Oligonucleotide Mediated Mutagenesis

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Mutations are heritable changes in genomic sequences caused by radiation, viruses, transposes and mutagenic chemicals, as well as errors that occur during meiosis or DNA replication. On the basis of site of action mutation is of two kinds: random mutation and site-specific mutation. Oligonucleotide mediated mutagenesis deals with site specific mutation. G. Ruvkun and Fredrick M. Ausubel firstly developed a general method of site directed mutagenes is in prokaryotes. Oligonucleotide directed mutagenesis, is a molecular biology technique used to create mutation at a defined site in a DNA molecule. OMM is a technique used to correct or to introduce specific mutations at defined sites of the genome by incorporating a desired amino acid (of one's choice) in place of a specific amino acid in a protein or a polypeptide. It is referred under different names such as targeted nucleotide exchange, chimeraplasty, oligonucleotide mediated gene editing, chimeric oligonucleotide dependent mismatch repair, oligonucleotide-mediated gene repair, triplex-forming oligonucleotides induced recombination, oligodeoxynucleotide-directed gene modification, therapeutic nucleic acid repair approach, targeted gene repair.

OMM requires a synthetic oligonucleotide typically 20-100 bp in length, complimentary to the area of the gene of interest having desired nucleotide change. It makes use of different types of oligonucleotides: single-stranded DNA oligonucleotides containing 5_ and/or 3_ modified ends to protect the molecule against cellular nuclease activities, chimeric RNA/DNA or DNA/DNA., RNA oligonucleotides, and triplex forming oligonucleotides with homology to the target gene except for the nucleotide(s) to be changed. Introduction of the oligonucleotides in the cells do not require any vector delivery system, zinc-finger nucleases (ZFNs) to generate double-strand breaks at specific sites and also independent of any chemical modification in oligonucleotide.

It does not involve the integration of foreign genetic Oligonucleotide itself used as a vector for introduction of mutagenic agent in DNA site specific manner. Different techniques such as electroporation, lipofection, transfection or particle bombardment (biolistic) are used for delivery of oligonucleotides within the cell. A synthetic oligonucleotide encoding the desired mutation is annealed to target region of wild type template DNA where it serves as primer for initiation of DNA synthesis. Extension of oligonucleotide by DNA polymerase generates a double stranded DNA that carries the desired mutation. The mutated DNA is then inserted at appropriate location of the target gene.

OMM is based on the principle that small specific change in amino acid sequence at some critical site within a protein is responsible for change in performance of the protein and correspond ding phenotypic trait. It generates amino acid coding changes in the DNA by incorporating a desired amino acid in place of a specific amino acid in a polypeptide chain. By this approach specific site-directed mutagenesis can be made in the base (or bases) of the gene to produce a desired enzyme. Here, the DNA repair mechanism plays a vital role, involving the activation of the mismatch repair and/or nucleotide excision repair pathway. The oligonucleotide hybridizes at the targeted location in the genome to create a mismatched base-pair(s), which acts as a triggering signal for the cell's repair enzymes. The gene modification is induced directly and exclusively via the effect of the oligonucleotide itself. It means the process is a type of gene repair and not homologous recombination. OMM lead to in situ i.e. site-specific mutation targeting any nucleotide (regulatory, coding or non-coding) to inactivate deleterious gene effect through local modification in expression by changing an amino acid in the corresponding protein resulting a protein with possible new properties.

OMM has been used successfully in bacteria and yeast mainly as a tool to perform fundamental research on gene expression and regulation, aiming at

better understanding of the possible mechanisms underlying the genetic modification. It has been successful in restoring or knocking out wild-type genes in animal cells, creating mouse mutants by modification of embryonic stem cells, and in directing genetic improvement of livestock animals and also it seems to offer the potential to correct point mutations in human gene therapy. In case of plants *in vivo* gene modification has been demonstrated notably in maize, rice, tobacco and wheat, *e.g.* to create plants insensitive to the action of a specific herbicide. The efforts have been made to developed CLEARFIELD Production System plants in Brassica winter oilseed rape and spring canola using Rapid Trait Development System (RTDSTM) to enhance the tolerance levels of spring canola plants to CLEARFIELD herbicides.

OMM is conceptually an improved technique over conventional breeding and traditional mutagenesis techniques. It is theoretically more precise than other mutational techniques such as irradiation or chemical treatment and recombinant DNA technology which makes the risk to generate unintended effects in the genome of the recipient cells. The final product produced is similar and, in many cases, indistinguishable from conventionally bred or traditional mutagenesis products. Plants carrying the specific mutation are subsequently regenerated by tissue culture techniques and traditional breeding is deployed to efficiently breed the desirable trait into elite plant varieties while eliminating undesirable characterizes. OMM products are free from foreign nucleotides and have some safety corners as compared to other GMOs.

References

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