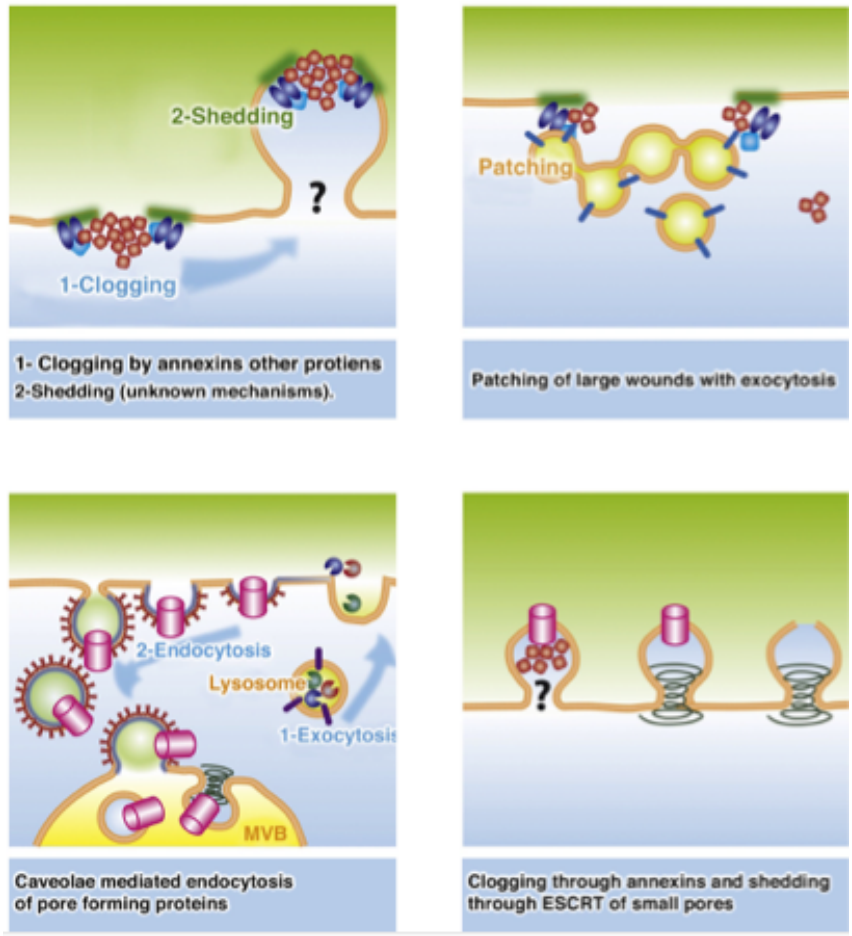
The Calcium signaling lab - Research

Currently, we focus mostly on the role of **ALG-2 and the related Peflin** protein in **plasma membrane repair**.

Plasma membrane repair is a Ca^{2+}-dependent process essential for cell survival upon membrane damage. Metastasizing cancer cells need to have especially efficient membrane repair systems to cope with continuous mechanical stress leading to membrane damage. Inhibiting the membrane repair process may help to eliminate unwanted cells e.g. cancer cells. Partially or temporally interfering with membrane repair mechanisms may help to deliver drugs into the cells to be removed.
Several repair mechanisms are known (1), one of which is the macro-vesicle shedding model. The Ca^{2+}-binding protein ALG-2 has been found to be implicated in this mechanism by mediating the assembly of the ESCRT machinery at membrane wound sites (2). We found that knocking out ALG-2 in chicken DT-40 cells leads to increased sensitivity to electroporation treatment, a phenotype that could be reversed by reintroducing ALG-2. In addition, overexpression of ALG-2 in HeLa cells leads to partial protection against digitonin-caused membrane damage (3). Our project will further focus on the involvement of ALG-2 in membrane repair. Besides wt ALG-2 we will test a number of ALG-2 mutations for their potential to repair cell membrane damage induced by different means. For these experiments we will use wt and ALG-2 knock out cells, recently established in our lab by CRISPR/Cas9. A role in membrane repair of Peflin, another calcium binding protein that dimerizes with ALG-2 has yet to be established and this project will try to elucidate this. This will be followed by investigations of ALG-2 in membrane repair of metastasizing cancer cells.

