The transcription factor MtSERF1 may function as a nexus between stress and development in somatic embryogenesis in Medicago truncatula

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In Medicago truncatula high rates of somatic embryo formation can be induced in the Jemalong genotype 2HA by application of the hormones auxin and cytokinin. Biosynthesis of the stress-related hormone ethylene is also necessary for somatic embryogenesis (SE) and is most likely a response to wounding and the presence of auxin in the medium. We have demonstrated that expression of a gene designated Mt SOMATIC EMBRYO RELATED FACTOR 1 (MtSERF1) induced by ethylene, in the presence of auxin plus cytokinin, is essential for SE. The promoter region of this transcription factor, a member of the ERF sub-family of the AP2/ERF super family, contains putative binding sites relating to auxin and cytokinin in addition to ethylene. An additional finding was the presence of WUSCHEL (WUS) binding sites in the MtSERF1 promoter region, which is discussed. Here we also discuss the Medicago data in the context of embryogenesis studies in Arabidopsis and suggest that MtSERF1 has a key developmental role, possibly in conjunction with WUS, in regulating downstream genes required for the initiation of SE.

Attempts to decipher the mechanisms underlying the induction of somatic embryogenesis (SE) have resulted in identification of several genes and transcription factors whose overexpression has been shown to effectively enhance the regenerative competence of plant cells for somatic embryogenesis, including SERK1, LEC1, LEC2, BBM, AGL15 and WUSCHEL. Nonetheless, the precise molecular mechanisms involved in the induction of SE from cultured tissue are not well understood.

In Medicago truncatula high rates of somatic embryo formation can be induced in the Jemalong genotype 2HA by application of the hormones auxin and cytokinin. In addition to the application of hormones to induce somatic embryogenesis there is the stress component, induced by the excision and culture of the explant, to consider. In Medicago truncatula there are many stress-related proteins associated with SE. A number of these proteins are differentially expressed between 2HA and wild-type Jemalong. The synthesis of the stress-related hormone ethylene can be rapidly evoked in response to a variety of biotic and abiotic stresses including wounding. The wound effect would most likely be augmented by the presence of auxin in the medium.

We have recently demonstrated an important role for an AP2/ERF transcription factor designated the MEDICAGO TRUNCATULA SOMATIC EMBRYO RELATED FACTOR 1 (MtSERF1) in somatic embryogenesis in the model legume Medicago truncatula. The idea that MtSERF1 is embryo specific and required for SE in M. truncatula is supported by several lines of evidence. Its expression is increased during the culture period and reaches a peak at approximately 3 weeks. In our study explant culture this is the time embryos are forming. In wild-type Jemalong where SE is rare there is no increase in MtSERF1 expression. Furthermore, in situ mRNA localization analysis reveals that its transcripts are preferentially localized in embryos and not in callus cells. Finally, RNAi silencing of MtSERF1 results in virtually total suppression of somatic embryo formation.

In our study we demonstrated that ethylene was required for the transcription of MtSERF1. The importance of ethylene for its upregulation is highlighted by the following findings: first, qRT-PCR experiments showed that when either the inhibitor of ethylene biosynthesis or perception was added to the medium MtSERF1 was downregulated; second, in silico analysis of its promoter region revealed the presence of three ethylene regulatory regions; and third, no signal was detected by in situ RNA localization when explants cultured on medium containing the inhibitor of ethylene perception were hybridized with either antisense or sense MtSERF1 RNA probes. As in our previous studies auxin and cytokinin were present in the medium to induce SE. Both auxin and cytokinin are necessary for MtSERF1 transcription as qRT-PCR experiments reveal that when either auxin or cytokinin is absent MtSERF1 is downregulated. Furthermore the MtSERF1 promoter region contains putative binding sites relating to auxin and cytokinin in addition to ethylene. These data overall support the idea that the stress hormone ethylene produced in response to the stress of the explant excision and culture interacts with the growth and differentiation hormones auxin and cytokinin to promote SE. MtSERF1 transcription may act as a nexus for auxin, cytokinin and ethylene action to promote SE. It has been
suggested that transcription factors interconnect different hormone pathways and play an important role in hormone signal transduction. Furthermore, it is well established that transcription factors mediate key effects of hormones in development. The finding of a relationship between an ERF subfamily gene and the formation of somatic embryos in vitro is consistent with an emerging picture of the involvement of ERF proteins in developmental processes studied in vitro. The **ENHANCER OF SHOOT REGENERATION 1 and 2 (ESR1 and ESR2)**, and **RAP2.6** have a role in shoot regeneration in Arabidopsis. **BABY BOOM (BBM)**, a member of the AP2 family possessing two repeated AP2/ERF domains, has been shown to induce somatic embryogenesis when overexpressed in Arabidopsis and **Brassica napus**. Recently a gene called **ERF REQUIRED FOR NODULATION (ERN)**, has been identified in *Medicago truncatula*. Furthermore ethylene and **PLETHORA**, other member of the AP2/ERF superfamily are involved in stem cell maintenance in the root apex. Although, all these genes were studied in the context of developmental roles, most of the ERF proteins have been studied in relation to biotic and abiotic stress.

Therefore, it appears that the AP2/ERF superfamily has a mix of transcription factors involved in growth and development as well as abiotic and biotic stress. This may relate to the need to link development and stress in the evolution of sessile plants.

Another interesting finding from this work is the presence of two WUSCHEL binding sites in the promoter region of *MtSERF1*, which raises the possibility that there is a link between *WUS* and *MtSERF1*. This is further supported by the fact that both genes are found to similarly localize in the embryo of *M. truncatula* and *WUS* (the *M. truncatula* homolog) expression is maximal prior to *MtSERF1* expression (our unpublished data). It has been well appreciated that *WUS* plays a dominant role in regulating the stem cell population in shoot and flower meristems, however, *WUS* has also been shown to have the ability to be a somatic embryo organiser in Arabidopsis. The almost total suppression of the embryogenic capacity of *MtSERF1* knockdown suggests a failure to promote or maintain an embryonic stem cell fate in the cultured leaf explants. It is therefore possible that *MtSERF1* functions downstream of *WUS* as a positive regulator in promoting or maintaining an embryonic stem cell fate.

The downstream targets of the *MtSERF1* transcription factor are not known. One possibility is that *MtSERF1* interacts with HD-Zip III genes involved in the determination of cell fate and embryonic patterning, including **PHABULOSA (PHB), PHAVOLUTA (PHV), and REVOLUTA (REV)**, as these genes are also expressed throughout the early globular embryo in Arabidopsis and are also required for normal shoot apical meristem (SAM) development. The interaction between one of these genes with a member of the ERF subfamily has been demonstrated recently. *Chandler and co-workers demonstrated that a heterodimeric complex between ESR1 or ESR2 and PHV or related HD-ZIP III proteins is necessary to control the transcription of target genes required for embryo patterning and normal cotyledon development.*

Therefore, it is also possible that cross-coupling between *MtSERF1* and one of these genes is needed in mediating the induction of SE. Further, there is also the possibility that *MtSERF1* is involved in a regulatory interaction with *WUS* and these HD-ZIP III proteins. Sieber et al. have demonstrated that *WUS* and *PHB* regulate processes during the transition from proximal-distal to adaxial-abaxial ovule development. The overlapping expression of all these genes at the early globular-stage embryo make this idea an attractive hypothesis (Fig. 1).

Understanding precisely how *MtSERF1* is regulated by different hormone and stress factors and how *MtSERF1* interacts with other genes or transcription factors involved in the promotion and maintenance of stem cells and embryo patterning will provide a useful contribution to understanding how a single somatic cell can become a whole plant.

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