STUDIES OF PLANT GENES USING MAIZE TRANSPOSONS

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INTRODUCTION

Without previous knowledge of the molecular and biochemical properties of a gene, the phenotypic expression of a mutant is very informative in understanding how a gene plays a part in plant development. Moreover, loss of function mutants could lead to a better understanding of existing biochemical and molecular pathways. The generation of mutants using physical, chemical and insertional agents is a common strategy for genetic approaches to biological problems. For example γ-irradiation and alkylating agents such ethylmethane sulfonate (EMS) typically lead to physical and chemical mutagenesis, respectively. Chemical mutagens can be very effective considering that the frequency of recessive mutations per locus can be raised as high as 1 in 800 for EMS treated gametes in maize (Neuffer, 1982). The highest spontaneous mutation rate of a maize gene is 0.55×10^{-6} (Stadler, 1946), even though there is some variation. Due to

the high mutation frequencies, it is relatively easy to obtain mutations in genes of interest through proper selection schemes . However, studies with chemically or physically induced mutations have a significant limitation. Due to a large genomic size and lack of proper molecular markers it can be extremely difficult and time-consuming to clone genes of interest. 'Map based cloning' has led to some success in the small Arabidopsis genome of ca. 150 Mb (Mindrinos et al., 1994; Petter et al., 1994). T-DNA elements and maize transposons are most commonly used in plants as insertional mutagens. T-DNAs delivered by Agrobacterium into plant cells are integrated into the plant genome through illegitimate recombination. The effectiveness of T-DNA mediated insertional mutagenesis has been evaluated in Arabidopsis by Feldman (Feldmann, 1991). In Arabidopsis, a number of genes have been cloned by T-DNA tagging strategy (Yanofsky et al., 1990; Deng et al., 1992; Wei et al., 1994; Castle and Meike, 1994) although the efficiency of this method is limited by immobility of the element once inserted into a genome. Endogeneous transposable elements from maize and snapdragon have likewise been used for 'tagging' and cloning genes of interest. Mutagenesis and gene cloning using transposon tagging and T-DNA insertional mutagenesis has been reviewed in detail (Walbot, 1992). The transposable elements Ac/Ds (Activator/Dissociator) and Spm/dSpm (Suppressor-mutator/defective Spm) have been extensively characterized in higher plants (for review, see Federoff, 1989). The mobility and utility of maize Ac/Ds and Spm/dSpm as insertional agents have been demonstrated in heterologous plants (Murai et al., 1991; Scofield et al., 1992; Swinburne et al., 1992; Caron et al., 1993). The scope of this review is limited to new advanced strategies of insertional mutagenesis in plants using the maize transposon (Ac/Ds) mediated enhancer and gene trapping system. Also, the new strategy of 'site selection cloning' for genes from a pool of insertion mutants will

be introduced. It should be emphasized that these strategies are not novel but were originally developed in Drosophila, C. elegans and mice. Recently these systems have been successfully applied in plants.

DISCUSSION

1). Transposon mediated enhancer and gene trapping systems in plants

While conventional transposon-aided gene tagging strategy offers an opportunity to get molecular access to genes responsible for phenotypic expression of interest, most of the tagging transposons do not give spatial and temporal information on the expression of host genes. Maize Ac/Ds and Spm/dSpm transposons can be genetically engineered without affecting their ability to transpose. Enhancer and gene traps are variants of conventional transposable elements. An enhancer trap transposon contains a weak or minimal promoter fused with the open reading frame of a reporter gene and a genetic marker. In some cases insertion will result in cis-activation of the weak or minimal promoter by enhancer sequences of host genes. P element and 35S CaMV (cauliflower mosaic virus) minimal promoters are commonly used for this system in Drosophila and plants, respectively (Bier et al., 1989; Wilson et al., 1989; Benfey and Chua, 1989). The reporter genes usually encode b-galactosidase (lacZ) in animals and b-glucuronidase (gusA) in plants. A gene trap transposon contains endogenous or artificial splicing acceptor sites fused to a reporter gene in three different frames and a genetic marker (Kerr et al., 1989). A fusion protein of the reporter gene with the N-terminal portion of a host gene will be produced if the elements is inserted into either an exon or intron of the host gene in the same orientation. Recently, plant scientists at Cold Spring Harbor Laboratory have successfully applied the enhancer/gene trapping system to Arabidopsis using the maize Ac/Ds

transposable element (Springer et al., 1995). In enhancer trap Ds or dSpm, a minimal promoter fused with a reporter gene and a genetic marker are inserted into the elements. The Ds genetic marker is expressed by a promoter that will not transactivate the minimal promoter. An enhancer trap Ds construct is inserted into or next to the Ds excision marker. In the case that the Ds is inserted into the excision marker, the function of the excision marker will be restored once Ds excises from the original site. The Ds is inserted next to a negative selection marker, which enables selection against plants containing the original Ds insertion locus. In gene trap system, an intron with three alternative splicing sites but no splicing donor site, is fused to GUS at the end of gene trap Ds. The three splice acceptor sites are in different frames relative to the coding region of a reporter gene. Therefore, one of the three splicing products from fusion transcripts of host gene and reporter should lie in the right context to a reporter gene.

