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Article Addendum

Membrane-Mediated Salt Stress Signaling in Flowering Time Control

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Addendum to:

A Membrane-Associated NAC Transcription Factor Regulates Salt-Responsive Flowering via FLOWERING LOCUS T in Arabidopsis

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ABSTRACT

More than 10% of the plant-specific NAC (NAM, ATAF1/2, CUC2) transcription factors have been predicted to have alpha-helical transmembrane (TM) domain in their C-terminal regions, among which at least three members have been proven to be membrane-associated and play a role in cell cycle control and stress responses. These observations suggest that membrane-mediated regulation would be an important molecular mechanism mediating rapid transcriptional responses to internal and external stimuli in plants. Recently, we showed that a salt-responsive NTL (NTM1-Like's) transcription factor NTL8 is localized primarily in plasma membranes as dormant form and subsequently processed into transcriptionally active, nuclear form. Overexpression of an active NTL8 form exhibited delayed flowering as well as reduced growth with small curled leaves. Consistent with this, expression of *FLOWERING LOCUS T* (*FT*) and its downstream genes was significantly reduced in the transgenic plants. Furthermore, *FT* was notably repressed by high salt. These results indicate that NTL8 mediates salt-responsive flowering via *FT* in Arabidopsis and that membrane-mediated transcription regulation underlies the salt signaling in mediating flowering initiation.

In a fairy story, a sleeping princess was awakened by the kiss from a prince. Recent studies demonstrate that a similar episode is occurring in the cell. A group of transcription factors is tethered to cellular membranes as inactive forms (sleeping). When it is kissed by the ubiquitin/proteasome machinery (RUP) or by membrane-associated proteases (RIP), an active form is released from the membranes and translocated into the nucleus, where it turns on target genes.[1] This activation mechanism guarantees rapid transcriptional response to internal and environmental changes in yeast, animals and plants.[2-4] Recent studies have revealed that a considerable portion of the NAC transcription factors are associated with the intracellular membranes.[5,6] These NAC members have been collectively termed NTLs for NTM1-Like's.[7]

NTL8, one of the NTLs, is consisted of 335 amino acids with a predicted TM domain in its far C-terminal region.[6,8] To confirm the membrane localization of NTL8, a full size NTL8 (8F) and a truncated NTL8 (8ΔC) lacking the TM domain was translationally fused with GFP, and the fusion constructs were transiently expressed in onion epidermal cells. Whereas the 8F signal was predominantly detected in association with the plasma membranes, the 8ΔC signal was exclusively located in the nucleus, confirming the membrane association of NTL8.

The 8F-overproducing transgenic plants (*35S::8F*) exhibited a moderate degree of growth reduction but with apparently normal leaf morphology. In contrast, those transformed with the *8ΔC* construct (*35S::8ΔC*) exhibited two distinct phenotypes with severe morphological and developmental alterations. One line (*35S::8ΔC-1*) was late flowering with normal leaf morphology and the other line (*35S::8ΔC-2*) exhibited severe phenotypic changes, including reduced growth with small, curled leaves, in addition to late flowering, which looks like a plant grown under stress conditions. These observations demonstrate that membrane release is essential for NTL8 function, as has been proven with NTM1.[5] It is also envisaged that *NTL8* might be related to plant stress responses. Meanwhile, a knockout *ntl8-1* mutant did not exhibit any discernible phenotypic changes, although it was slightly different from wild type plants in lateral root growth and flowering time. This may be due to a functional redundancy among the NTL members. Although primary root growth was normal in the transgenic and mutant plants, lateral root growth was significantly accelerated in the *35S::8ΔC* transgenic plants but discernibly reduced in the *ntl8-1* mutant. It is well known that lateral root growth is greatly affected by abiotic stresses, such as high salinity and drought.[9] The *NTL8* expression is markedly

influenced by salt. The previous and our observations suggest that *NTL8* may also be involved in root development in response to salt stress.

