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AtFtsH4 perturbs the mitochondrial respiratory chain complexes and auxin homeostasis in *Arabidopsis*

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Mitochondrial AtFtsH4 protease is one of four inner membrane-bound FtsH proteases in *Arabidopsis*. We found that the loss of *AtFtsH4* regulates *Arabidopsis* development and architecture by mediating the peroxidase-dependent interplay between hydrogen peroxide (H_2O_2) and auxin homeostasis. These morphological changes were correlated with elevated levels of both hydrogen peroxide and peroxidases, which suggested that *ftsh4–4* plant was related to the oxidative stress, and that the architecture was caused by the auxin homeostasis perturbation. This view was supported by the expression levels of several auxin signaling genes and auxin binding and transport genes were decreased significantly in *ftsh4–4* plants. Taken together, our data published in the May issue of Molecular Plant suggests a link between the lack of AtFtsH4 protease, oxidative stress,s and auxin homeostasis to regulate plant growth and development. However, the detail molecular mechanisms of AtFtSH4 regulating oxidation stress and auxin homeostasis is unclear. Here, we present evidence that the high level accumulated of H_2O_2 in *ftsh4–4* may correlates with the decreased mitochondrial respiration genes. We also showed that the decreased auxin level and auxin transport may caused by the inhibition of mitochondrial respiratory chain complexes.

In *Arabidopsis*, there are 4 mitochondrial located FtsH proteases which belong to the ATP-dependent metalloprotease family protein. FtSH3 and FtSH10 are considered to be m-AAA proteases,[1] while FtSH4 and FtSH11 showed i-AAA protease characteristics.[2] The function of plant mitochondrial AAA protease is still very unclear at present but complementation experiments show that the important functions of m-AAA protease in fungi and plants are conserved.[3] With the exception of AtFtSH11, the *Arabidopsis* mitochondrial AAA proteases may be related to the plant oxidative phosphorylation system.[4] AtFtSH4 influences the vegetative growth of *Arabidopsis* late rosette leaves formation under short day conditions, depending on preventing the accumulation of oxidized proteins.[5] Numerous studies on the chloroplast FtSH protease have found that chloroplast FtSH proteases act mainly through ROS to control the leaf morphological changes.[6] There has, however, been little study into the function of plant mitochondrial FtSH protease. The integrity of the mitochondrial inner membrane depends on a number of membrane localized FtSH/AAA proteases, and these proteases can specifically degrade badly folded or unassembled inner membrane protein.

Using one new T-DNA null mutant we investigated the function of AtFtsH4 in peroxide hydrogen accumulation and plant development. The results indicated that the loss-function of AtFtSH4 significantly affects morphogenesis of *Arabidopsis*. The *ftsh4–4* mutation caused the decrease of free IAA concentration, the perturbation of auxin signaling, and the elevation of H_2O_2 and peroxidase levels. The dwarfism and increased in axillary branching of the *ftsh4–4* mutant could be reversed by expressing the *iaaM* gene or by knocking down the peroxidase genes *PRX34* and *PRX33*, both of which elevated auxin level in *ftsh4–4* mutant. Moreover, the microarray data showed that several auxin transport genes downregulated significantly in the *ftsh4–4* mutant. Collectively, our data indicate that the mitochondrial ATP-dependent protease, FtSH4, is a modulator between H_2O_2 and auxin homeostasis to regulate plant growth and development.

However, though the significant H_2O_2 accumulation and auxin homeostasis perturbation were caused by the loss-function of AtFtsH4, whether these results are relate to the mitochondrial dysfunction in *ftsh4–4* is not clear. It has been reported that the aging *ftsh4* under SD conditions accumulated high level of reactive oxygen species (ROS) and carbonylated proteins, and the aging *ftsh4* plants was suffered from oxidative stress.[5] In the mitochondrial matrix of phenotype displayed *ftsh4* plants, elevated electron-dense material, and aggregates of oxidized proteins were accumulated.[5] But why the lack of AtFtSH4 can cause the accumulation of ROS and oxidized proteins, and the

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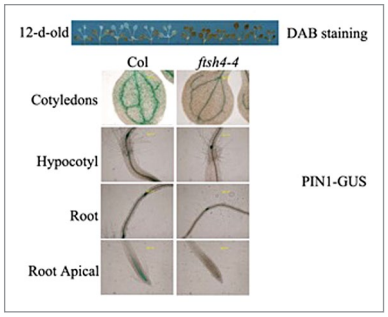

Figure 1. Soared hydrogen peroxide perturbs the auxin transportation in *ftsh4–4* mutant. GUS staining for 6 h.

