# Whirly proteins as communicators between plant organelles and the nucleus?

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## Abstract

Whirly proteins belong to a small family of proteins with a characteristic secondary structure and a conserved DNA binding domain that is found mainly in angiosperms. At least one member of the Whirly protein family, Whirly1, is dually targeted to the nucleus and to the chloroplasts and it was shown that apart from its initially described function as a transcriptional regulator of nuclear disease resistance genes, this protein comprises many more functions. It seems to fulfil roles in nuclear telomere homeostasis and possibly chloroplast rRNA metabolism as well as chloroplast intron splicing.

A homologous protein with a mitochondrial presequence, Whirly2, in contrast, is presumably involved in the replication of the mitochondrial genome and in mitochondrial gene expression. In addition, it seems to affect the expression of a small subset of nuclear genes. Both Whirly proteins show an antagonistic effect on leaf senescence. Although direct evidence for a nuclear localisation of Whirly2 has yet to be obtained, we hypothesise that all members of the Whirly family are intriguing candidates for organelle-to-nucleus crosstalk with an intricate interaction between each other.

## Introduction

In recent years, it has emerged that nuclear gene expression is controlled by regulators that temporarily also can be located elsewhere in the cell. Some of these regulators are membrane-bound transcription factors (MTF) sequestered at the cytoplasmic faces of the ER (SEO et al. 2008) and the organelle envelope (LAGRANGE et al. 2003; HAM et al. 2006). Other nuclear regulators have a second home within the plastids or mitochondria (KRAUSE and KRUPINSKA 2009). As these compartments contain their own DNA, their communication with the nucleus by retrograde signalling is critical for normal plant development. Although many compounds such as reactive oxygen species, chlorophyll biosynthesis intermediates and redox system components are discussed as retrograde signals (see for example the recent review by WOODSON and CHORY 2008), a direct contribution of proteins in transferring information from plastids or mitochondria to the nucleus has not been envisaged so far. The reason for this is most likely that only a small number of dually targeted proteins with a verified subcellular distribution in the nucleus and these organelles are known. Strikingly, however, all of them seem to play a role in gene expression or the maintenance of DNA (SMALL et al. 1998; KRAUSE and KRUPINSKA 2009). Recent computer-assisted analyses of the targeting prediction of all eukaryotic transcription factors in Arabidopsis and rice have indicated that the currently known few dually targeted nuclear/organellar proteins could be just the tip of the iceberg (WAGNER and PFANNSCHMIDT 2006; SCHWACKE et al. 2007).

The analysis of dual localisation and the unequivocal identification of dually targeted

**Table 1:** Physicochemical properties of Whirly proteins

| Protein | loc. pred. | # AA | identity with StWhy1 | identity with StWhy2 | MW in kDa | pI |
|---|---|---|---|---|---|---|
| StWhy1 | p | 274 (220) | --- | 32.5 % | 30.3 (24.6) | 9.7 (8.9) |
| VvWhy1 | p | 268 (210) | 67.2 % | 36.4 % | 30.2 (23.5) | 10.2 (9.1) |
| RcWhy1 | p | 271 (217) | 64.2 % | 38.1 % | 30.2 (24.2) | 10.0 (8.2) |
| AtWhy3 | p | 267 (192) | 60.0 % | 51.1 % | 29.6 (21.3) | 10.1 (9.1) |
| AtWhy1 | p | 263 (216) | 56.9 % | 36.4 % | 29.0 (24.0) | 9.9 (8.2) |
| SbWhy | p | 266 (230) | 54.4 % | 36.4% | 29.5 (25.6) | 10.0 (9.7) |
| **PtWhy3* | | *(187)* | *(54.4 %)* | *(30.7 %)* | *(20.8)* | *(8.2)* |
| OsWhy1 | p | 272 (222) | 54.0 % | 35.9 % | 30.0 (24.9) | 9.8 (9.5) |
| ZmWhy1 | p | 266 (229) | 52.2 % | 35.1 % | 29.4 (25.6) | 9.9 (9.7) |
| **PtWhy1* | | *(189)* | *(51.8 %)* | *(31.2%)* | *(21.0)* | *(8.2)* |
| AcWhy | p | 226 (191) | 51.5 % | 32.0 % | 25.2 (21.3) | 9.9 (9.8) |
| TaWhy1 | p | 248 (225) | 48.5 % | 33.8 % | 27.4 (24.9) | 9.7 (9.3) |
| **HvWhy1* | | *(194)* | *(45.6 %)* | *(32.9 %)* | *(21.9)* | *(7.3)* |
| StWhy2 | m | 231 (216) | 32.5 % | --- | 25.8 (24.0) | 10.7 (10.4) |
| AtWhy2 | m | 238 (209) | 37.2 % | 51.1 % | 26.3 (23.0) | 10.3 (10.1) |
| **PtWhy2* | | *(181)* | *(31.4 %)* | *(48.1 %)* | *(19.9)* | *(10.1)* |
| VvWhy2 | m | 230 (183) | 36.9 % | 47.6 % | 25.7 (20.8) | 9.9 (9.3) |
| OsWhy2 | m | 228 (181) | 35.0 % | 45.9 % | 25.2 (19.9) | 10.3 (10.0) |
| HvWhy2 | m | 233 (191) | 38.0 % | 44.6 % | 30.1 (20.9) | 10.4 (9.9) |
| ZmWhy2 | m | 230 (198) | 33.9 % | 43.7 % | 25.1 (21.4) | 10.0 (9.8) |
| TaWhy2 | m | 224 (184) | 33.6 % | 43.3 % | 24.4 (20.1) | 10.2 (9.9) |
| PpWhy | m | 257 (243) | 35.0 % | 31.6 % | 28.6 (27.1) | 9.3 (8.1) |
| CrWhy | p,m | 238 (203) | 27.0 % | 29.0 % | 24.9 (21.4) | 10.1 (9.7) |
| OtWhy | p,m | 240 (232) | 25.5 % | 25.5 % | 25.9 (25.1) | 10.8 (10.8) |

