Enhancing the possibilities of promoter research

Rainer Melzer
School of Biology and Environmental Science, University College Dublin, Ireland
rainer.melzer@ucd.ie

Developmental regulatory genes have played a pivotal role during the evolution and domestication of plants. From a molecular genetics perspective, our understanding of those regulators is largely driven by the analysis of full loss-of-function mutants. This has provided profound insights into gene regulatory circuits governing developmental processes. However, from the analysis of evolutionary and domestication processes it is also evident that in many cases not loss-of-function mutations but variations in gene expression patterns and expression strength caused phenotypic change (Meyer and Purugganan, 2013). This is at least partly due to the pleiotropic functions of many developmental regulators: complete loss-of-function mutations often yield so dramatic phenotypes that the fitness of the plant is severely impaired, hence the relevance of null mutants during evolution and domestication is limited.

From the analysis of a few exemplary cases we know that the modularity of promoter architectures is one important component of pleiotropic gene functions. For example, APETALA3, a transcription factor coding gene that controls petal as well as stamen development, has specific enhancer regions that are required for AP3 expression in stamens (Hill et al., 1998). Studying promoter functions at the molecular level is therefore extremely valuable for our understanding of evolutionary and domestication processes. However, detailed promoter studies are usually quite laborious and are largely limited to a few genetic model plants. Promoter studies often encompass the identification of putative enhancer elements using 
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decreases and the expression of a reporter gene under control of a mutated promoter version lacking those elements (or containing multiples of them). Altered expression pattern of the reporter gene informs about the function of putative enhancer elements (Hernandez-Garcia and Finer, 2014). Although this approach has been very successful, 
in silico(predictions do not always identify critical promoter elements (Hong et al., 2003). In many cases, a more unbiased approach to study promoter functions might be at least equally promising. A recent publication by Rodriguez-Leal et al. (2017) presents a method that will substantially facilitate such an unbiased molecular genetic analysis. Using tomato fruit size as a model system, the authors target the promoter of Solanum lycopersicum CLAVATA3 (SCLV3), a gene known to be involved in fruit size regulation, using CRISPR-Cas9. However, unlike more
conventional CRISPR-Cas9 approaches that aim to generate full loss-of-function mutants, the authors designed not just one but eight guide RNAs that spanned a 2 kb range of the putative promoter region of *SlCLV3*. Because of variations in guide RNA directed cleavage activities and subsequent repair processes, many types of mutations can be induced, ranging from large promoter deletions to inversions, small deletions and single nucleotide substitutions (Rodríguez-Leal *et al.*, 2017).

As the analysis of the mutant plants proved difficult because different mutations are introduced in the two alleles of the target gene, the authors devised a genetic screen in which they crossed a wild-type plant with a transgenic plant that expressed the eight guide RNAs and Cas9 and also carried a full loss-of-function allele of *SlCLV3*. In the resulting progeny, mutations were induced by CRISPR-Cas9 in the wild-type allele. In this set-up, the phenotypic effects of even mutations causing only subtle phenotypic changes are relatively easy to detect as the second *SlCLV3* allele is a null allele. Thus, Rodríguez-Leal and colleagues essentially developed an elegant yet simple procedure to randomly mutagenize one particular locus and screen for phenotypic consequences of the mutations. Indeed, using this system, it was possible to create an allelic series of 14 *SlCLV3* mutant alleles that showed a continuous variation in carpel number (thus leading to a variation in fruit size) (Rodríguez-Leal *et al.*, 2017).

This method is potentially of huge importance for dissection promoter functions. It may be possible to quickly generate a large number of random mutations and analyse their phenotypic effect in a streamlined way. This will prove to be very powerful to untangle the functional elements in promoters. In turn, our understanding of how master regulators of development may have contributed to morphological evolution may be substantially increased. For example, it has been proposed that alterations in the expression of floral homeotic transcription factors contributed to floral diversity (reviewed by Theißen and Melzer, 2007). The approach presented by Rodríguez-Leal *et al.* (2017) constitutes a promising avenue to test whether and how promoter mutations can induce such phenotypic changes. Theoretically, a large number of mutant alleles can be generated from one transgenic plant. Thus, the approach may also be suitable for phylogenetically informative non-model plants that are difficult to transform.

Last but not least, plant domestication and crop improvement often proceed via variations in quantitative traits. Rodríguez-Leal *et al.* (2017) demonstrated that a substantial variation in tomato fruit size can be obtained in just a few generations, bypassing years of breeding efforts. It will be interesting to see to which extent the same method can be applied to other quantitative traits in crops.

References


