REMI: A novel method for tagging genes

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Mutagenesis and gene cloning have traditionally been two separate tasks for the geneticist. But the realization that a transposon can be used both as a mutagen and also as a 'gene tag' now enables one to identify and isolate genes at one shot, particularly in systems such as bacteria, yeast, *Drosophila*, *Caenorhabditis* and maize.

But moving transposons around has not been possible in some systems like cellular slime moulds and filamentous fungi. The cloning of genes associated with aberrant phenotypes still remains an ardous task. However the work of Schiestl and Petes[1] has kindled a ray of hope in this direction. Much to their surprise, they found that the transformation frequencies increased considerably when the yeast *Saccharomyces cerevisiae* was transformed with a linearized plasmid together with the restriction enzyme used for linearization. In other words, the restriction enzyme stimulated the integration of the plasmid into cognate restriction sites in the genome.

The evidence suggested a 'simple' mechanism. The restriction enzyme enters the cell along with the linearized vector and cuts at the restriction sites in the genome. The host repair system does its job but at a low frequency, the sticky ends of the vector pair up with the compatible ends generated in the genome by the restriction enzyme. DNA ligase ties the loose ends up.

Encouraged by this finding, Kuspa and Loomis explored the possibility of restriction enzyme mediated integration (REMI) in the obstinate cellular slime mold, *Dictyostelium discoideum*. Much to their delight, they found that the efficiency of transformation increased more than 20-fold and the plasmid integrated more than 70% of the time at the appropriate restriction site[2].

This raised the prospect of using REMI as a mutagenesis-cum-gene tagging tool in *Dictyostelium discodeum*. The plan of action was simple. Transform the cells with a piece of sticky DNA carrying a selectable marker in the presence of the appropriate restriction enzyme and select for the transformants. Among the set of transformants generated, hunt for the one with the desirable mutant phenotype. If one is lucky to get a mutant, then isolating the gene into which the vector has inserted is not difficult. Cut the genomic DNA with a rare restriction enzyme. The fragment from the digest carrying the inserted vector can be rescued in *E. coli* and reintroduced into the wild type strain to disrupt the cloned gene by homologous recombination. The high frequency of mutants among the transformants would confirm that the phenotype was indeed because of a mutation in the cloned gene.

Kuspa *et al.* and Adachi *et al.* have executed the plan successfully by cloning the genes involved in cell–cell interaction and cytokinesis in *Dictyostelium discoideum* respectively[3,4]. REMI has been attempted in other systems, as well. Shi *et al.* have reported REMI enhanced transformation in the rice blast fungus *Magnaportha grisea*[5]. Success has also been reported in the maize pathogenic fungus *Cochleobolus heterostrophus*[6]. However, the presence of restriction enzyme seemed to have had no effect on transformation efficiency in *Neurospora crassa*[7]. There are no explanations as of now, why REMI works in some systems and not in others.

A new technique for physical mapping has been possible by using REMI in league with RFLP mapping. Kuspa and Loomis did REMI with a vector carrying a rare site Apa I within to generate a set of 147 transformants in *Dictyostelium discoideum*. An Apa I digest of the genomic DNA of the transformants yielded 2 fragments carrying the regions of the vector flanking the internal Apa I site. The probing of these 2 fragments with probes from the cloned genes enabled them to map the genes relative to the Apa I sites. Kuspa and Loomis published the detailed maps of the six chromosomes of *Dictyostelium discoideum* using this technique, REMI-RFLP mapping[8,9]. This method obviates the need to go in for other genetic data necessary for RFLP mapping.

REMI as a tool for insertional mutagenesis should enable one to identify genes which are not present in functionally redundant forms and not required for viability. The encouraging sign is that restriction enzymes have had no effect on the viability of the cells presumably because of an efficient host-repair system.

There is a point of doubt whether the integration is random[1] or non-random[2]. Despite the possible non-random nature of insertions, the number of transformants could be increased by using different restriction enzymes. REMI also holds a lot of promise in physical mapping in creating chromosomal aberrations and inducing mitotic recombination.

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