The sequences of Whirly proteins were extracted from the databases and pairwise sequence alignments with the potato proteins StWhy1 and StWhy2 were done using Needle (www.ebi.ac.uk/Tools/emboss/align/index.html). For each protein, sequence identity values were calculated respective to StWhy1 and StWhy2. Predictions of localisation, target peptide length, molecular weight (MW) and isoelectric point (pI) were done with TargetP and WinPep, respectively. Values in brackets refer to the mature, cleaved organellar protein. Asterisks (*) denote proteins where the N-terminus is not yet known. All proteins are listed in descending order of their identity relative to StWhy1 and StWhy2, respectively.
(Ac, *Allium cepa*; At, *Arabidopsis thaliana*; Cr, *Chlamydomonas rheinhardtii*; Hv, *Hordeum vulgare*; Os, *Oryza sativa*; Ot, *Ostreococcus taurii*; Pp, *Pinus pinea*; Pt, *Populus trichocarpa*; Rc, *Ricinus communis*; Sb, *Sorghum bicolor*; St, *Solanum tuberosum*; Ta, *Triticum aestivum*; Vv, *Vitis vinifera*; Zm, *Zea mays*)

proteins was prevented mainly by a biased or mono-targeted localisation of many GFP fusion proteins. As recently outlined in several comprehensive studies, the position of the fluorescent tag, upstream regulating sequences but also the tissue in which the protein is analysed, have an influence on the localisation, and often one of the two localisations is masked in a given experiment (REGEV-RUDZKI and PINES 2007; SCHMIDT VON

BRAUN et al. 2007; CARRIE et al. 2008a, 2008b).

One dually targeted protein that has been under intense investigations over the last ten years is Whirly1. As with other dually targeted proteins, this protein was initially described as an exclusively nucleus-located regulator of transcription (DESVEAUX et al. 2000, 2004). The predicted dual targeting of Whirly1 has been finally confirmed by *in vitro* import assays into isolated chloroplasts (KRAUSE et al. 2005) and by immunological identification of the native Whirly1 protein in the nucleus and the chloroplasts of the same cell (GRABOWSKI et al. 2008).

Among the nucleus-and-chloroplast dually targeted proteins, the Whirly1 protein occupies a special position as in no other example the functions of the respective protein have been analysed in so many different systems and in such detail. The aim of this review is to summarise the wealth of recent information gathered on the Whirly1 protein and its homologues. We further discuss that the dual targeting potential of these proteins has interesting implications for the communication between plastids, mitochondria and the nucleus.

## The Whirly protein family

Whirly1 belongs to a small family of DNA binding proteins found in many angiosperms. In most species, the family seems to consist of only two proteins (Whirly1, Whirly2) whereas *A. thaliana* and a few other species possess a third Whirly protein (Whirly3) (KRAUSE et al. 2005; DESVEAUX et al. 2005; Table 1). All Whirly proteins identified so far possess putative N-terminal target sequences that would direct them to either the plastids or the mitochondria (KRAUSE et al. 2005; SCHWACKE et al. 2007). While Whirly1 and Whirly3 proteins are predicted to be imported into chloroplasts, all Whirly2 proteins have a predicted mitochondrial presequence. In organellar import assays, these predictions were confirmed, but showed also that *Arabidopsis thaliana* Whirly2 can be imported likewise into chloroplasts as into mitochondria (KRAUSE et al. 2005).