1. Jimenez, A. J., Perez, F., *Plasma membrane repair: the adaptable cell life-insurance,*
   Curr Opin Cell Biol, 2017, **47:** p.99-107
2. Scheffer, L. L., Sreetama, S. C., Sharma, N., Medikayala, S., Brown, K. J., Defour, A. &
   Jaiswal, J. K. (2014) *Mechanism of Ca(2)(+)-triggered ESCRT assembly and regulation*
   of cell membrane repair, *Nat Commun.* **5**, 5646.
3. Berchtold, M. W., et al., *ALG-2 participates in recovery of cells after plasma membrane*
   damage by electroporation and digitonin treatment, PLoS One, 2018, **13**(9): p. e0204520
4. Maki, M., Takahara, T., Shibata, H., *Multifaceted roles of ALG-2 in Ca^{2+}-regulated*
   membrane trafficking, Int J Mol Sci, 2016, **17**(9)



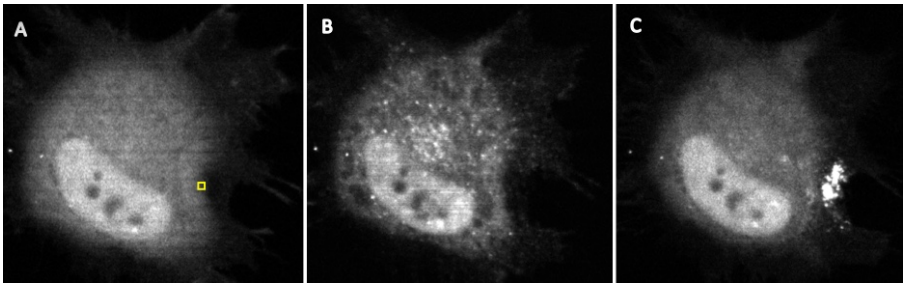
The four modes of mechanisms of membrane repair

Key points of investigation - challenges:

This project will focus on investigating the following main questions:

- Can the observed phenotype of ALG-2 KO cells of diminished plasma repair potential be reversed and thus provide evidence for the effect actually being due to the absence of ALG-2?
- Is binding of Ca^{2+} to ALG-2 necessary for its effect?
- Will an ALG-2 isoform with a different target preference show a similar effect as ALG-2?
- Do peptides with ALG-2 binding sequences or α-ALG-2 antibodies entering the cells through membrane lesions inhibit the function of ALG-2?
- What is the effect of Peflin on membrane repair?
- Can inhibition of ALG-2 expression/function help to the inhibit metastatic potential of cancer cells?
- Is there a correlation between ALG-2 expression and metastatic potential of cancer cells?

Major findings from our group:
In 2018, we published a paper investigating the recovery of cells after membrane damage in relation to ALG-2 (3). In DT-40 cells, ALG-2 was knocked out and the cells were subjected to electroporation. Viability after treatment was used as a measure of recovery, and it was found that ALG-2 KO cells had significantly lower viability compared to wt. Reintroduction of ALG-2 rescued the phenotype (to almost wt level). HeLa cells transfected with ALG-2 expressing constructs, thus overexpressing ALG-2, were subjected to digitonin treatment. Measurements of cell viability revealed that 1) overexpression of ALG-2 showed a protective phenotype against membrane damage treatment, 2) ALG-2.1 overexpression showed no effect on viability and 3) ALG-2 incapable of binding Ca^{2+} showed decreased viability. Thus, ALG-2 appeared to be important for membrane repair in a Ca^{2+}-dependent manner. As these experiments were measuring viability 24 hours after membrane damage, it cannot be concluded that ALG-2 is an important player in the repair process that takes place in a few minutes after damage. It is thus of interest to perform these investigations in the time frame of membrane repair.



Hela cell visualized through its expression of GFP-tagged ALG-2 (A-C). **A.** The cell is laser damaged using a focused scan (yellow insert) by a pulsed multiphoton laser **B.** Immediately after laser damage cellular Ca^{2+} is increased due to the compromised membrane integrity causing ALG-2 to accumulate in speckles as described previously (la Cour et al. 2007). **C**. Following a recovery period (2 min) ALG-2 accumulates at injury site (la Cour et al., unpublished).

For a short **master project description** see:

https://www1.bio.ku.dk/english/education/project-proposals/details/?obvius_proxy_url=https://bio2.science.ku.dk/cms/projekter/detaljer.asp?ID=159