Insertional mutants: a foundation for assessing gene function

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The abundance of *Arabidopsis* insert mutants portends the day when null alleles in every gene will be obtained. Once these are created, all plant scientists can become geneticists. However, this brief technical highlight of genetic concepts cautions against ascribing gene function based exclusively on phenotypic analysis of null alleles.

The cornerstone of FORWARD GENETICS (see Glossary) is to create mutants, analyze phenotypes and then stalk the genes. In REVERSE GENETICS, genes are known and phenotypes hunted. The lack of efficient HOMOLOGOUS RECOMBINATION has been the arrow that plant reverse genetics has lacked in the quiver. Recently, methodologies have advanced to alleviate this impediment. Currently, there are several large collections of insertion mutant lines available in *Arabidopsis* where the insertion sites have been sequenced [1]. Researchers can use computers to scan these 'sequence banks', and often the only thing separating a plant scientist from becoming a geneticist is a materials transfer agreement. Here I review a standard tenet of genetics: create and analyze multiple ALLELES before ascribing gene function.

Moving quickly in reverse

Once it has been determined that a given PHENOTYPE is caused by a T-DNA or transposon INSERTION, the *Arabidopsis* sequences flanking the insertion site can be characterized quickly [2]. The simplicity of this approach is magnified by the ease with which these insertion mutants are now being created. Many laboratories are using this mutagenesis approach for isolating second site SUPPRESSOR MUTATIONS [3]. This technology has devalued the tracking of mutants and has placed an increased emphasis on phenotype analysis.

Many of these gene knockouts do not appear to produce a phenotype even in the presence of severe environmental stresses [4]. However, regardless of phenotype, plant scientists should exercise caution when using insertions as the sole means of assigning gene function.

Functional overlap–redundancy and compensation

In spite of the compact size of the *Arabidopsis* genome, functional overlap within gene families is well documented [5]. However, functional overlap can be explained by two distinct mechanisms [6]. A tissue that expresses more than one gene family member might require inactivation of each of the proteins to visualize a phenotype. The continual presence of an unambiguous 'backup' system is referred to as genetic redundancy. Alternatively, the loss of a specific gene family member might result in another gene product inappropriately 'stepping in'. This scenario is best described as functional compensation [6]. A useful analogy would be a gourmet restaurant using paper cups to serve wine when all the wine glasses are in the dishwasher. Although the outcome might be the same as using wine glasses, it is an abnormal occurrence and represents a departure from standard procedures.

Examples of functional compensation

Although functional compensation has been demonstrated among mice retinoblastoma genes [6], the most-detailed

Glossary

General terms:
Allele: one of the different forms of a gene or DNA sequence that can exist.
Forward genetics: this process begins with a mutant phenotype but says nothing about the nature of the gene.
Homologous recombination: specific gene disruptions are made by replacing the endogenous gene with an altered version of that gene.
Phenotype: the characteristics of the organism, usually with respect to the traits a particular gene controls.
Reverse genetics: this process initiates from the gene sequence and tries to generate a mutant phenotype.
Making mutant alleles:
Co-suppression and RNAi: transgenes that can be used to achieve gene silencing by overexpressing a copy of a gene of interest or expressing a double-stranded RNA.
Ethyl methane sulfonate (EMS): a mutagen that preferentially induces G-to-A transitions throughout the genome.
Insertions: T-DNA or other transposons that cause large insertions and frequently cause loss of gene function.
TILLING (Targeted Induced Local Lesions In Genomes): a PCR mutagenesis protocol to create single-base mutations in particular genes. This method allows the creation of multiple alleles.
Consequence of the mutagen:
Hypermorphic: an allele that confers more function than the wild-type protein does.
Missense mutation: a base substitution that results in amino acid substitutions.
Neomorph: an allele that causes the protein to have a new function that is not present in the wild type.
Null Alleles: an allele that makes no gene product or whose product has no activity.
Suppressor mutation: a mutation that counteracts the effects of another mutation. A suppressor maps at a different site than the mutation it counteracts.
Wild type: a genotype or phenotype that is found most commonly in nature or the standard laboratory stock.

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example that has been documented is the yeast mitogen-activated protein kinases (MAPKs). Fus3p and Kss1p are MAPKs that when analyzed as null mutants appear to be functionally equivalent in the yeast mating response [7,8] (Fig. 1). In *fus3* null mutants where Fus3p is absent, cells still mate. In *kss1* null mutants, cells also mate; but *fus3 kss1* double mutants are sterile. Apparently, in the absence of the Fus3p protein, Kss1p can replace Fus3p function in the mating response. However, when particular point mutations are made in Fus3p, which appear to disrupt FUS3 activity, Kss1p cannot replace the inactive allele, and cells are sterile. This example shows that the use of NULL ALLELES and numerous point mutations in *FUS3* and *KSS1* must be analyzed to disentangle the relationship between these kinases.

