

# A Membrane Protein in the Nucleus

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## Research Area: The Prion Protein Family

/Prion: infectious agent primarily composed of a protein without nucleic acid/

### Prion diseases:

- Creutzfeldt-Jacob disease (CJD)
- Mad-cow disease (BSE), etc.
- Neurodegeneration - incurable
- „Infectious” nature → primary importance in public health and food safety.
- Prion protein family members: **Prion (PrP)**, **Shadoo (Sho)** and **Doppel**



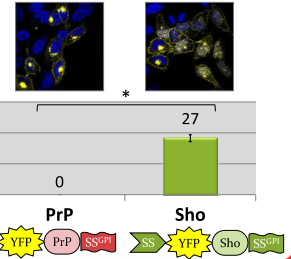
## Background

- PrP and Sho are **functionally analogue membrane proteins**. They are both directed to the **secretory pathway** and possess a **GPI anchor** which induces **plasma membrane localization**.
- However, the localization of the proteins is different:

**PrP**  
exclusive  
plasma membrane (PM)  
localization

**Sho**  
dual  
nuclear and PM  
localization

Nuclei - blue  
Fusion YFP - yellow



## Methods

### Plasmid constructs:

- Backbone: pEYFP-C1 vector (+the cDNA of mouse PrP & Sho proteins)

Nascent chain marks: ER signal peptide → yellow fluorescent protein → core sequence → GPI-signal

### Cell culture work:

- Transfection of plasmids into mammalian cells (HeLa)
- Localization analysis with **fluorescent microscope**
- Counting: cells with nuclear staining/all transfected cell (in percentage)
- Experiments repeated at least three times
- Statistics (Mann-Whitney, \* p<0,05)

What causes the difference?  
How can this secretory protein, Shadoo  
appear in the nucleo-cytoplasmic  
compartment?

Answer:

- NOT the core sequence,
- NOT the different efficiency of the secretion signals,
- PRIMARILY the 'rescue' function of PrP GPI-signal.

## Conclusions

A fraction of Sho exits to the cytosol after entering the ER.

There may be a pathway for proteins to transit from the secretory pathway to the cytosol.

Major importance concerning proteins which are involved in signal transduction or which have dual functions.

## Results

### 1. What is the role of the core protein?

- We examined a YFP with signals of either PrP or Sho to direct it to the secretory pathway.

SP → YFP → SS<sup>PrP</sup> or SP → YFP → SS<sup>Sho</sup>

- Only membrane staining was visible in both cases.

- The nuclear presence disappears in the absence of the core protein. However, ...

...both proteins were proved to have nuclear localization signal (NLS) and to enter the nucleus when directed to the cytosol.[1, 2, 3]

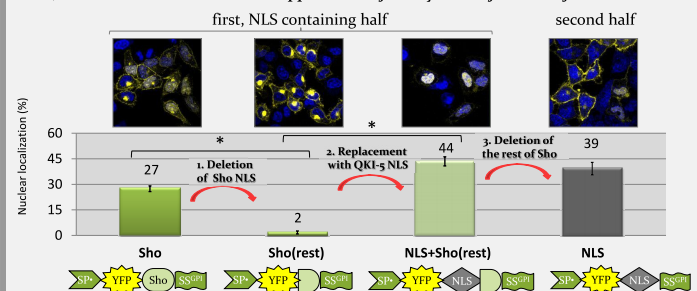
So the difference is that Sho appears in the cytosol while PrP does not.

Which part of the proteins is responsible for the different cytosolic appearance?

### 2. The core sequence of Shadoo?

- Replacement of the core sequence with QKI-5 NLS.

- NO, it has no contribution to the appearance of the cytosolic fraction of Shadoo.

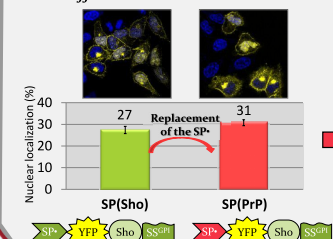


Replacing the core Shadoo with the NLS of the QKI-5 protein gives similar nuclear localization results → it has a role only in the nuclear transport, no contribution to the cytosolic occurrence. The concentration of the protein in the nucleus by an NLS makes the detection of the nucleo-cytoplasmic appearance more sensitive.

### 3. The secretion signal? Different SP efficiency → cytosolic protein?

- Constructs differ only in SP.

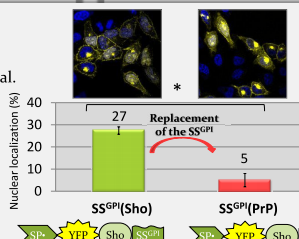
- NO, there is no significant difference in the efficiency of the two SPs. It cannot cause the different localization.



### 4. The GPI-signal?

- Constructs differ only in GPI-signal.

- YES, the disparate GPI-signals can cause the different localization.



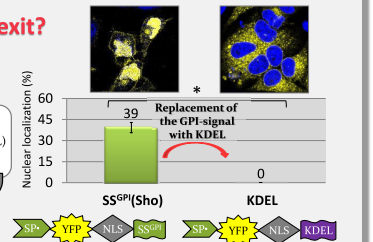
### 6. No translocation to the ER or exit?

- Constructs with eighter GPI-signal or KDEL (ER-retention signal)

If no translocation → cytosol → nuclear localization (NLS)  
If translocation → retention in the ER (KDEL) → no nuclear staining

- EXIT from the secretory pathway.

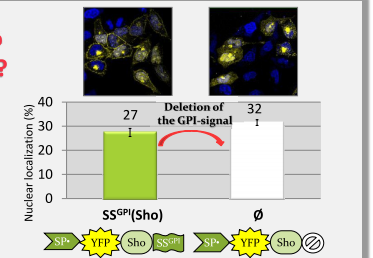
How does the cytosolic fraction emerge?



### 5. Does the GPI-signal of Shadoo induce the cytosolic presence?

- Constructs with and without the GPI-signal.

- NO, the GPI-signal is not necessary to the appearance of the cytosolic fraction.



Does the Sho GPI-signal induce the antagonistic process?

PrP GPI-signal induces posttranslational translocation to the ER. [4] It is like a 'rescue' function - protects the cell from cytosolic occurrence of Prion protein, which is toxic for the cell. However, the efficiency is not too high (5%).  
Is it enough to explain our results?

## References

- [1] A. Jaeger, F. Mouton, J.M. Peyrin, B. Camugli, J.P. Deslys, D. Dormont: Search for a nuclear localization signal in the prion protein. Mol Cell Neurosci 11 (1998) 127-133.
- [2] Y. Gu, J. Huterwisch, R. Friedrich, S. Kalops, K.S. Mishra, N. Singh: Identification of cryptic nuclear localization signals in the prion protein. Neurobiol Dis 12 (2003) 133-140.
- [3] E. Tóth, P.L. Kulcsár, E. Fodor, Ph.D. F. Ágyó, L. Kalmár, A.E. Bory, L. László, E. Welker: The highly conserved, N-terminal (RXXXX)<sub>1</sub> motif of mouse Shadoo mediates nuclear accumulation. Biochim Biophys Acta 1773 (2003) 199-210.
- [4] C. Höltscher, U.C. Bach, B. Dobberstein: Prion protein contains a second endoplasmic reticulum targeting signal sequence located at its C-terminus. Biol Chem 276 (2001) 13388-13394.

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