SCAN FOR MORE INFORMATION

PROTEIN STABILITY TRACKED WITH A LIVE CELL REPORTER

TIM SIVERTSEN, EVGENY ONISHCHENKO
DEPARTMENT OF BIOLOGICAL SCIENCES, UNIVERSITY OF BERGEN, BERGEN, NORWAY

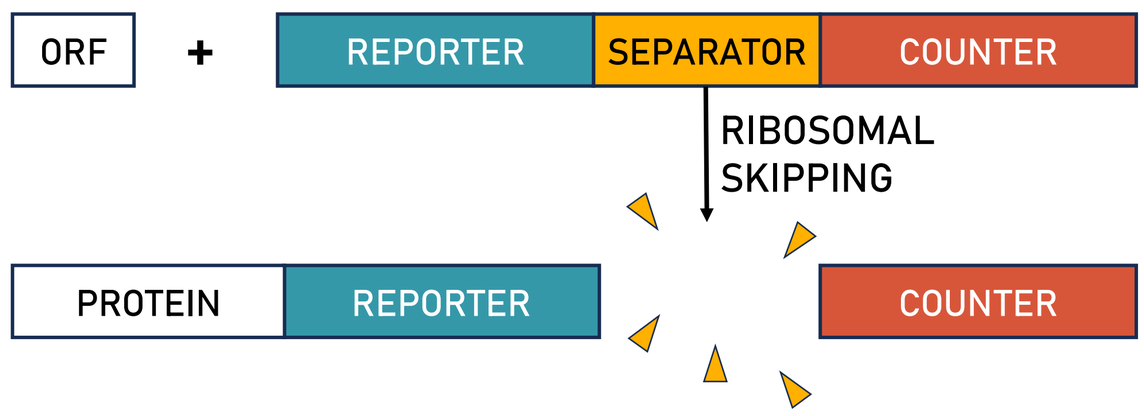
WHY IS THIS REPORTER NEEDED?

Knowing how fast proteins are made and degraded says a lot about the cell's behavior and dynamics

Traditional bulk methods miss cell-specific differences in protein stability or adaptive responses to changing environment.

The aim for this study was to create a fluoresce-based reporter enables determining endogenous protein stability in real time and in individual cells.

The reporter concept is based on a genetic construct that C-terminally attaches two fast-folding, stable and bright fluorophores[1]: ymNeonGreen and ymScarletI
Separated by a high efficiency cleaving peptide[2]: ERBV1



IT WORKS!

The “reporter” and “counter” split well

The construct was attached to mitochondrial membrane protein TOM70 and analyzed with microscopy and western blotting to ensure splitting

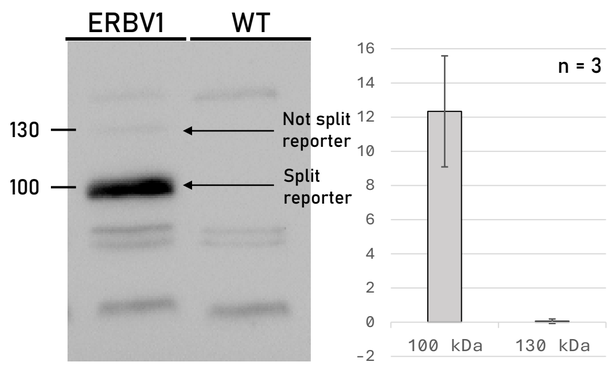


FIGURE 2: Tom70-FLAG-mNG-ERBV1-mScar (ERVB1) and WT (WT) yeast cell extracts were analyzed by WB with anti-FLAG antibodies. Quantification of relative intensities of the full-length (130 kDa) and split (100 kDa) product bands (left) Percentage of band volume for the differing sizes (right).