The basic genetic strategy of the enhancer/gene trapping systems is the following:

Using the maize non-autonomous transposons (Ds and dSpm) and enhancer/gene detection system, random insertions will be generated throughout a plant genome with aid of non-mobile transposase sources (Ac and Spm). Plants carrying translocated transposons will be selected using genetic markers present along with the elements at the original insertion sites. Plant genes trapped by these systems can be identified by in situ activity staining of the reporter gene present in Ds or dSpm.

The following list is a brief description of unique features of enhancer/gene trapping systems:

1. Since a reporter gene (gusA) is a dominant trait, the expression

patterns of trapped cellular genes can be examined throughout a life cycle by in situ activity staining. This is a very valuable technique for the study of homozygous lethal mutations that lead to death during embryogenesis or seedling stage.

2. Since the expression of a reporter gene is driven by the transcriptional machinery of a host gene, these systems are extremely powerful to evaluate the interactions of the host gene with exogeneous factors (e.g., drought or pathogen), and to understand the relationship with other known genetic factors.
3. Since a reporter gene can tolerate a large N-terminal fusion of a cellular protein that often contain subcellular localization signals, a gene trapping system can help identify intracellular destiny of the gene products.
4. Previous knowledge of the sequence of the inserted element greatly facilitates the cloning of trapped genes using techniques such as RACE-PCR and IPCR.

2) Site-selected transposon mutagenesis.

Since maize transposons are mobilized and inserted without insertion site specificity, random insertions of any given transposon can be generated throughout the genome. It is clearly necessary to generate a large number of independent insertions such that any given gene is tagged. Theoretically, the total number of independent insertions that are necessary for a transposon to jump into any gene of a plant genome with 95% probability can be calculated. For example, consider the rice genome of roughly 450 megabases and a plant gene of average size 10 kb. The total number of independent insertions into the rice genome required for 95% saturation can be estimated in the following way: $P(0.05) = (1-(10/450,000))n$, where n is the number of independent insertions. Based

on this formula, ca. 135,000 independent insertions are needed. If a single plant carries 50 copies of a transposon, at least 2,700 (135,000/50) lines will have to be propagated. Transposon insertions into genes that have already been cloned can easily be isolated. However, transposons in maize are preferentially inserted into transcriptionally active genes. Therefore, the number of independent insertions necessary for 95% saturation can be lower 2 - 3 fold. It has been shown in Drosophila and C. elegans that such insertions can be detected by PCR using primers from both the cloned gene and terminal sequences of the transposable element (Ballinger and Benzer, 1989; Kerr and Goodwin, 1990; Rushforth et al., 1993). This technique is called 'site-selected transposon mutagenesis'. Recently, such a scheme has been successfully applied to the maize hcf106 locus using Mu (Mutation) element lines (Das and Martienssen, 1995). This is an extremely powerful technique, what with the abundance of the randomly sequenced cDNAs and little known of their function and phenotypic expression.

CONCLUSION

The expression of genes is temporally and spatially controlled throughout life cycle of higher organisms. In higher plants, such developmental programs are triggered or altered by environmental cues and foreign agents (e.g., pathogens). To better understand the relationships between genes and environmental factors, it is informative to obtain mutations in genes of interest and to monitor the expression patterns of the genes in planta. Maize transposon systems, Ac/Ds and Spm/dSpm, have successfully been utilized to 'tag', 'trap', and 'knock-out' genes of interest. The gene trapping systems developed aim not only to generate phenotypic mutations but also detect expression patterns of genes. Furthermore, through the reporter systems in transposons, responses of

'trapped' genes to external factors can be monitored in planta. This offers an opportunaty to study the interactions between genes and external factors. These genetic approaches should be an excellent tool to study plant genes at the developmental and phenotypic levels. Recently, efforts have been made to sequence expressed cDNA and to map DNA markers and mutants on chromosomes. Random cDNA sequences offer little information on biological function of these genes except for inferences made by sequence homolgy. Gene tagging and trapping systems should be excellent tools to further understand the biological significance of genes. Also, these systems provide us with the genetic and molecular tools to conduct more efficient genomic mapping and sequencing projects. Furthermore, once sufficient numbers of lines harboring transposons are developed in any given species, site-selected mutagenesis will be a powerful tool to obtain phenotypic information about mutations in genes of interest.

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