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# Regulation of senescence under elevated atmospheric $CO_2$ via ubiquitin modification

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Elevated atmospheric $CO_2$ concentration is a serious global environmental problem. Elevated $CO_2$ affects plant growth by changing primary metabolism, closely related to carbon (C) and nitrogen (N) availability. Under sufficient N conditions, plant growth is dramatically promoted by elevated $CO_2$. When N availability is limited, however, elevated $CO_2$ disrupts the balance between cellular C and N (C/N). Disruption of the C/N balance is regarded as an important factor in plant growth defects. Here we highlight the regulation of senescence in higher plants by atmospheric $CO_2$ and N, and the physiological function of C/N-related ubiquitin ligase ATL31 under condition of elevated $CO_2$. We also provide an overview of the ubiquitin ligases and related enzymes involved in regulating senescence in plants.

## Plant Growth Regulation in Response to $CO_2$/N Balance

Nutrient availability, in particular the availability of carbon (C) and nitrogen (N), is important in the regulation of plant metabolism and development. In addition to their independent utilization, the ratio of C to N metabolites in the cell, referred to as the C/N balance, is also important for the regulation of plant growth.[1,2] In nature, the availability of C and N changes in response to environmental conditions, such as atmospheric $CO_2$, light availability, diurnal cycles, seasonal effects, rainfall and factors influencing microbial activity.[3,4,5,6] Since atmospheric $CO_2$ is the sole C source for plants in nature, the cellular C/N balance is affected by the balance between atmospheric $CO_2$ and the available N amount taken up from soil ($CO_2$/N). The increasing concentration of atmospheric $CO_2$, however, is a serious environmental problem worldwide, causing not only elevated temperatures but having major ecological consequences, such as changes in plant growth.[7,8,9] Increases in atmospheric $CO_2$ concentration are generally thought to promote plant growth, since higher amounts of $CO_2$ can provide more sugars to plants as energy sources. Under certain conditions, however, elevated $CO_2$ may reduce plant growth by altering the primary metabolism of plants.[10,11] This, in turn, can disrupt the cellular C/N balance, resulting in plant growth defects. Elevated atmosphere $CO_2$ concentration can cause excess carbohydrates to accumulate in plants,[12,13] altering nitrogen metabolism and partitioning in plants.[10,11] In addition, elevated $CO_2$ concentration can inhibit the assimilation of nitrate, the predominant N source in soils, causing N depletion and inhibiting plant growth.[11,14] Thus, elevations in atmospheric $CO_2$ concentration alter the C/N balance, increasing C and reducing N, repressing the expression of photosynthesis-related genes and promoting the expression of stress-responsive genes. These alterations also affect root architecture and biomass allocation between roots and shoots. Conditions of elevated $CO_2$ and limited N have been reported to promote lateral root formation, a process mediated by sugars and the phytohormone auxin signaling pathway.[15]

Our recent study demonstrated that conditions of elevated $CO_2$ combined with limited N promote the progression of plant senescence, such as leaf yellowing and anthocyanin accumulation.[16] Under these conditions, the expression of the senescence regulator *WRKY53* and genes that respond to N-starvation, including cytosolic glutamine synthase (*GS1.4*) and high affinity nitrate transporter (*NRT2.4*),[17,18,19] were upregulated compared with their levels in plants grown under normal $CO_2$/N conditions. The senescence phenotype and the upregulation of senescence-related genes were not observed in plants grown at elevated $CO_2$/normal N or normal $CO_2$/limited N conditions, indicating that the senescence phenotype was not due to either $CO_2$ or N level alone, but was dependent upon the $CO_2$/N balance.[16] According to previous studies, the amounts of several sugars were found to increase, while the amounts of nitrogen compounds decreased, in senescent leaves.[20,21,22,23] Taken together, these results indicated that elevated $CO_2$ and limited N availability mutually affect each other, markedly disrupting the cellular C/N balance and leading to the promotion of leaf senescence in mature plants.

