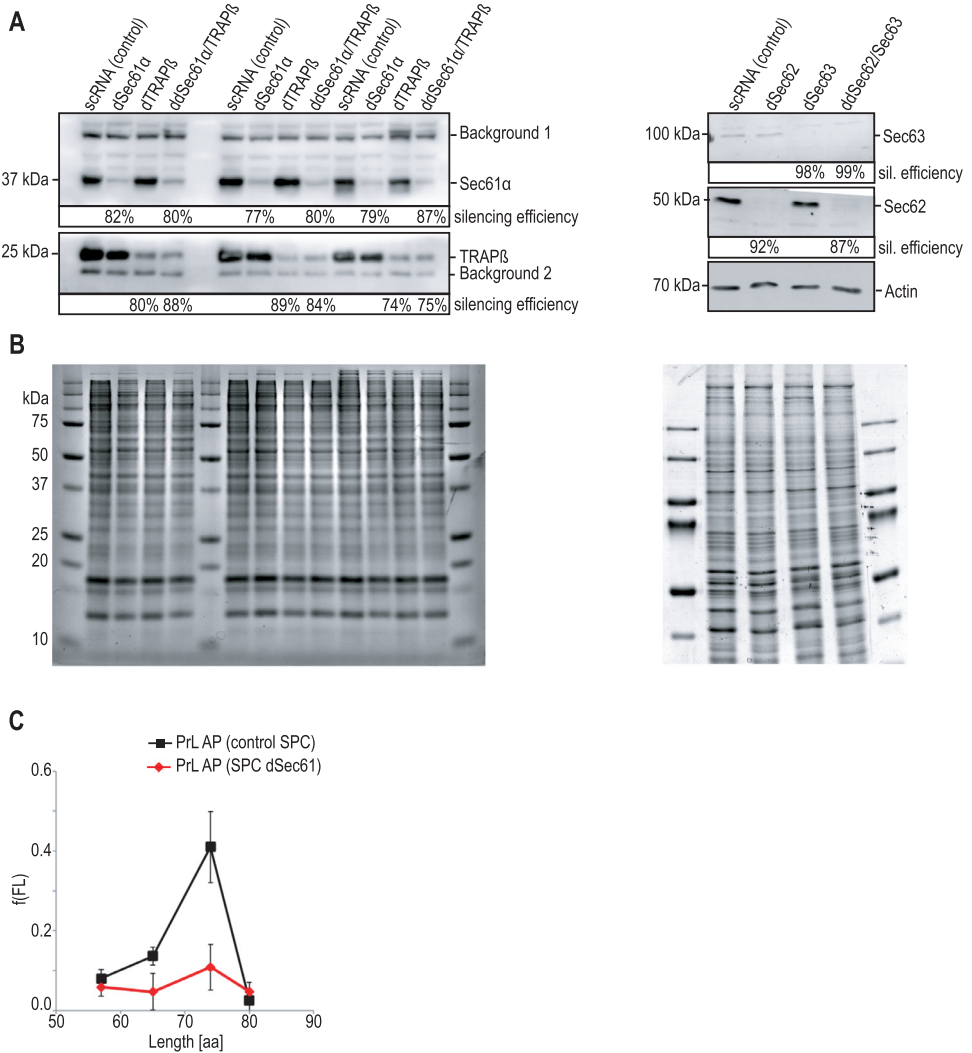


Fig. 3. Semi-permeabilized cells (SPCs) can be treated with siRNAs to knockdown components of the ER translocation machinery and used to measure force profiles. Cells were transfected with siRNA against the indicated target or with scrambled control siRNA (scrNA). Transfected SPCs were prepared as described (see Fig. 2 and materials and methods). (A) Demonstration of silencing efficiency. The levels of the silenced targets in the prepared SPCs were analyzed by Western blotting. Silencing efficiency was calculated based on the relative amounts of quantified protein in silenced and control SPCs normalized to either prominent background bands or actin. (B) Coomassie stain of the total protein content of SPCs separated by SDS-PAGE to confirm equivalent concentrations (40 000 cells/ μ l) between different SPC preparations. (C) Pulling force profile for Prolactin (PrI) after Sec61 α silencing. The fraction of full length protein f(FL) generated in control SPCs (black) or SPCs exhibiting decreased levels of Sec61 α (red) is plotted against the length in amino acids of the arrested protein for each of the constructs. Experiments have been performed three times and error bars represent the standard deviation between the independent experiments.

