The contribution of an *RCO* enhancer to the diversification of leaf shape

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Abstract

***Cardamine hirsuta REDUCED COMPLEXITY* (*RCO*) originated from a duplication of *LATE MERISTEM IDENTITY1* (*LMI1*) and acquired a novel role as a key regulator of leaf complexity. Recently, transgenic approaches demonstrated that discrete paralogous enhancer sequences upstream of *RCO* (*ChRCOenh*^{500}) and *LMI1* (*ChLMI1enh*^{500}) are sufficient to drive the respective expression domains of the two genes. Transgenic approaches also indicated that these sequences are necessary for *ChRCO* and *ChLMI1* expression. Nevertheless, the specific functionality and properties of these regulatory sequences are difficult to infer on the basis of transgenes alone. To resolve this issue, it is essential to understand to what extent these regulatory elements are necessary to correctly express the endogenous *RCO* and *LMI1* and define more specific sequences that contribute to their distinctive functions. Here, I aim to address this question using a CRISPR/Cas9-based targeted genetics approach. The results show that mutations in the *LMI1* enhancer frequently cause loss-of-function phenotypes and reduction in *LMI1* transcript levels. On the contrary, mutations in the *RCO* enhancer cause a much broader spectrum of phenotypic variation, frequently correlated with increased leaf dissection and *RCO* expression levels and only in a few cases with weak loss-of-function phenotypes. These observations suggest that the *RCO* enhancer evolved more negative regulatory elements than its *LMI1* paralogues and that its regulatory activity has been buffered, probably by redundantly *cis*-acting elements. In addition, I present evidence that the negative regulation of *RCO* was at least partially evolved to suppress *RCO* expression in the *LMI1* domain. Thus, *RCO* stands as an excellent example of neofunctionalization after duplication, obtained mainly by recasting its *cis*-regulatory elements.**