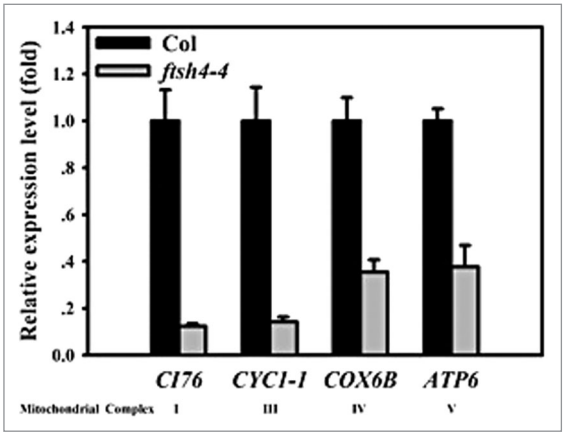

Figure 2. Mitochondrial respiratory chain complexes genes were downregulated in *ftsh4–4* mutant. *CI76*, At5g37510; *CYC1–1*, At3g27240; *COX6B*, At4g37830; *ATP6*, AtMG00410. 21-d-old seedlings were used for RNA extraction and gene expression detection.

relationship between AtFtSH4 and the mitochondrial oxidative phosphorylation, was not researched.

In this communication, we show additional data to support the function of AtFtsH4 in regulating the expressions of mitochondrial respiratory chain complexes genes and auxin transportation. First, the auxin transportation in the *ftsh4–4* mutant was tested to elucidate whether the auxin transportation is also disturbed by the accumulation. In the 12-d-old seedlings in long day conditions, the H_2O_2 accumulation was soared in the ftsh4–4 mutant tested by the DAB staining. As consequently, the expression of auxin efflux carrier PIN1 was perturbed in the *ftsh4–4* seedlings, including in the cotyledons, hypocotyls, root, and root apical (**Fig. 1**). These results further implied that the disturbed auxin signaling is relate to the H_2O_2 accumulation, and confirmed our results in the Molecular Plant paper. Currently, the generation of ROS is considered resulting from the respiratory activity of mitochondria, mainly from the Complex I, Complex III activities, and the respiratory chain complexes V which responsible for ATP generation through the process of oxidative phosphorylation (OxPhos).[7,8] The mitochondrial respiratory chain is consisted of 5 protein complexes named as Complex I (NADH dehydrogenase), Complex II (succinate dehydrogenase), Complex III (cytochrome *c* reductase), Complex IV (cytochrome *c* oxidase), and Complex V (ATP synthase).[9] We have detected a series number of these complexes genes, and found that *CI76* in the Complex I,[10] *CYC1–1* in the Complex III,[11] *COX6B* in the Complex IV,[12] and *ATP6* in the Complex V[11] were downregulated significantly by the knock out of *AtFtSH4* under the long day condition (**Fig. 2**). These results indicated that the mitochondrial respiratory chain complexes may be controlled by the AtFtSH4. In order to demonstrate whether the auxin homeostasis could be perturbed by the dysfunction of respiratory chain in *Arabidopsis*, the seedlings were treated by the inhibitors of mitochondrial respiratory chain complexes. After treated by the rotenone (inhibitor for Complex I), cyazofamid (inhibitor for Complex III), and NaN3 (inhibitor for Complex IV and V) synchronously, the expressions of auxin responsive marker gene DR5, auxin influx carrier AUX1, and auxin efflux carrier PIN1 were downregulated or inhibited significantly (**Fig. 3**). These results were identified that in the *ftsh4–4* mutant. And these results combined with the results that in **Figure 2** implied that AtFtSH4 affect the ROS accumulation may through control the integrity of mitochondrial respiratory chain complexes.