3.2. siRNA treatment and preparation of semi-permeabilized cells (SPCs)[2,6,8]

The day before silencing was initiated, cells were split 1:3 to ensure robust cell growth during seeding. On the following day we seeded 5.6×10^5 cells contained in 4 mL complete DMEM (as above) in a 6-cm culture plate and treated them with a transfection mix containing 76 μl OptiMeM (Gibco), 20 μl HiPerFect (Qiagen) and 4 μl siRNA (final concentration 20 nM) for each siRNA (see [2] Table 4 for sequence). The mix was incubated at room temperature for 10 min before transfection. Cells were cultured for 24 h under normal conditions then the media was renewed and cells were transfected in the same way a second time. Total incubation time for the depletion was 96 h.

The efficiency of the silencing was monitored by Western blotting using rabbit antibodies raised against human Sec61 α , Trap β , Sec62 and Sec63, all at a dilution of 1:500. The antibodies were provided generously by Richard Zimmermann. For visualization a goat anti-rabbit peroxidase conjugated IgG (1:10,000) was used, the signal was developed with SuperSignal West Femto Maximum Sensitivity Substrate kit from Thermo Scientific and detected in an Azure biosystems c600 imaging system (with cSeries Capture Software, version 1.9.8.0403). The resulting bands were analysed with Fiji open source image processing software (version 2.0.0-rc-65/1.51 u) and quantified with EasyQuant (in-house developed quantification software based on QtiPlot). The calculations of silencing efficiency were performed in Excel (Microsoft Excel for Mac, version 16.34).

Preparation of SPCs was performed as has been published previously [5–7]. Briefly, cells were harvested and counted then suspended thoroughly in 6 ml of cold KHM-buffer (“potassium-Hepes-magnesium” buffer: 20 mM Hepes pH 7.2 at 2 °C, 110 mM KAc, 2 mM MgAc₂). Per 10⁶ cells 1 μl of digitonin (40 mg/ml DMSO) was added to the cell suspension and incubated for 5 min on ice. The volume was adjusted to 14 ml with cold KHM buffer and the cell sedimented by centrifuging for 3 min at 500x g and 4 °C. Cells were resuspended in 14 ml of cold Hepes buffer (90 mM Hepes pH 7.2, 50 mM KAc) and placed on ice for 10 min. Following collection, the cells were suspended in 300 ml cold KHM buffer and subjected to nuclease treatment (2 μl of S7 nuclease 4000 U/ml activated by 1 μl CaCl₂ (200 mM)) for 12 min at room temperature. Nucleic acid digestion was stopped by adding 4 μl EGTA solution (200 mM) and 3 ml of cold KHM buffer. Cells were collected as before and counted four times for accurate dilution to the final cell number of 40 000 cells/ μl in KHM buffer.

3.3. In vitro transcription/translation (see also [2])

PrP constructs were expressed in a SP6 coupled transcription/translation system derived from Rabbit Reticulocyte Lysate (RRL) (Promega). Reactions were performed in a total volume of 10 μl for 30 min at 30 °C and labeled with 2.5 μCi [³⁵S]-methionine. If indicated, 0.5 μl column washed microsomes (RM) from dog pancreas (tRNA products) were added to the reaction. The reactions were stopped by addition of sample buffer (final concentration: 62.5 mM Tris, pH 6.8, 2% SDS, 10% glycerol, 100 mM DTT and 0.01% bromophenol blue) and incubation at 95 °C for 5 min. After cooling the tRNA was digested from the arrested nascent chains by addition of RNaseA (final concentration of 0.2 $\mu\text{g}/\mu\text{l}$, 15 min, 37 °C).

If SPCs were used, reaction volume was reduced to 5 μl including 1.5 μl SPC suspension (40 000 cell/ μl) or Potassium-Hepes-Magnesium (KHM) buffer for controls. Reaction conditions were as before but the reaction was stopped by incubation on ice, followed by pelleting the samples (benchtop centrifuge: 15,000 rpm, 20 min and 4 °C). Pellets were suspended in warm SDS-PAGE sample buffer and incubated (5 min at 95 °C and 1200 rpm). Samples were analysed by SDS-PAGE (12% or 10% Tris-glycine polyacrylamide gels) and autoradiography.

