Characterisation of the Arabidopsis mid-SUN proteins

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The Plant Nuclear Envelope

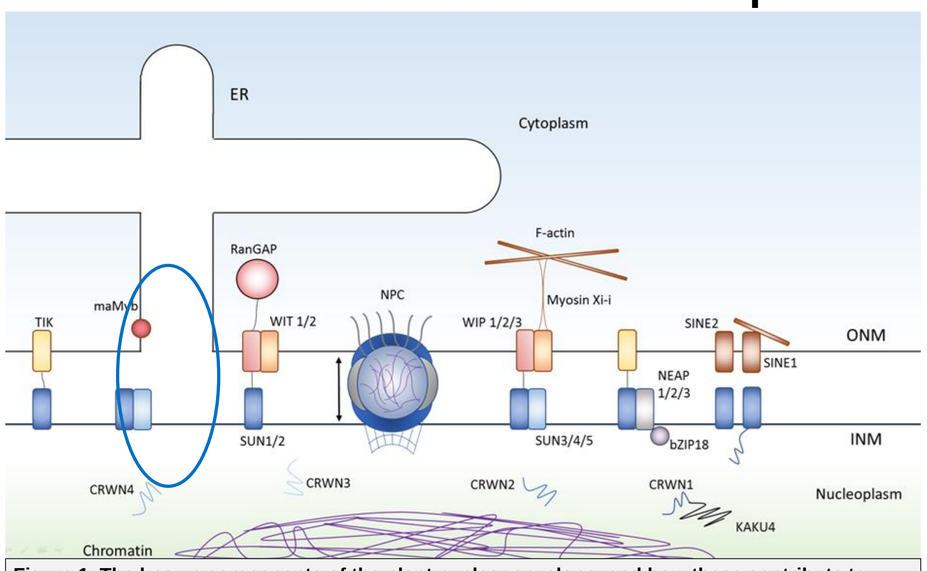


Figure 1. The known components of the plant nuclear envelope, and how these contribute to nuclear bridging complexes, outlined in blue.

The Nuclear Envelope (NE) consists of two separate bilayers: the outer nuclear membrane (ONM) and inner nuclear membrane (INM). INM-localised sad1/UNC-84 (SUN) proteins interact with ONM-localised Klarsicht/ANC-1/Syne homology (KASH) proteins; the SUN and KASH proteins form protein-protein bridges across the periplasm known as nuclear bridging complexes

mid-SUN proteins; a novel sub-family of nuclear envelope proteins

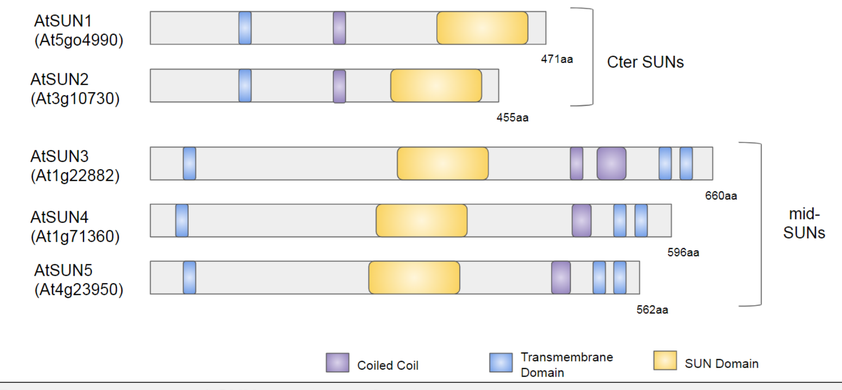


Figure 2. Domain architecture of SUN proteins in *Arabidopsis thaliana*, outlining how there are two different plant SUN subfamilies.