All family members with a putative plastid target peptide (Whirly1, Whirly3) share also some typical sequence elements in the mature protein, making orthologues from different species more homologous to each other than paralogues from the same species (Table 1). Apart from these typical differences, all Whirly proteins are remarkably uniform with respect to their sizes and isoelectric points (Table 1).

## Evolution of the Whirly structure

As one of few plant transcription factors, the Whirly1 protein and recently also the Whirly2 protein were successfully subjected to crystal structure analysis (DESVEAUX et al. 2002; CAPPADOCIA et al. 2008). These analyses showed that the Whirly proteins form tetrameric complexes with melted double stranded DNA in such a way that each monomer binds to part of a symmetrical inverted repeat sequence of one DNA strand. The quaternary structure of these tetramers exhibits the typical whirligig appearance for which the Whirly proteins were eventually named (see Figure 1). The interaction between the individual monomers is mediated by a helix-loop-helix motif located at the C-terminus of the protein (DESVEAUX et al. 2002). The characteristic structure determining motif, however, is the so-called Whirly domain that consists of two times four ß-strands that are each followed by an α-helix (ß-ß-ß-ß-α-ß-ß-ß-ß-α) (Figure 2). The two sets of ß-strands form two antiparallel ß-sheets that are organized perpendicular to each other (Figure 1). On the ß1 and ß2-strands of the protomer, an amino acid motif consisting of six amino acids, Lys-Gly-Lys-Ala-Ala-Leu (KGKAAL) is found that is critical for the interaction of Whirly1 with the DNA (Figure 2) (DESVEAUX et al. 2002).

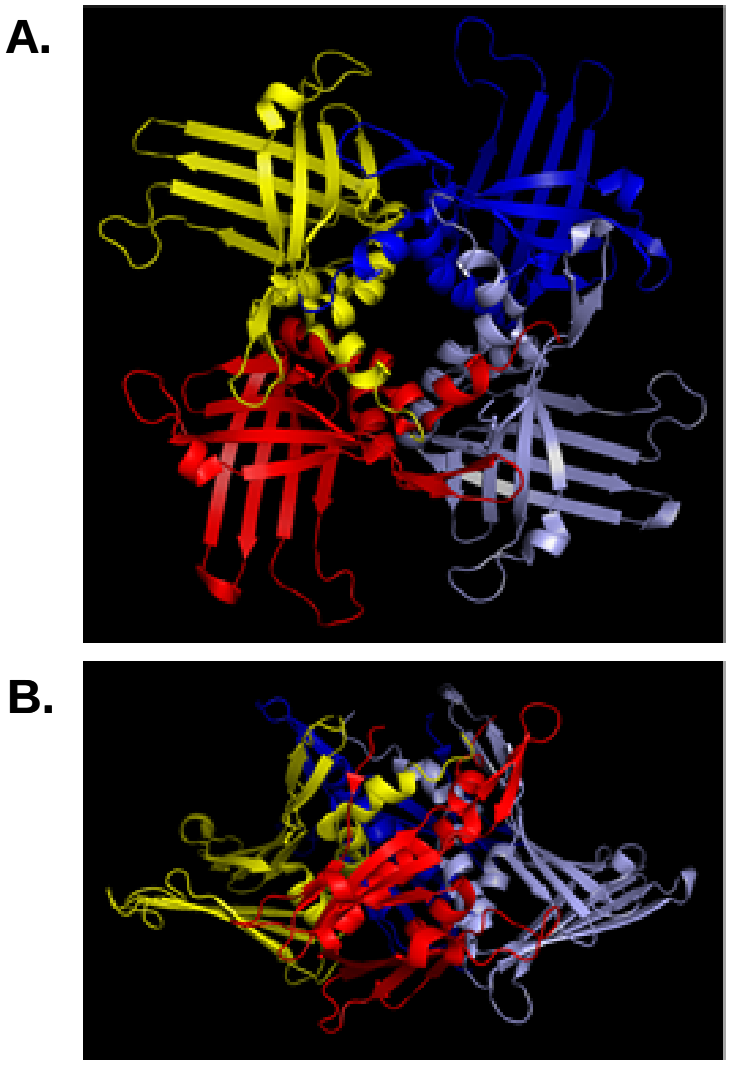


**Figure 1:** Comparative modelling of the tetrameric structure of AtWhy1. The three-dimensional model was built based on the structure of the homologue StWhy1 (DESVEAUX et al. 2002) and visualised using using 3D-JIGSAW (BATES et al. 2001) and PyMOL. (A) Top view showing the central pore and the outwards radiating ß-sheets. (B) Lateral view of the same molecule. The four protomers are shown in different colours.

