Functional characterization of the Whirly protein family in Arabidopsis by using

„Separation-of-localization“ mutants

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Introduction: Whirly proteins are regulatory proteins localized to plant organelles.

Whirly proteins are a small family of nucleic-acid-binding proteins that were first described as nuclear transcription factors in potato. In *Arabidopsis thaliana* three Whirly proteins, At-Why1, At-Why2 and At-Why3, can be found, whereas most other species merely possess two Whirly proteins (Desveaux et al. 2005, Krause et al. 2005, 2009). Here we present results which suggest that At-Why1 is a regulator of senescence and that the impact that the protein has on the senescence process is dependent on it`s subcellular localization.

Despite their assigned function as nuclear regulators, Whirly proteins are predicted to be localized to the organelles.

workflow

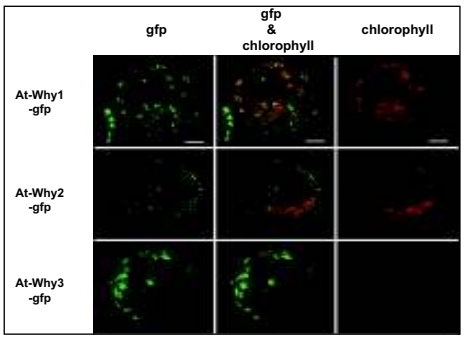
Whirly1 was shown to be translocated to chloroplasts, while Whirly2 was shown to be imported into mitochondria.

workflow

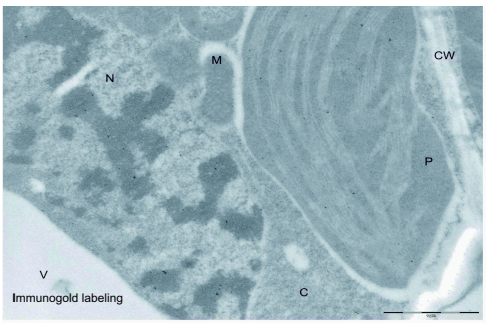
Whirly1 of barley was shown to be located in chloroplasts and the nucleus of the same cell.

program	At-Why1	At-Why2	At-Why3
iPSORT	chloro	mito	chloro
WoLFPSORT	chloro	nucleus	chloro
SubLoc	mito	mito	nucleus
TargetP	chloro	mito	chloro
ChloroP	chloro	chloro	chloro

Krause et al. 2005



Krause et al. 2005

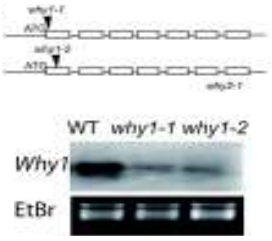


Grabowski et al. 2008

Results: „Separation-of-localization“ mutants display opposed senescence.

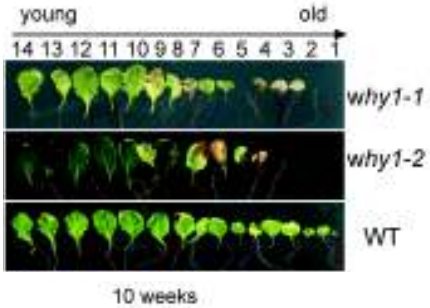
1.

Two T-DNA-insertion lines of *At-Why1* (*why1-1* and *why1-2*) show decreased amounts of *At-Why1* transcript in Northern Blot experiments.



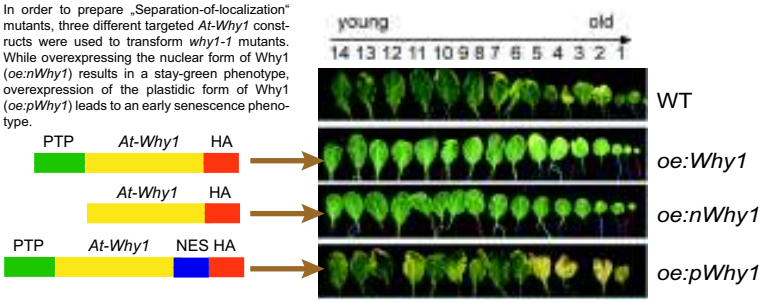
2.