## Ubiquitin Ligase ATL31 Regulates Senescence When the $CO_2$/N is Disrupted

We previously identified ATL31 as a novel C/N regulator in *Arabidopsis*. In the post-germination stage, *ATL31*-overexpressing plants (*ATL31 OX*) were insensitive to the high C/low N stress medium containing excess sugar and limited

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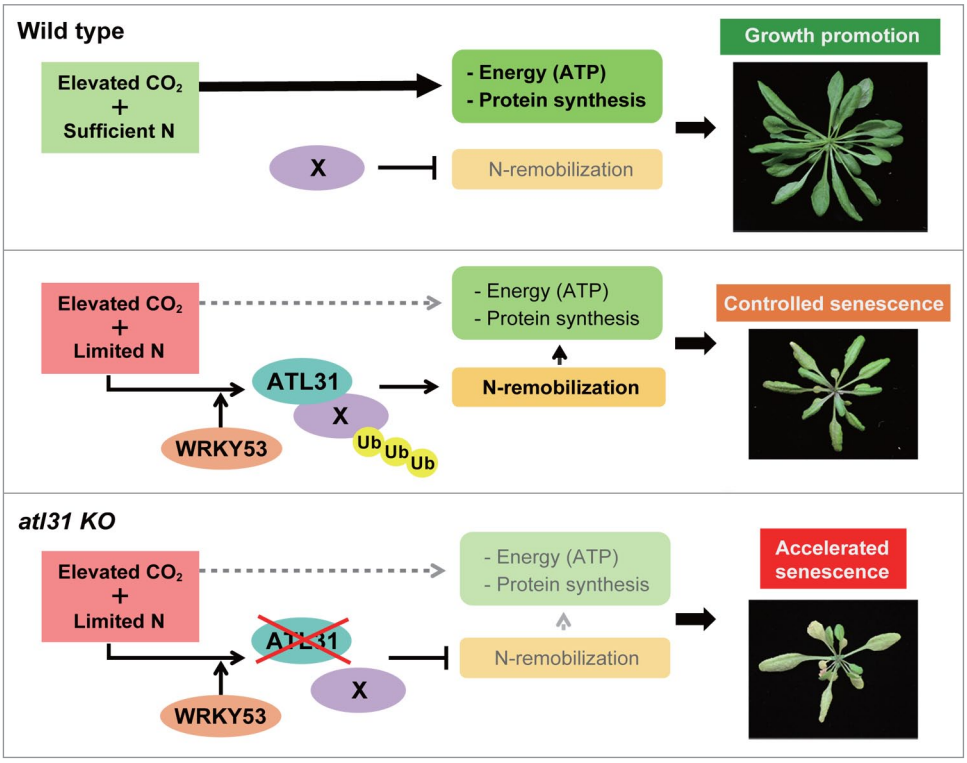


**Figure 1.** Proposed model of plant growth regulation by elevated $CO_2$ concentration and the role of the ubiquitin ligase ATL31. (Upper panel) When both C and N sources are abundant, organic compounds such as ATP production and protein synthesis are upregulated, promoting vegetative growth and increasing plant biomass. (Middle panel) When N supplies are limited, the C/N balance in plants is disrupted, resulting in high C and low N in plant cells. Under these conditions, plants remobilize cellular organic compound and control the progression of senescence, enabling successful reproductive growth of wild-type *Arabidopsis* plants. ATL31 is transcriptionally promoted by the transcriptional factor WRKY53 under conditions of elevated $CO_2$ and limited N. This process mediates the control of N remobilization sources via the ubiquitination and degradation of target protein (X) in order to control the progression of senescence. (Lower panel) Plants with an *atl31* loss of function mutation (*atl31 KO*) are unable to adapt to disrupted cellular C/N when $CO_2$ is elevated and N is limited, resulting in accelerated senescence.