Proteins with the KGKAAL motif were only found in angiosperms (KRAUSE et al. 2005; DESVEAUX et al. 2005) leading to the initial assumption that the Whirly proteins are specific for this plant lineage. However, with the annotation of several complete genome sequences from green algae such as *Chlamydomonas* (MERCHANT et al. 2007) and *Ostreococcus* (PALENIK et al. 2007), related putative proteins in both unicellular algae were discovered. Despite a low sequence homology, the typical structure of these proteins is conserved (Figure 3). Interestingly, only one single gene coding for a Whirly-like protein was found in the genomes of the green algal species. The gene product possesses putative target sequences for both, chloroplasts and mitochondria. In conjunction with this, there is no elevated sequence homology with either the plastid or the mitochondrial group of Whirly proteins (Table 1). One possible conclusion is that in green algae a single Whirly protein could serve both,

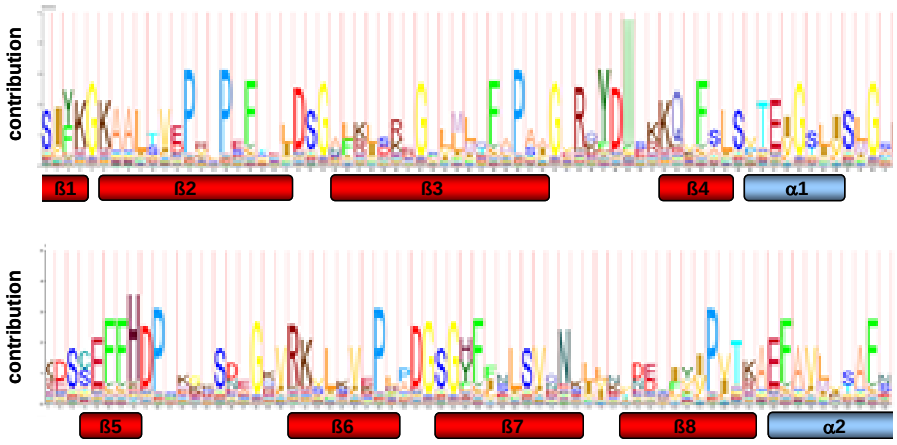


**Figure 2:** HMM logo of the Whirly domain. The mapping was obtained as described (SCHUSTER-BÖCKLER et al. 2004) by aligning the sequences of 21 known Whirly domains. Below the logo, the elements responsible for the secondary structure are indicated by red boxes (ß-sheets) and blue boxes (α-helices). The contribution of different amino acids at individual positions is indicated by the height of the amino acid symbol.

A.

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StWhy1     MSNFSLSPSPTSGFSLNLQNPTKTSYLSFSSSINTIFAPLSSNTTNSFSGLTHKAALPRN 60
StWhy2     ----------------------------MLKVSRLLHPRNQLLHKKLPGECVNGSIWQH 31
OtWhy      ------------MRAIGTTCAATARALRRASTSADVARADRARDRRPRPRDGDRRERHHR 48

StWhy1     LSLTCRHSDYFEPQQQQQQQQQQPQG-ASTPKVFVGYSIYKGKAALTVEPRSPEFSPLDS 119
StWhy2     AINTFAG-------FSTVRQNVVADG-KREGRVEAPYSVFKGKAALSAEPRLPTFNRLDS 83
OtWhy      WTSSTAYGASTPPPRSWDDGARRDDGRAPPPTTPVDFGVYKTRGPMKMKAVRPTLGAPGE 108

StWhy1     QAPKLSNEGMDMLQPAPAAGVNQDWSRAQVFSLPVTEIGSIIS-LQAKDSCFFHDFNK 178
StWhy2     GGVKLNNRGVIMLTPWPSVQEKKIDWEKRQLPALSATEVGSLIS-MPTRDSSFFHDFSM 142
OtWhy      NAVVR-PPGGMLLELANATAPPTTDWQNNGSPMLPGTPAAELADRMANGPCSFFHDSAK 167