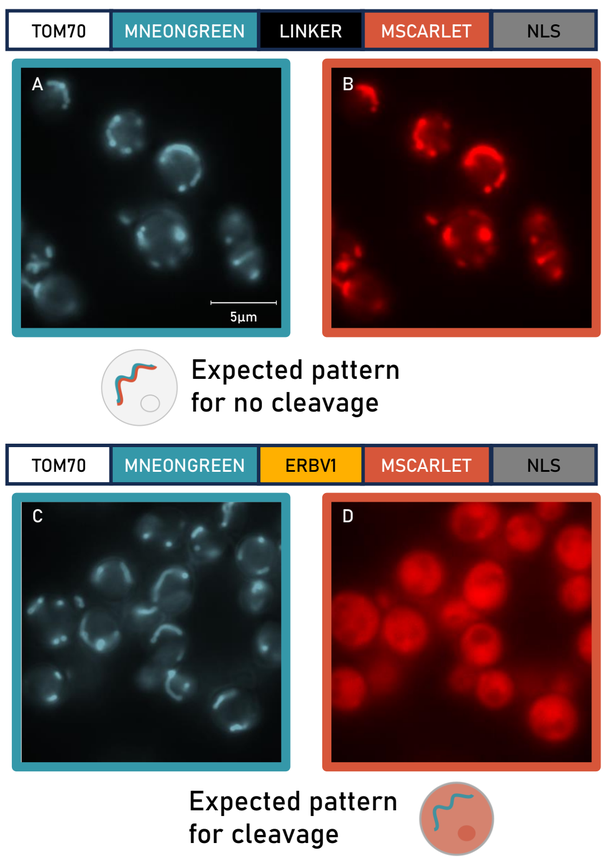


FIGURE 3: (A and B) green fluorescence (GFP) (A) and red fluorescence (RFP) (B) for TOM70 tagged using uncleavable linker between two fluorescent proteins in the reporter. (C and D) The same as in (A and B) but using ERBV1 as a linker.

The “counter” is stable

The construct or its “counter” and “reporter” parts separately were attached to a stable proteasomal subunit PRE4

The effect of separation on the “counter” and “reporter” was determined by comparing fluorescence intensity between the trails

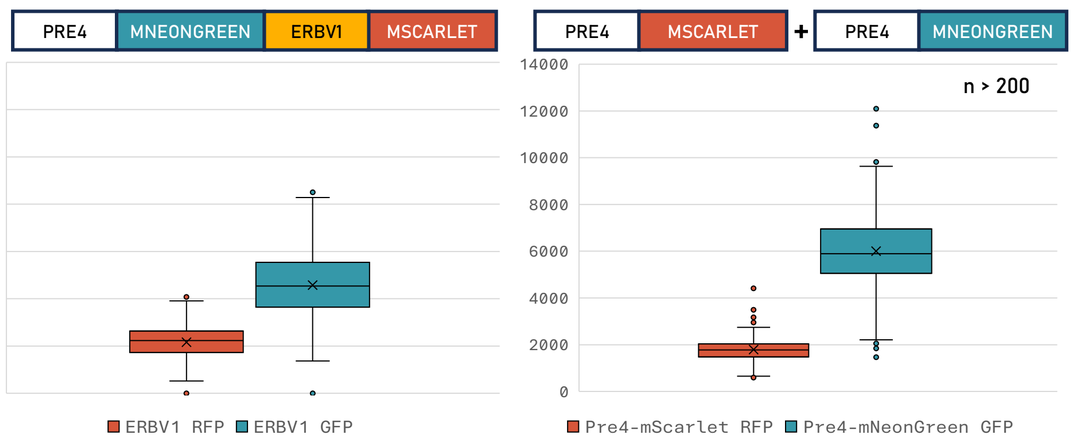


FIGURE 1: Intensity of green and red fluorescence in cells expressing PRE4 tagged with the reporter (left) and of PRE4 tagged with each fluorophore separately (right).

IT’S USEFUL!