nitrogen (300 mM glucose/0.1 mM nitrogen), whereas an *atl31* loss-of-function mutant (*atl31 KO*) showed a hypersensitive phenotype.[24] ATL31 is a member of the plant-specific ubiquitin ligase ATL family, which is comprised of proteins that contain a transmembrane-like hydrophobic region at the N-terminus, a region rich in basic amino acids, a RING-H2 type zinc finger domain and a non-conserved C-terminal region.[25,26] Subsequent analysis revealed that ATL31 targets 14-3-3 proteins for ubiquitination and degradation, which regulates the post-germinative growth of *Arabidopsis* plant in response to C/N status.[27] In evaluating the physiological function of ATL31 under different atmospheric $CO_2$ and N conditions, we found that the promotion of senescence under conditions of elevated $CO_2$ and limited N was suppressed in *ATL31 OX* but enhanced in *atl31 KO* plants.[16] Moreover, under these conditions, the expression of *ATL31* was transcriptionally upregulated in senescent leaves. Interestingly, the promoter region of the *ATL31* gene contains several putative W-box sequences, a DNA motif directly recognized by WRKY transcription factors.[19] Public microarray database analysis also indicated that the expression of *ATL31* highly correlated with that of *WRKY53*, suggesting a close relationship between ATL31 and WRKY53 in plants. Protoplast reporter analysis confirmed that WRKY53 could directly activate *ATL31* transcription and that *ATL31* mRNA expression was promoted in *Arabidopsis* plants that overexpress *WRKY53*.[16] These results indicated that ATL31 functions in senescent leaves, that ATL31 negatively regulates senescence progression in response to $CO_2$ and N availability, and that ATL31 is under the control of WRKY53 by regulating C/N signaling and/or metabolism in plants (**Fig. 1**).

## Senescence is Regulated by Post-Translational Ubiquitin Modification

In addition to ATL31, several ubiquitin ligases and enzymes related to ubiquitin modification have been reported to function in regulating senescence in *Arabidopsis* (**Table 1**). A member of the HECT ubiquitin-protein ligase (UPL) family, UPL5, was identified as a ubiquitin ligase targeting the senescence regulator WRKY53 in *Arabidopsis*.[28] WRKY53 is a transcription factor that promotes the expression of several senescence-associated genes (SAGs) and other transcription factors.[19] UPL5 interacts with WRKY53 in cytoplasm and was able to ubiquitinate WRKY53 in vitro. *UPL5* overexpression decreased the amounts of WRKY53, whereas a loss-of-function mutant of *UPL5* promoted senescence, in a manner similar to that of a

**Table 1.** Ubiquitin ligases and related enzymes involved in senescence regulation

| Protein name | Type | Function | Substrate | Ref |
|---|---|---|---|---|
| UPL5 | HECT E3 | Repression of SAGs transcription | WRKY53 | 28 |
| SAUL1 | U-box E3 | Inhibition of ABA biosynthesis | AAO3 | 29 |
| NLA | RING E3 | Adaptation to low N and Pi homeostasis | PHT1s | 32, 35 |
| ATL31 | RING E3 | Adaptation to disrupted $CO_2$/N | 14-3-3/? | 16, 27 |
| PHO2/UBC24 | E2 | Pi homeostasis | PHT1s | 33 |
| AMSH1 | MPN+ DUB | Membrane trafficking and autophagic degradation | PIN2* | 37 |

*AMSH1 is thought to target multiple cargo proteins on the endosome, including the autophagosome

*WRKY53* overexpressor. Taken together, these findings indicated that UPL5 negatively regulates senescence progression via WRKY53 degradation. Although the detailed regulatory mechanisms underlying these protein-protein interactions remain unclear, the expression of UPL5 and WRKY53 mRNAs were regulated reciprocally by hydrogen peroxide, jasmonic acid and plant development, suggesting that WRKY53 activity is regulated at the transcriptional and post-transcriptional levels.[28]

SENESCENCE-ASSOCIATED UBIQUITIN LIGASE (SAUL1) is a U-box type ubiquitin ligase that prevents premature senescence in *Arabidopsis*.[29] SAUL1 was identified as one of the U-box proteins transcriptionally regulated by ABA treatment. A *SAUL1* loss of function mutant showed an earlier senescence phenotype than wild-type, with the levels of ABA increased in the mutant. Assessments of the stability of ABA biosynthesizing enzymes showed that SAUL1 directly regulates AAO3 degradation in plants.[29] The *AAO3* gene encodes the aldehyde oxidase isoform that catalyzes the last step in ABA biosynthesis, suggesting that SAUL1 functions in senescence via the ABA pathway.