StWhy1     GRSDEG-RVREVLKVEPLPDGSG-HPFMLSVQNKLINLDENIYIPVTKAEFAVLVSAFNF 236
StWhy2     LSSNAG-QVRKSLSIKPNADGSG-YPISLSVVN--NNLKPMIVLLFLSLLPNLLLCEQLS 198
OtWhy      ANGGGGGTLGPAFKVEPMPDGSGGGMPVMLTPTLSENRGQQRFSVPVSYGPSPAIRQLLVY 227

StWhy1     VMPYLLGWHTAVNSFKPEDASRSNNANPRSGAELEWNR 274
StWhy2     VLRCLTSWEWDRFTNRPSESISQSPSKVVPQLM----- 231
OtWhy      LVPRVLGFDEVFK------------------------- 240
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B.

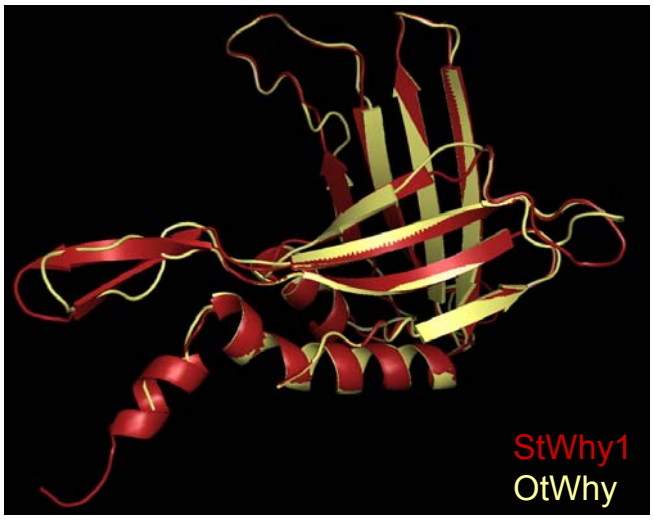


**Figure 3:** (A) Sequence alignment of Whirly1 and Whirly2 sequences from *Solanum tuberosum* (St) and the single Whirly protein from *Ostreococcus tauri* (Ot). Green shading depicts residues that are conserved in all three proteins (dark green) or only between the two potato proteins (light green) whereas residues conserved between OtWhy and StWhy1 are shaded red and residues conserved between OtWhy and StWhy2 are shaded yellow. (B) Structural modelling of the protomer of OtWhy (yellow) based on the structure of StWhy1 (red). The models were built using 3D-JIGSAW (BATES et al. 2001). PyMOL was used to visualise both structures and to generate the overlay.

plastids and mitochondria. Whether a triple targeting to all three DNA-containing compartments exists remains to be investigated. Alternatively, this single Whirly protein could be specific for only one organelle and the gene duplication that must have arisen in higher plants, could have led to the development of a second class of Whirly proteins with a localisation in the other organelle.

Whirly-like proteins have so far not been found in cyanobacteria or other prokaryotes. However, SCHUMACHER and coworkers detected a conspicuous structural similarity between Whirly proteins and two mitochondrial RNA binding proteins of *Trypanosoma brucei* when they managed to resolve the crystal structure of these proteins that form the Mrp1/Mrp2 guide RNA binding complex (SCHUMACHER et al. 2006). The Mrp1/Mrp2 complex is involved in mitochondrial kinetoplastid RNA editing. The striking structural similarity to the Whirly proteins was observed despite a very low degree of sequence conservation. Likewise as the Whirly proteins, MRP1/MRP2 exhibits a nucleic acid binding surface on the antiparallel ß-sheet, but in contrast to the Whirlies they bind to RNA and not to single stranded DNA. This is perhaps not surprising as the ssDNA-binding amino acid motif, KGKAAL, is not present in MRP1 or MRP2. It has been thus speculated that both protein classes have evolved separately to bind to different nucleic acids (SCHUMACHER et al. 2006).

## StWhy1, a transcriptional activator involved in pathogen response, was initially found only in the nucleus

The first described member of the Whirly protein family is the p24 protein of potato, also known as the DNA-binding component of the nuclear transcriptional activator PBF-2. PBF-2 was one of two complexes that were shown to mediate elicitor-induced gene expression of the pathogenesis-related nuclear gene *PR-10a* (DESPRÉS et al. 1995). The interaction of this complex with the *PR-10a* promoter was initially mapped to an area approximately 105 to 135 nucleotides upstream of the ATG start codon which was termed elicitor response element (ERE) by virtue of its importance for elicitor-induced gene expression (DESPRÉS et al. 1995). An inverted repeat (IR) sequence in the ERE motif as well as several nucleotides downstream of the second half of the IR seem to be the target of the Whirly1 protein of potato (DESVEAUX et al. 2000). Although no sequences outside the IR are specifically needed for binding, the interaction seems to be stabilized by a GC-rich environment (DESVEAUX Et al. 2000).