One distinct phenotype of the *35S::8ΔC* transgenic plants is late flowering. Plants decide very carefully when to initiate flowering because it is essential for reproductive propagation. Therefore, plants constantly monitor internal and external environment, such as daylength, temperature, salinity, moisture, and so on.[10-12] Since the *35S::8ΔC* transgenic plants exhibit delayed flowering we examined the expression patterns of various flowering time genes in the transgenic plants. The expression of *FT* and of genes that act later in the flowering process, such as *AP1*, *CAL*, *FUL* and *LEY*, was greatly reduced in the transgenic plants, indicating that *NTL8* regulates flowering initiation by modulating *FT* expression.

The expression of *NTL8* is significantly induced by high salt, and the *35S::8ΔC* transgenic plants is late flowering due to the repression of *FT*. Furthermore, it is known that high salt delays flowering in Arabidopsis, although the underlying molecular mechanisms are unknown.[13] Therefore, an interesting question was whether *FT* is influenced by high salt or not.

A previous study has shown that *FT* is not affected by salt stress.[13] However, it was still possible that daily rhythm or amplitude of the *FT* expression would be altered in the presence of high salt. To examine this possibility, wild type plants were treated with 100 mM NaCl and *FT* expression was measured during the time course of 24 hours under long day condition. Surprisingly, the *FT* transcript level was significantly reduced in plants treated with NaCl, especially during the period of 12–20 hours after dawn, when the *FT* transcript level is most high when grown under normal conditions.[14] These results strongly support that high salt delays flowering by repressing *FT*. This view is also consistent with the salt responsiveness of *NTL8* and the delayed flowering of the *35S::8ΔC* transgenic plants in which *FT* is markedly repressed. Altogether, our observations suggest that controlled processing of the membrane-associated NTL8 transcription factor is important for its function and mediates salt stress responses in flowering time control via *FT* in Arabidopsis.

Although the expression of *NTL8* is induced by high salt, there is no experimental evidence for the effects of high salt on the NTL8 protein processing. One possibility is that molecular components (or certain proteases) mediating NTL8 protein processing would exist only during specific developmental stages or in specific plant tissues. Actually, induction of *NTL8* by high salt was higher in the roots rather than in the aerial plant parts. In addition, when a *NTL8* promoter-GUS fusion was examined in transgenic plants, the GUS activity was primarily distributed in the vascular tissues of inflorescence stems and roots. It is well known that membrane-associated transcription factors are activated either by RUP or RIP.[1] Our result showed that the stability of 8F and 8ΔC proteins was controlled by the ubiquitin/proteasome-mediated processing, but NTL8 protein processing might be regulated by unidentified intramembrane protease(s), like NTM1. We are currently under way to identify NTL8 processing protease(s).

Another interesting observation is that NTL8 may regulate salt stress signaling during seed germination. We recently found that the level of *NTL8* transcript is extremely high in imbibed seeds. Seed germination is delayed in the presence of high salt. However, the *ntl8-1* seeds were insensitive to high salt (unpublished). It will be interesting to investigate how the two developmental processes, flowering initiation and seed germination, are interrelated. It is possible that NTL8 may mediate the signaling crosstalks between these two processes (Fig. 1).

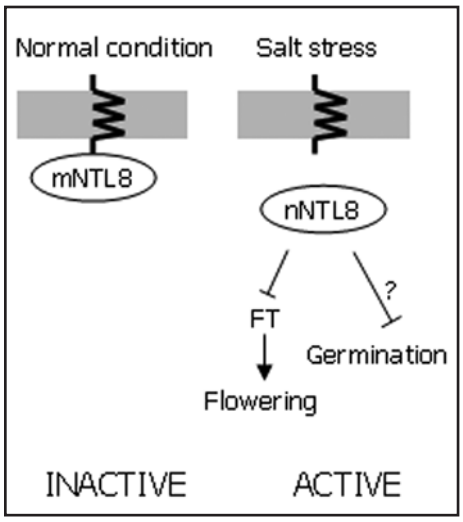

Figure 1. A proposed working scheme for NTL8 function in flowering time control and seed germination. NTL8 is liberated from the membranes triggered by an unidentified salt-induced molecular mechanism and enters the nucleus. In the nucleus, it represses *FT* expression, resulting in late flowering. NTL8 may also mediate seed germination through a molecular signaling.

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