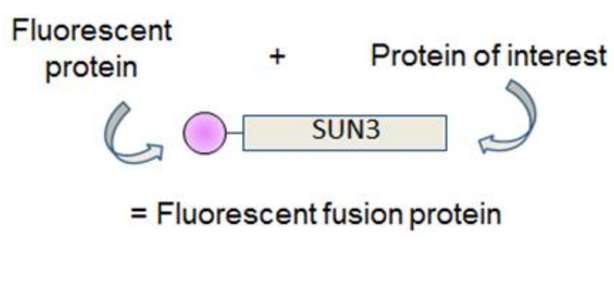
Arabidopsis thaliana has five SUN proteins but only two (AtSUN1 and AtSUN2) have a characteristic C-terminal SUN domain; the other three (AtSUNs3-5) possess a more centrally-located SUN domain[1,3]. These are mid-SUN proteins and are poorly characterised throughout all phylogenetic kingdoms.

Previous work has shown that mid-SUNs are localised to the NE and endoplasmic reticulum (ER), with the expectation that they are specifically located at the INM like their Cter-SUN counterparts; however, a potential role in the ER has yet to be explored.

Aims of the overall project:

- **To investigate protein-protein interactions using confocal imaging**
- **To confirm the exact location of the mid-SUN proteins at the NE**

Using confocal microscopy to localise the mid-SUN proteins *in planta*



Fluorescent fusion proteins are regularly used in cell biology to observe protein dynamics in live cells[5]. These are used to not only identify which compartments of the cell that they localise to, but to also provide quantitative data about protein activity. This includes identifying novel protein-protein interactions, and the protein domains that are required for them do so.

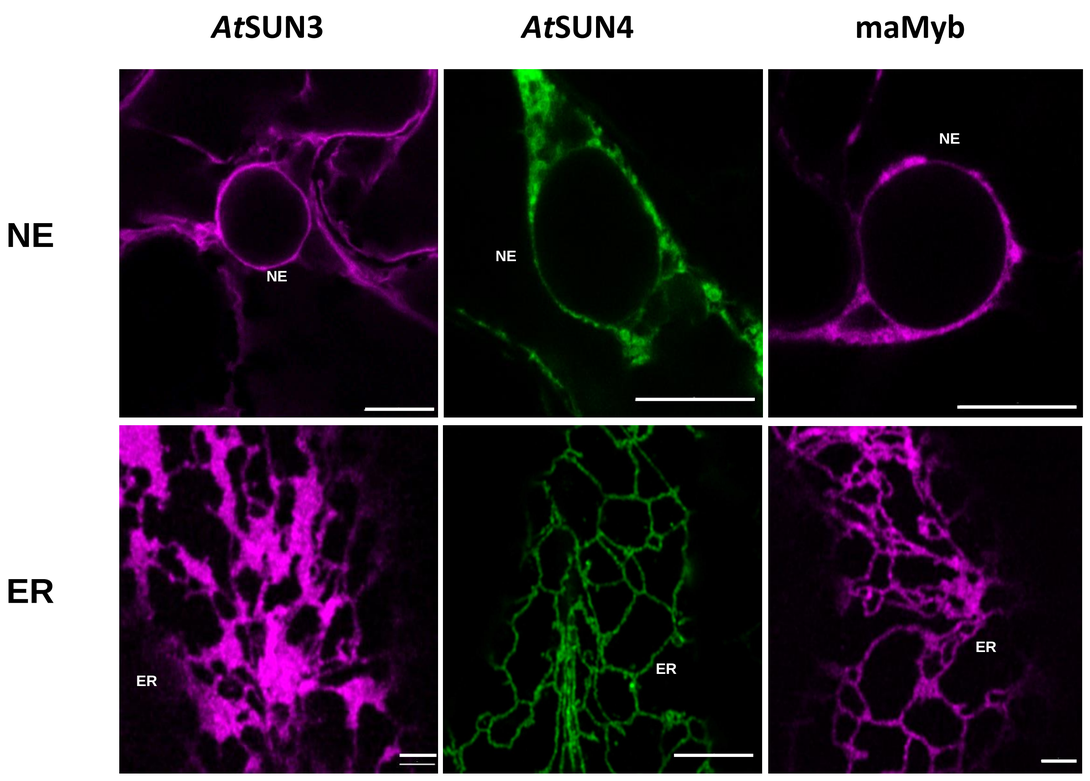
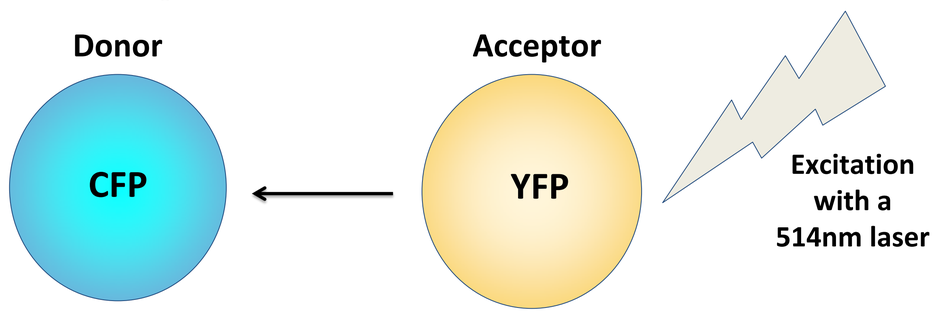