Transcriptional regulation of the *PR-10a* gene by the StWhy1 tetramer, alias PBF-2, is dependent on a protein kinase, implying that phosphorylation of the protein plays a role in the regulation of its activity (SUBRAMANIAM et al. 1997). It has been suggested that it is stored in the nucleus in an inactive, dephosphorylated form and becomes available for binding to the *PR-10a* promoter by phosphorylation (DESVEAUX et al. 2000).

## Whirly proteins are more versatile than originally believed and possess functions in the nucleus and the organelles

Subsequent work on a range of Whirly proteins brought evidence for additional functions in the nucleus on the one hand, and confirmed on the other hand that the Whirlies play a functional role in the plastids or mitochondria (Table 2). Like StWhy1, the *Arabidopsis* Whirly1 protein was also shown to be involved in pathogen resistance responses (DESVEAUX et al. 2004). Two tilling mutants of AtWhy1 with reduced DNA binding activity showed severe defects in salicylic-acid mediated defence reactions. Interestingly, the ERE binding motif, that was shown to be critical for StWhy1 binding in the potato *PR-10a* gene, is also enriched in the promoters of *Arabidopsis* genes that are co-regulated during systemic acquired resistance (SAR) (DESVEAUX et al. 2005). The

**Table 2:** Whirly protein functions

| Protein | Functions | References |
|---|---|---|
| StWhy1 | - transcriptional activator, involved in pathogen response; | DESPRES et al. 1995; SUBRAMANIAM et al. 1997; DESVEAUX et al. 2000; |
| StWhy2 | - mitochondrial ssDNA or RNA binding protein | VERMEL et al. 2002 |
| AtWhy1 | - transcriptional activator, involved in SA-mediated pathogen response; | DESVEAUX et al. 2004; |
| | - regulator of telomere homeostasis; | YOO et al. 2007; |
| | - repressor of WRKY 53 and AtWhy2; upstream regulator of plant senescence and cell death | MIAO et al., manuscript submitted |
| AtWhy2 | - mitochondrial biogenesis; affects DNA copy number, mitochondrial transcript levels and mitochondrial respiration; | MARECHAL et al. 2008; |
| | - involved in regulation of senescence and cell death | MIAO et al., manuscript submitted |
| ZmWhy1 | - plastid biogenensis; involved in ribosomal RNA metabolism; possibly involved in RNA splicing? | PRIKRYL et al. 2008 |
| HvWhy1 | - regulator of senescence | KRUPINSKA et al., manuscript submitted |

mode of interaction between DNA and the Whirly1 protein seems to be very similar, therefore.

AtWhy1 binds also to the single-stranded telomeric repeat TTTAGGG indicating a second, independent function of nucleus-located Whirly1 in telomere length homeostasis (YOO et al. 2007). AtWhy1 deficient plants were shown to contain increased lengths of telomere tracts over generations and these correlated with increased activities of telomerase. Plants overexpressing AtWhy1 showed the opposite effect, i.e. a decrease in telomerase activity resulting in shortened telomeres (YOO et al. 2007).

*Arabidopsis* mutants with a T-DNA insertion in the Whirly1 gene were also shown to have an accelerated senescence phenotype (MIAO et al., manuscript submitted). In the same study, Whirly1 was shown to act as a repressor of the *WRKY53* gene encoding a central transcriptional activator of senescence-associated genes in *A. thaliana* (MIAO et al. 2004, 2008) which explains why these mutants have a premature senescence phenotype. In addition, Whirly1 appeared to be a negative regulator of the gene encoding Whirly2 and a number of other genes involved in senescence or cell death (MIAO et al., manuscript submitted).

Independently, it has been also shown in barley that Whirly1 is a negative regulator of senescence (KRUPINSKA et al., manuscript submitted). The barley Whirly1 protein has been isolated by binding to an ERE containing motif in the promoter of the senescence associated gene *HvS40*. Binding was observed in non-senescent leaves but not in senescent leaves. In accordance with this observation, RNAi lines of barley resulting in a knockdown of Whirly1 show expression of the *HvS40* gene already in non-senescent leaves (KRUPINSKA et al., manuscript submitted).