Addition of the tripeptide Asn-Tyr-Thr (NYT) at 0.1 mM during the coupled reaction was used to inhibit N-glycosylation.

If indicated, samples were further analysed by sedimentation on sucrose cushion as described in the related article [2].

3.4. Quantification and analysis

Quantification was as described in [2]. Fractions of full length (FL) protein were calculated as previously described [3, 4] and as performed in the main article [2] by using:

$$f(FL) = \frac{(full\ lengths\ protein + cleaved\ protein + glycosylated\ FL\ protein)}{(FL + cleaved + glycosylated\ FL + arrested + cleaved\ arrest + glycosylated\ arrest)}$$

Fractions of processed protein was calculated by using:

$$f(processed) = \frac{(f\ cleaved\ protein + glycosylated\ FL\ protein)}{(FL + cleaved + glycosylated\ FL + arrested + cleaved\ arrest + glycosylated\ arrest)}$$

Fractions of cleaved protein as for prolactin that does not contain any glycosylation sites was calculated by using:

$$f(CL) = \frac{(f\ cleaved\ protein)}{(FL + cleaved + arrested + cleaved\ arrest)}$$

Experiments were performed at least three times unless indicated differently. In the line graphs the average of the experiments is given, standard deviation is presented as error bars (if not indicated differently).

Ethics statement

The authors declare that to the best of their knowledge they have followed the ethical principles for scientific publishing in *Data in Brief*.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships which have, or could be perceived to have, influenced the work reported in this article.

Acknowledgments

We would like to thank Prof. Richard Zimmermann for the generous gift of the antibodies Sec61 α , TRAP β , Sec62 and Sec63. This work was supported by grants from the Swedish Foundation for Strategic Research to TH and the Deutsche Forschungsgemeinschaft (SFB 894 and IRTG 1830) to SL.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.dib.2020.105931](https://doi.org/10.1016/j.dib.2020.105931).

References

- [1] O. Chakrabarti, A. Ashok, R.S. Hegde, Prion protein biosynthesis and its emerging role in neurodegeneration, *Trends Biochem. Sci.* 34 (6) (2009) 287–295.
- [2] T. Kriegler, S. Lang, L. Notari, T. Hessa, Prion protein translocation mechanism revealed by pulling force studies, *J. Mol. Biol.* (2020) In press.
- [3] N. Ismail, R. Hedman, N. Schiller, G. von Heijne, A biphasic pulling force acts on transmembrane helices during translocon-mediated membrane integration, *Nat. Struct. Mol. Biol.* 19 (10) (2012) 1018–1022.
- [4] T. Kriegler, A. Magouloupoulou, R. Amate Marchal, T. Hessa, Measuring Endoplasmic Reticulum Signal Sequences Translocation Efficiency Using the Xbp1 Arrest Peptide, *Cell Chem. Biol.* (2018).
- [5] S. Lang, et al., Different effects of Sec61, Sec62 and Sec63 depletion on transport of polypeptides into the endoplasmic reticulum of mammalian cells, *J. Cell Sci.* 125 (8) (2012) 1958–1969 Apr..
- [6] A. Ziska, et al., The signal peptide plus a cluster of positive charges in prion protein dictate chaperone-mediated Sec61 channel gating, *Biol. Open* 8 (3) (2019) bio040691, Mar..
- [7] R. Wilson, A.J. Allen, J. Oliver, J.L. Brookman, S. High, N.J. Bulleid, The translocation, folding, assembly and redox-dependent degradation of secretory and membrane proteins in semi-permeabilized mammalian cells, *Biochem. J.* 307 (Pt 3) (1995) 679–687 May.
- [8] J. Dudek, S. Lang, S. Schorr, J. Linxweiler, M. Greiner, R. Zimmermann, Analysis of protein translocation into the endoplasmic reticulum of human cells, *Methods Mol. Biol.* 1033 (2013) 285–299.
- [9] K. Yanagitani, et al., Translational pausing ensures membrane targeting and cytoplasmic splicing of XBP1u mRNA, *Science* 331 (6017) (2011) 586–589 Feb..
- [10] V. Shanmuganathan, et al., Structural and mutational analysis of the ribosome-arresting human XBP1u, *Elife* 8 (2019) Jun..