Taken together, our results elucidate the relationship between AtFtsH4 gene, oxidative stress, and auxin homeostasis. Our results indicate that the loss-function of AtFtsH4 has impaired the mitochondrial oxidative phosphorylation system. And this deficiency my caused by instability of respiratory complexes. The impaired oxidative phosphorylation system may cause a soared accumulation of ROS and oxidized proteins to perturb the function of mitochondrial. It is well known that ROS has the ability to impair the auxin signaling, and then caused the change of *Arabidopsis* architecture. Recent studies have shown that elevated ROS-induced oxidative stress results in a severe auxin deficiency phenotypes, referred to as stress-induced morphological responses, in *Arabidopsis* seedlings with perturbation in auxin levels, and/or distribution.[13-17] Redox signaling pathways play important roles in modulating the plant development to adapt to their growth environment.[18] Auxin homeostasis could be altered by H_2O_2 induced changes to *PINOID* gene expression, which affects polar auxin transport.[13] In addition to the influence on auxin homeostasis through the regulation of enzymes involved in auxin biosynthesis and conjugation,[19,20] oxidative degradation of auxin through H_2O_2-dependent peroxidases occurs as well.[19,21] However, the molecular basis of mitochondrial genes or proteins playing the ROS and auxin interaction need more evidence to elucidate. Diverse experiments performed on mutants deficient in mitochondrial i-AAA and m-AAA proteases indicate that these membrane embedded proteases are crucial components of the defense against mitochondrial oxidative protein damage at least in *Arabidopsis* and mammals. AtFtSH4 may degrade carbonylated membrane proteins and protect mitochondrial membrane proteins against oxidation, like the chloroplast FtsH proteases degrade oxidatively damaged proteins.[22] Furthermore, our results have proved that mitochondrial membrane are likely associated with instability of the mitochondrial

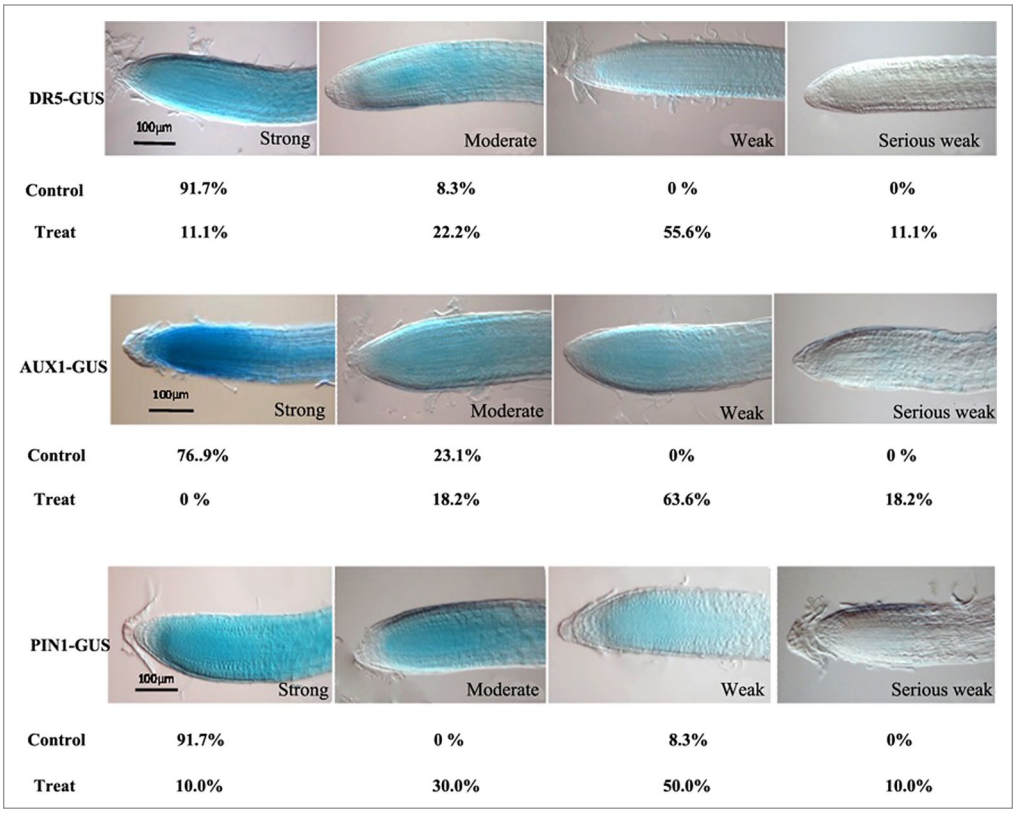

Figure 3. Dysfunction of mitochondrial respiratory chain complexes destroyed the auxin homeostasis. Control and Treat, seedlings were treated without or with 1.25 mM rotenone, 0.1 mM cyazofamid, and 1 mM NaN3, synchronously. All kinds of seeds germinated in the MS plates for 5 d, and then transferred to the plates with or without inhibitors for further more 3 d. After treatment, the seedlings were staining by GUS solution overnight.

respiratory chain complexes leading to their dysfunction which results in an increase in ROS production and progression of oxidative damage, and AtFtSH4 may play important roles in this biological process. However, the exact relations between mitochondrial respiratory chain complexes and AtFtSH4 and auxin homeostasis require further investigations.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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