Ubiquitin ligases responsive to nitrogen nutrient conditions have also been reported to affect senescence progression in *Arabidopsis*, a process designated NITROGEN LIMITATION ADAPTAION (NLA).[30] An *nla* mutant showed a delay in senescence phenotype under limited nitrogen conditions, affecting the accumulation of inorganic phosphate (Pi) in plants.[31] NLA is a RING-type ubiquitin ligase carrying an SPX domain.[30] Recently, the NLA was shown to interact directly with a member of the phosphate transporter (PHT1) family at plasma membrane and to ubiquitinate this protein.[32] The amounts of PHT1 protein were increased in *nla* mutants, resulting in the overaccumulation of Pi. In addition, the ubiquitination of PHT1s by NLA was reported to triggers clathrin-dependent endocytosis followed by endosomal sorting to vacuoles.[32] The E2 enzyme UBC24, designated as phosphate 2 (PHO2) was also reported to localize to the plasma membrane and to regulate the stability of PHT1s.[33] Intriguingly, NLA and PHO2 are the targets of the microRNAs miR827 and miR399, respectively, both of which are induced by Pi starvation, suggesting that feedback regulation of the amounts of PHT1 are mediated by NLA and PHO2 activity in response to Pi availability.[34] In addition, NLA was shown to regulate the stability of PT2, a member of the PHT1 family, by proteasomal degradation following poly-ubiquitination with PHO2.[35] Further studies are expected to reveal the complex mechanisms by which ubiquitin-mediated transporter regulation modulates Pi homeostasis and senescence.

In addition to attachment of ubiquitin molecule, a recent study found that deubiquitinating enzymes (DUB) also play important roles in regulating senescence. The ASSOCIATED MOLECULE WITH THE SH3 DOMAIN OF STAM (AMSH) proteins are DUBs widely conserved in eukaryotes and are involved in intracellular trafficking through the deubiquitination of Lys-63 type ubiquitinated targets on the endosome, such as receptors and transporters.[36] The *Arabidopsis* AMSH1 protein was found to directly interact with the endosomal complex required for transport-III (ESCRT-III), which is part of the endocytosis machinery, and to mediate autophagic degradation in the dark.[37] An *AMSH1* loss of function mutant exhibited an early senescence phenotype under dark conditions due to a deficiency in autophagy. The ubiquitin ligase ATL31 localized to the plasma membrane and the endosome is also involved in intracellular trafficking via its interaction with the SNARE protein, SYP121, in response to pathogen attack.[38] These findings suggest that the function of ATL31 in endosomal degradation, including in autophagy, be examined under C/N nutrient stress conditions.

## Perspectives

Our recent study showed that the ubiquitin ligase ATL31 functions in the progression of leaf senescence in response to $CO_2$ and N availability. Although we previously showed that the 14-3-3 proteins were targets of ATL31 ubiquitination during the post-germination growth stage, the target proteins have not yet been determined in senescence regulation. It is important to clarify whether or not the 14-3-3 proteins are related to senescence regulation, as well as to identify the detailed upstream signaling cascade that modulates ATL31 activity under disrupted $CO_2$/N conditions.

A recent study demonstrated that a sugar metabolite, trehalose 6-phosphate (T6P), is an essential signaling molecule for the initiation of senescence in plants grown in high sugar medium.[22] T6P has also been reported to be a regulatory molecule that functions during flowering transition in *Arabidopsis*.[39] Future studies are required to clarify the role of T6P in the C/N signaling cascade.

At present, atmospheric $CO_2$ concentrations are increasing steadily while soil N availability to plants is often limited in

nature. Thus, the combination of elevated $CO_2$ and limited N for plants is a serious global problem, affecting crop production and forest maintenance. Clarification of the physiological effects and the detailed molecular mechanism of C/N response would be important for adaptation to the consequences of future increases of atmospheric $CO_2$ concentrations.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.


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