Figure 3. Sub-cellular localisation of Arabidopsis mid-SUN fluorescent fusion proteins when transiently expressed in leaf epidermal cells of *Nicotiana benthamiana*. *At*SUN3, *At*SUN4, and maMyb both localise to the NE and ER; Nucleus, scale bar 10µm; ER, scale bar 2µm.

maMyb is an ER-bound membrane protein[4] that has been shown in previous research to interact with mid-SUN proteins. These interactions, and the location of them, have yet to be confirmed *in planta*.

Investigating protein interactions between SUN3 and MaMyb

Protein-protein interactions were tested by using a quantitative imaging technique known as acceptor photobleaching fluorescence resonance energy transfer (apFRET), a proven method used to identify *in vivo* protein interactions in living cells[2].



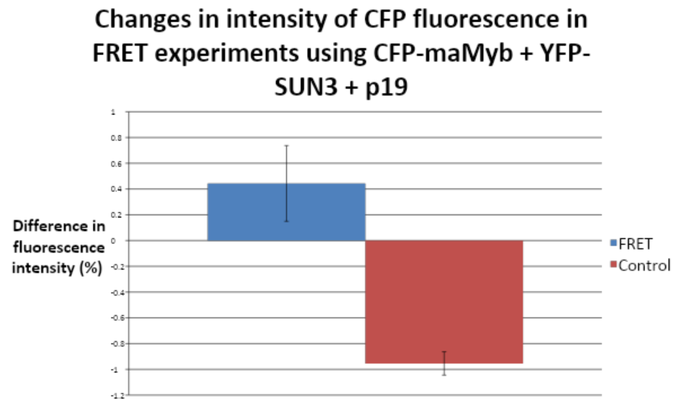


Figure 4. SUN3-maMyb interaction confirmed at the NE based on changes in CFP-fluorescence when conducting apFRET analysis on two constructs (E_F = 0.44 ± 0.29 SEM; n = 58; p = 2.3862E-05).

Table 1. FRET efficiency as detected by FRET demonstrates interaction between maMyb and SUN3. Positive interactions highlighted in green

	Construct Combinations	Experimental E_F	Control E_F	P-value (t-test)
Full-length mid-SUN and maMyb	CFP-maMyb + p19 + YFP-SUN3	**0.44±0.294**	-0.95±0.09	2.39 x10^{-5}
	maMyb-CFP + p19 + YFP-SUN3	**0.35±0.33**	-1.01±0.13	1.14 x10^{-3}
	YFP-maMyb + p19 + CFP-SUN4	-1.99±0.98	-0.71±0.3	0.22

Conclusions and Future Work: maMyb was found to interact only with SUN3 at the NE, suggesting a protein specific function and confirming what has been found in previous studies. Further work needs to be carried out to see which domain in SUN3 is required for this interaction. This work provides evidence that mid-SUNs are active at the plant NE and contribute to NE dynamics.

This work is being done in collaboration with the laboratory of Professor Christophe Tatout at the Université Clermont Auvergne.

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