In contrast to the reports on potato, *Arabidopsis* and barley, maize null mutants for the protein exhibited an ivory phenotype which was traced back to an impaired accumulation of plastid ribosomes (PRIKRYL et al. 2008).

ZmWhy1 was found to bind to both plastid DNA and RNA *in vitro*. While plastome-wide binding of ZmWhy1 to the plastid DNA was observed, there was a marked preference for some RNA sequences, including specifically introns of the *atpF* transcript or the *rps12* transcript (PRIKRYL et al. 2008). Binding to plastid DNA was also confirmed for AtWhy1 as this protein was among those identified in a fraction enriched for plastid transcriptionally active chromosomes (TAC) (PFALZ et al. 2006). PRIKRYL et al. (2008), however, argue against a function of Whirly1 in general plastid transcription as chloroplast run-on experiments with ZmWhy1 mutants showed no differences in transcription rates. Likewise, run-on transcription assays with chloroplasts derived from the T-DNA insertion mutants ΔAtWhy1 and ΔAtWhy2 did not show differences in plastid transcription patterns compared to the wildtype (KRUPINSKA and coworkers, unpublished results).

In contrast to the Whirly1 proteins, Whirly2 has so far been only found in the mitochondria. In 2002, an approach to identify mitochondrial RNA binding proteins in potato using ssDNA chromatography yielded StWhy2 among others (VERMEL et al. 2002). Recently, organellar immunoprecipitation confirmed an association of the AtWhy2 protein with the mitochondrial genome (MARÉCHAL et al. 2008). The association of AtWhy2 with the mtDNA seemed to occur along the entire genome, suggesting sequence-unspecific binding (MARÉCHAL et al. 2008) like it was also observed for the Whirly1 binding to plastid DNA (PRIKRYL et al. 2008). A role of AtWhy2 in mitochondrial gene expression has been proposed based on the fact that mitochondrial transcript levels are diminished in plants overexpressing this protein.

Interestingly, *AtWhy2* overexpression has, furthermore, been shown to induce early senescence and cell death by causing an imbalance in respiratory electron transport (MARÉCHAL et al. 2008). With respect to senescence, *Arabidopsis* Whirly1 and Whirly2, therefore, seem to have antagonistic roles.

**Whirly proteins might control the cell's master decision 'to die or not to die'**

Due to their unique subcellular distribution, Whirly proteins are ideal candidates for crosstalk between the two organelles on one hand and between the organelles and the nucleus on the other hand. As all three compartments share the genetic information of the plant cell, such communication is crucial to ensure normal plant development. It is well established that chloroplasts and mitochondria are interdependent at multiple levels (LEISTER 2005; PESARESI et al. 2007). Photosynthesis provides substrates for mitochondrial respiration but also depends on compounds synthesised by mitochondria (HOEFNAGEL et al. 1998; PADMASREE et al. 2002). Knowledge on organelle crosstalk is mainly based on mitochondrial mutations affecting photosynthetic performance and/or chloroplast development (NEWTON and COE 1986). Little is, however, known about the mechanisms of organelle communication and about the coordination of their activities (PESARESI et al. 2007).

Northern blot analyses and RLM-RACE assays did not reveal different Whirly transcripts (KRUPINSKA and coworkers, unpublished). In light of the similar sizes of nuclear and organellar isoforms of Whirly1, therefore, and of lacking evidence for alternative transcription start sites, translation start sites or processing, it is tempting to speculate that Whirly1 could be stored in the plastids and be released to the nucleus when the situation demands a nuclear location of the protein. It has already been demonstrated that organelle-based regulators can be attached to the surface of organelles as in case of a TFIIB-related protein (LAGRANGE et al. 2003) or in case of tobacco Tsip1 (HAM et al. 2006). Although no direct evidence is yet available for

