Transgene Inactivation in *Arabidopsis thaliana*

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1 Introduction

This review on transgene inactivation in *Arabidopsis thaliana* should not conceal the fact that—as in other plant species—there are numerous transformants which exhibit stable transgene expression and inheritance over many generations. Nevertheless, occasional loss of transgene expression in the progeny has been observed in many transformation experiments with *Arabidopsis*. In most cases, selection among the transgenic lines for those with a reliable gene expression is sufficient, and the exceptional lines can be treated as an experimental failure. However, the introduction of transgenes can be followed by silencing of foreign and endogenous genes, sometimes only in later generations, in attempts to achieve up- or downregulation of specific gene activity by overexpression or antisense inhibition as well as insertion mutagenesis. The interpretation of some results may therefore require consideration of the possibility of gene silencing.

Furthermore, and more importantly, the occurrence of gene silencing in
Arabidopsis thaliana offers the chance to study an important and interesting biological phenomenon in a plant species whose qualification as a model organism in other respects is beyond dispute. The main attributes, such as the small size and space requirement of the plant, its short generation cycle and ample seed production, the low chromosome number and the existence of various ecotypes, were emphasised by LAIBACH as long ago as 1943. Since then, numerous publications have referred to further advantages, especially for plant molecular genetics. Dense genetic maps based on restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), and morphological markers, transformation techniques and numerous mutations obtained by chemical and insertion mutagenesis are available, and a world-wide endeavour was made to characterise plant genes and their interaction in a genome with a size of only 100 Mb (for review see REDEI 1975, MEYEROWITZ 1989, KONCZ et al. 1992a). Although the model character does have its disadvantages (the amount of material and short lifetime of individual plants, the restricted tissue culture response, the tedious crossing procedure), there is no doubt that these are outweighed by the outstanding insights provided into the organisation of a plant genome. This contribution is intended to explain why this knowledge might turn out to be very helpful in understanding gene silencing in plants. I will therefore concentrate on the genetic perspective of transgene inactivation in Arabidopsis. For a more comprehensive discussion including biochemical and physiological aspects of possible underlying mechanisms the reader is referred to other contributions in this volume.

2 Unexpected Segregation of the Transgenic Phenotype

In the earliest reports on the introduction of marker genes into the genome of Arabidopsis thaliana, the analysis of genetic transmission was limited to three lines (LLOYD et al. 1986) or two lines (SHEIKHOLESLAM and WEEKS 1987). In both cases, the resistant phenotype segregated according to the expectation for single or multiple copies of the gene. The extension of genetic analysis to a larger number of independent transformants in later studies revealed that 10% of lines obtained after transformation with Agrobacterium tumefaciens exhibit reduced representation of the transgenic phenotype among their selfed progeny (13/124, FELDMANN and MARKS 1987; 2/20, SCHMIDT and WILLMITZER 1988; 1/10, VALVEKENS et al. 1988; 2/11, SANGWAN et al. 1991; 1/28, BOUCHEZ et al. 1993; 1/17, BECHTOLD et al. 1993). The frequency of this observation was similar for Agrobacterium infection of leaf disks (SCHMIDT and WILLMITZER 1988), roots (VALVEKENS et al. 1988), seeds (FELDMANN and MARKS 1987), zygotic embryos (SANGWAN et al. 1991) and whole plants (BOUCHEZ et al. 1993; BECHTOLD et al. 1993). The incidence of non-Mendelian segregation ratios in transformants obtained by direct gene transfer to protoplasts is even higher: figures of 25% (6/25, DAMM et al. 1989) and 50%