

THE EMBRYO MADS-DOMAIN PROTEIN AGL15 DIRECTLY REGULATES EXPRESSION OF A GENE ENCODING A GIBBERELLIN 2-OXIDASE

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ABSTRACT

AGL15 is a member of the plant MADS-box family that encodes transcriptional regulators. MADS proteins share a conserved ~60 amino acid residue region, termed the MADS-domain that mediates sequence-specific binding to DNA. The MADS family is relatively large in plants, with over 100 members in the model plant *Arabidopsis thaliana* (Parenicová et al., 2003). The better understood members have key roles in meristem and floral organ development (reviewed in Riechmann and Meyerowitz, 1997). Other members are expressed in other tissues, and their roles are less well understood. *AGL15* is currently the only identified member of the MIKC subgroup that is expressed preferentially in embryonic tissues. The MIKC subgroup includes a more weakly conserved K-domain that is involved in protein interactions, with an intervening linker (I) between the MADS (M) domain and K-domain. The carboxyl (C) terminal domain can be very divergent. *AGL15*-specific antiserum recognizes cross-reactive protein in a wide variety of angiosperm tissues developing in an embryonic mode, including zygotic, apomictic, microspore and somatic embryos (Perry et al., 1996; Perry et al., 1999). Additionally, constitutive expression of *AGL15* by a *35S:AGL15* transgene promotes development of tissue in an embryonic mode. When developing zygotic embryos containing the *35S:AGL15* transgene were placed into culture they produced secondary embryos more frequently than embryos lacking the transgene, and then, with subculturing, maintained development in embryonic mode for extended periods of time (over 7.5 years to date; Harding et al., 2003). The *35S:AGL15* transgene also enhanced production of somatic embryos from the shoot apical meristem (SAM) of seedlings that complete germination in liquid culture containing 2,4-D (Harding et al., 2003).

To better understand how *AGL15* contributes to embryo development, a chromatin immunoprecipitation (ChIP) approach was used to isolate DNA fragments that *AGL15* binds *in vivo* in order to identify genes directly regulated by *AGL15* (Wang et al., 2002). One DNA fragment isolated by ChIP corresponded to the 5' regulatory regions of *AtGA2ox6*. This gene encodes a gibberellin (GA) 2-oxidase that is predicted to be involved in catabolism of biologically active GAs. The ability of this gene product to convert GA₄ and GA₁ to GA₃₄ and GA₈ respectively was confirmed (Wang et al., 2004). Additionally, tests were performed to verify that *AtGA2ox6* was expressed in response to *AGL15* binding to its 5' regulatory elements (Wang et al., 2002; Wang et al., 2004).

During silique development, *AtGA2ox6* was expressed predominantly during the earlier stages of development and preferentially in the embryo (Wang et al., 2004). The amount of *AtGA2ox6* transcript declined as the siliques matured. This temporal and spatial pattern of expression coincides with presence of *AGL15* (Perry et al., 1996), supporting a role for *AGL15* in regulation of this GA 2-oxidase during embryogenesis. *AtGA2ox6* was also expressed after completion of germination predominantly in roots, but also in stems and rosette leaves, where it is likely that other factors regulate its expression. Plants that expressed *AtGA2ox6* ectopically and at higher amounts via a *35S:GA2ox6* transgene and plants that had decreased expression of

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AtGA2ox6 were obtained to help assess *in vivo* function. Similar to plants with a *35S:AGL15* transgene, plants carrying *35S:GA2ox6* transgenic constructs had shorter petioles, rounder leaves, were somewhat greener in color, flowered later than non-transgenic plants, and had increased number of inflorescence branches (Fernandez et al., 2000; Wang et al., 2004). Not all aspects of the *35S:AGL15* phenotype were present in the *35S:GA2ox6* plants however. For example, *35S:AGL15* plant showed delayed senescence and abscission of floral organs (Fernandez et al., 2000), but *35S:GA2ox6* plants did not. The shared phenotypes, and the function of GA 2-oxidases were consistent with deficiencies in biologically active GAs, therefore GA profiles in the ectopic expressing plants were compared to wild type. In both *35S:AGL15* and *35S:GA2ox6* leaf tissue, decreased amounts of GA₄ and GA₁ were present, while the catabolites for these GAs were present at higher amounts. Consistent with this finding, treatment of plants with biologically active GA₃ alleviated many aspects of the *35S* transgene phenotypes.

The loss-of-function mutation in *AtGA2ox6* caused a decrease in the amount of transcript, but there were no significant morphological phenotypes. However, *ga2ox6-1* knock-down mutants had decreased seed dormancy compared to wild type, while *35S:GA2ox6* seeds had increased dormancy. To test whether AGL15 requires *AtGA2ox6* to promote somatic embryo development from the shoot apical meristem of seedlings, the *ga2ox6-1* mutant was crossed into the *35S:AGL15* background. A significant decrease in percentage of *35S:AGL15, ga2ox6-1* seedlings that produce SAM embryos compared to *35S:AGL15* was observed, but the decrease was not to non-transgenic levels. This could be because the mutant is a knock-down rather than a null mutant, or due to regulation of other genes by AGL15. Conversely, the *35S:GA2ox6* transgene was able to promote SAM embryo formation. Because of the effects of *AtGA2ox6* expression on SAM embryo development, further experiments were performed to determine whether manipulation of biologically active GA amounts would impact on SAM embryo development. Addition of GA₃ to the media decreased the frequency of embryo production for both non-transgenic and for *35S:AGL15* seedlings, while inhibition of GA biosynthesis by addition of paclobutrazol increased the frequency of SAM embryo production.

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