TheT-DNA-insertion lines *why1-1* and *why1-2* display altered senescence compared to wildtype (WT). Rosette leaves were arranged according to their age.



3.

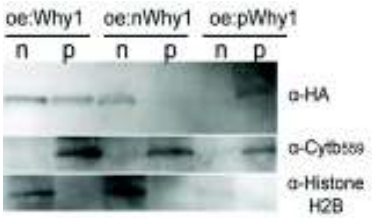
In order to prepare „Separation-of-localization“ mutants, three different targeted *At-Why1* constructs were used to transform *why1-1* mutants. While overexpressing the nuclear form of Why1 (*oe:nWhy1*) results in a stay-green phenotype, overexpression of the plastidic form of Why1 (*oe:pWhy1*) leads to an early senescence phenotype.



10-week-old plants

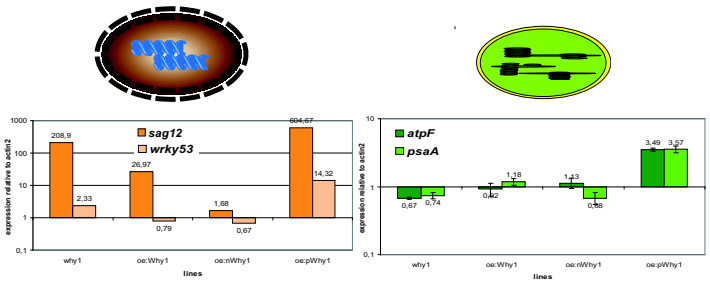
4

Subcellular localization of the overexpressed Whirly1 proteins was confirmed by Western Blot using an HA-antibody . Nuclei and plastids (n, p) were isolated from the three different mutant lines, purity of each fraction was shown by means of a Cytb559 antibody and a histone antibody, respectively



5

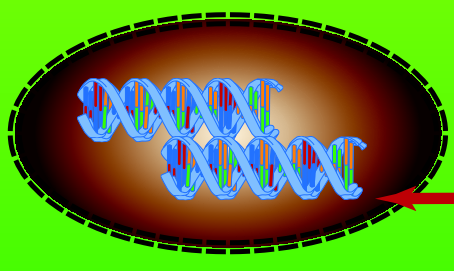
Expression of several nuclear and plastidic genes in 9-week-old rosettes of the T-DNA-insertion line *why1-1* and „Separation-of-localization“ mutants was compared to wild-type by quantitative Real-Time PCR.

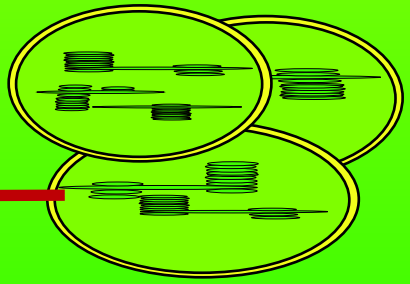


Discussion

Our data suggest that the ratio between the senescence repressor At-nWhirly1 and the senescence activator At-pWhirly1 is of pivotal importance for regulation of leaf senescence. Since overexpression of plastidic Whirly1 has not only effects on gene expression in plastids but also on nuclear transcripts, pWhirly1 has to be involved in plastidic control of senescene initiation by...

RETROGRADE SIGNALLING





Nuclear Whirly1 represses senescence!

Plastidic Whirly1 promotes senescence!

Outlook: What is the nature of retrograde signalling operated by pWhirly1?

Open question: What is the function of the third Whirly protein in Arabidopsis?

References: Desveaux et al. 2005, Trends Plant Sci 10: 95-102; Grabowski et al. 2008, Plant Physiol 147: 1800-1804; Krause et al. 2005, FEBS Lett 579: 3707-3712; Krause et al. 2009, Endocytobiosis Cell Res 19: 51-62