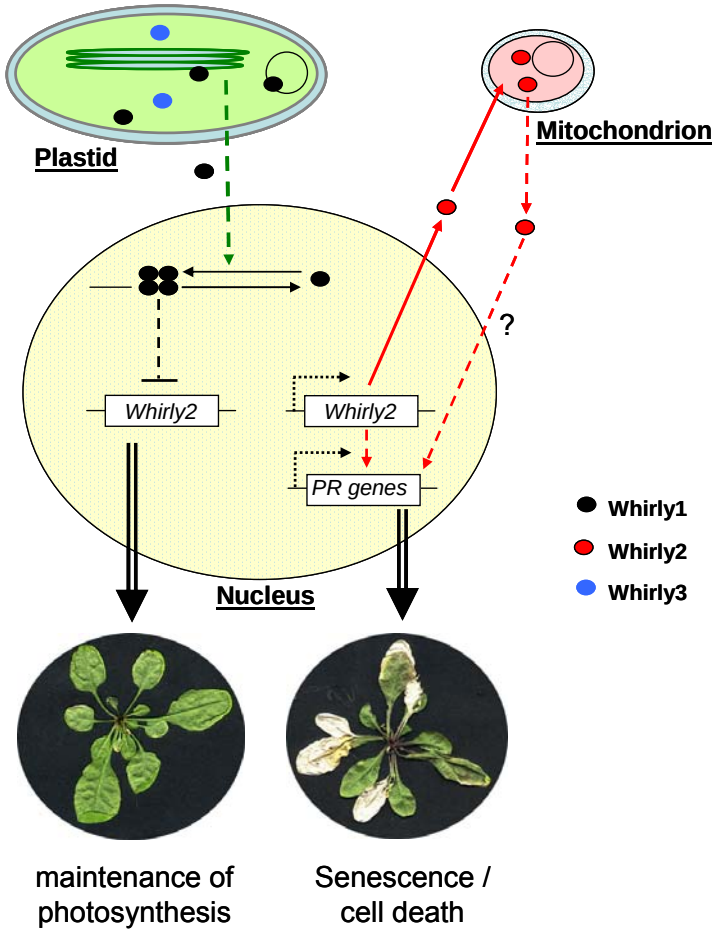


**Figure 4:** Hypothesis for the distribution of Whirly proteins and the relationship between Whirly1 and Whirly2. Whirly proteins are found in each of the three DNA containing compartments of the plant cell. In *Arabidopsis thaliana*, plastids contain Whirly1 (black) and Whirly3 (blue), mitochondria contain Whirly2 (red) and the nucleus contains at least Whirly1. While Whirly1 in plastids seems to be present as monomer, nuclear Whirly1 may form homooligomers (GRABOWSKI et al. 2008). Nuclear Whirly1 can act as a direct or indirect repressor on several nuclear genes, among them *Whirly2*, an effect that can be most probably attributed to the homotetramer. Reversible changes between the oligomeric and monomeric state of nucleus-located Whirly1 are proposed to be the key event in the repression of the *Whirly2* gene and regulation of the onset of senescence. These conformational changes may be controlled by the plastids and may involve phosporylation as described previously (SUBRAMANIAM et al. 1997). Derepressed *Whirly2* gene expression leads to the production of mitochondrial Whirly2 protein. Whirly2 itself seems to activate the expression of a small number of senescence- and cell-death-associated genes (MIAO et al., manuscript submitted) either by direct action of either the mitochondrial or the cytoplasmic form or indirectly through other proteins.

plants, it is possible that proteins sequestered inside the plastids or the mitochondria can be relocated to the nucleus upon reception of an appropriate stimulus (KRAUSE and KRUPINSKA 2009). Whether the export happens continuously and signals „well-being“ to the nucleus or whether the opposite is the case, is a matter that needs to be unravelled in the future.

One might further speculate that Whirly1 and Whirly2 function in parallel in retrograde control of the nucleus. However, results on the expression of the *Whirly* genes in *Arabidopsis* knockout mutants lacking either Whirly1 or Whirly2 (MIAO et al., manuscript submitted) argue against this assumption. While expression of the *Whirly1* gene is unaffected in the ΔWhirly2 mutant, expression of the *Whirly2* gene is enhanced in the ΔWhirly1 mutant. Overexpression of Whirly2 in *Arabidopsis* leads to premature senescence and cell death

(MARÉCHAL et al. 2008) while overexpression of Whirly1 results in a stay-green phenotype (MIAO et al., manuscript submitted). We conclude that at least in early developmental stages Whirly1 acts – directly or indirectly – as repressor of Whirly2 (Figure 4). This suggests that Whirly proteins have evolved in plants to enable a plastid dependent control of mitochondria- and nucleus-mediated cell death and senescence processes (Figure 4). Whether or not Whirly2 can be also relocated from the mitochondria to the nucleus and whether it can even form heterotetramers with Whirly1 to regulate a different set of genes remains to be shown in the future.

The mechanisms and regulation of putative redistribution of Whirly proteins in the plant cell as well the consequences of this redistribution will constitute an exciting field of research emerging from the data summarised here.

## Acknowledgements

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## Abbreviations

ERE – Elicitor response element; GFP – Green fluorescent protein; IR – Inverted repeat; ssDNA – single stranded DNA.

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