Functional compensation can also be seen when null alleles of calcium transporters from humans, yeast and plants are analyzed. In the human pathology of Brody disease, the functional knockout of a sarcoplasmic–endoplasmic reticulum calcium transporter leads to muscle stiffness and cramps [9]. In normal muscles, this calcium transporter is essential for pumping calcium back into the endomembrane to initiate muscle relaxation. However, Brody patients have partially compensated for the loss of this calcium transporter because muscle relaxation can occur, albeit at a reduced rate. This compensation is thought to be due to upregulation of calcium transporters at other membranes. A similar compensation among calcium transporters occurs in yeast. Yeast strains that contain a null allele of a Golgi calcium transporter show compensatory induction and altered localization of vacuolar calcium transporters [10]. In my laboratory, we recently isolated null mutants of a putative vacuolar calcium transporter, CAX1 [11]. In these mutants, we see increased activity of other calcium transporters and increased expression of genes similar to CAX1. In each of these examples, the mutant cells have altered the activity (and in some cases the localization) of proteins to compensate for loss of gene function.

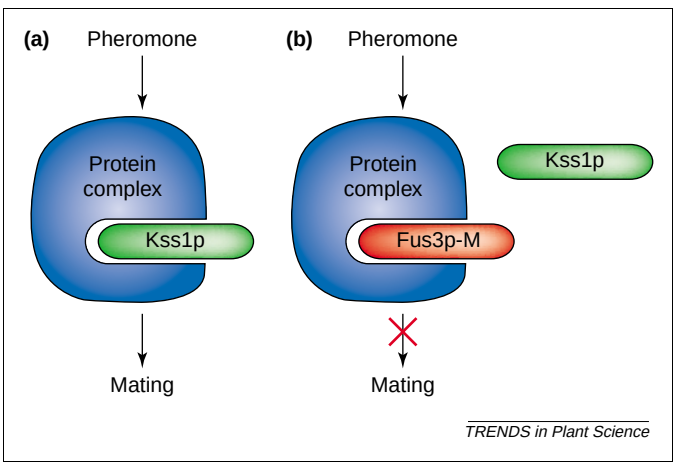

Fig. 1. A null allele and point mutation of Fus3 suggest different roles of Kss1p in haploid cells [7.8]. (a) *fus3Δ*: Kss1p functionally compensates for Fus3p by interacting with a protein complex that usually recognizes Fus3p. In this genetic background, Kss1p functions in the mating pathway. (b) *fus3-M*: a mutant form of Fus3 protein interacts with a protein complex and Kss1p is excluded. In this genetic background, strains are sterile.

No simple interpretations

Functional overlap aside, a WILD-TYPE phenotype for a null mutant is regularly interpreted as meaning a gene product does not function in the process that the phenotype measures [7]. However, even this straightforward conclusion might not be justified. For example, if a null allele disrupts both positive and negative activities of equal strength, its removal, via an insertion, will not significantly disrupt the normal process. Again, an example of these positive and negative effects can be seen in the dual roles of Kss1p in yeast invasive growth [7,8].

Always room for a classic

Classic mutagens such as ETHYL METHANE SULFONATE (EMS), fast neutron and radiation, along with emerging technologies such as RNA interference (RNAi), CO-SUPPRESSION and TILLING offer the opportunity to alter the dosage and function of a gene product to assess phenotypes [2]. An elegant example of classic genetics at work is the recent characterization of the *TED3* gene [12]. The *ted3* mutant was identified as an extragenic suppressor of *det1*, a mutant that develops as a light-grown plant in the dark. The *ted3* mutation is caused by a single MISSENSE MUTATION within the coding region of a peroxisomal protein. This mutation appears to be HYPERMORPHIC, and allows the investigators to draw a novel link between peroxisomes and light signaling. Certainly, if insertions had been used to generate the suppressors, the hypermorphic variant of *TED3* would not have been identified.

The function of TED3 in light signaling is not immediately clear and demonstrates why geneticists have typically preferred null mutants. Null alleles are not conclusive but the analysis of null alleles is easier to interpret than those obtained with missense mutations. In particular, the distinction between NEOMORPHS and hypermorphs is, in many cases, extremely difficult to make.

Conclusion

Individual mutations often only describe a subset of the functions of a gene [13], and only through molecular analysis of numerous alleles can the function of a gene be fully determined. The simplicity of using insertional mutations should not overshadow the power of other approaches, and T-DNA inserts should augment, not fill, a plant biologist's war chest.

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