The reporter detects nuclear degradation of the yeast proteasomal transport receptor STS1

The construct was attached to STS1, a protein that’s degraded by the proteasome after transporting it into the nucleus.[3]

Fluorescence ratios after 1.5h of treatment with the proteasomal inhibitor MG132 or control were compared showing a 139% increase in green fluorescence for MG132

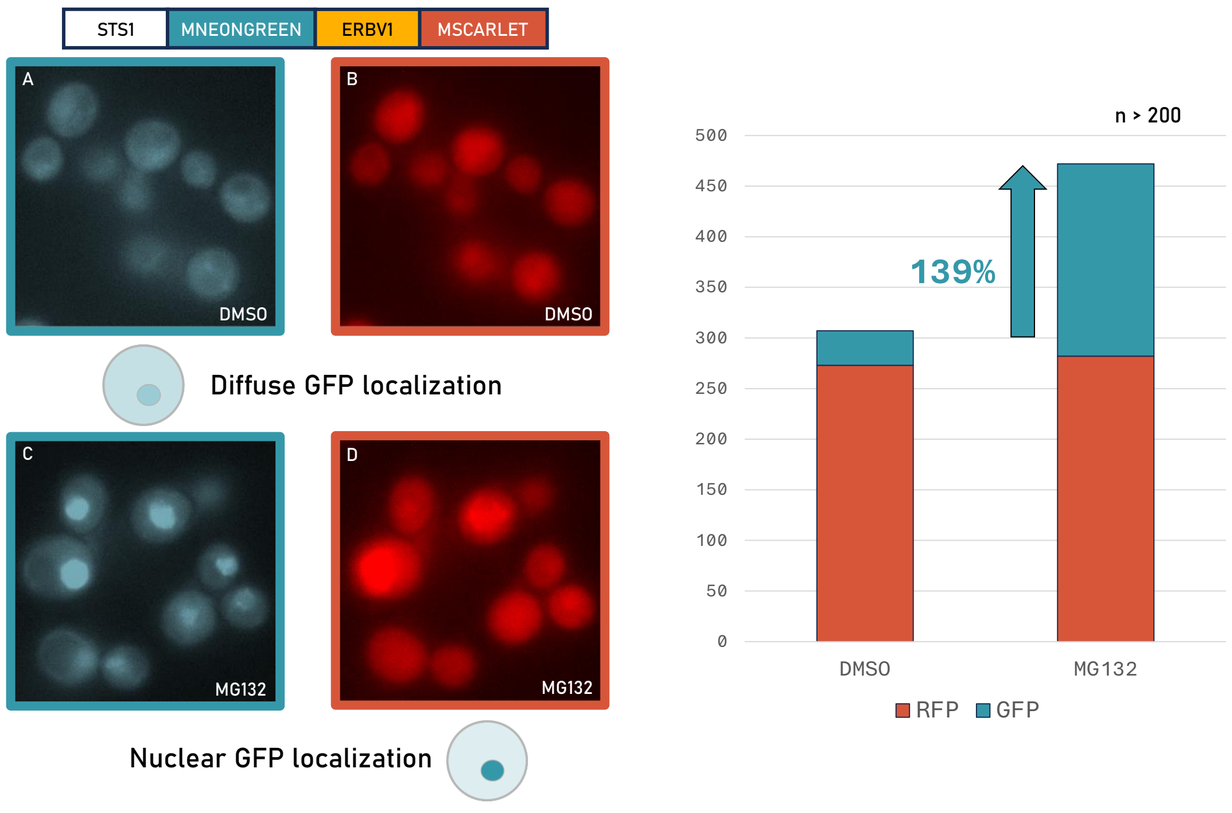


FIGURE 4: STS1 proteins tagged with ERBV1 construct, panels A and B show cells following 1.5 hours of treatment with DMSO (control), panels C and D show cells 1.5 hours of treatment with MG132 (left). Graph of RFP and GFP intensity differences between DMSO and MG132 treatment (right)

CONCLUDING REMARKS & FUTURE PERSPECTIVES

ERBV1 has shown promising results for cleavage in other fungi which legitimizes applications outside S. Cerevisiae. [4]

Flow cytometry would allow efficient application of the construct for:

- Tissue specific drug screening
- Organelle specific drug screening
- Larger comparative studies

The constructs modularity allows it to be tailored to specific needs such as:

- N-terminal tagging
- Epitope tags instead of fluorophores
- Alteration of the cleavage peptide

<u>Anything that can be tagged can be analyzed with this construct</u>

For more in-depth commentary on the results, raw data and references, scan the QR code at the top left

231.2